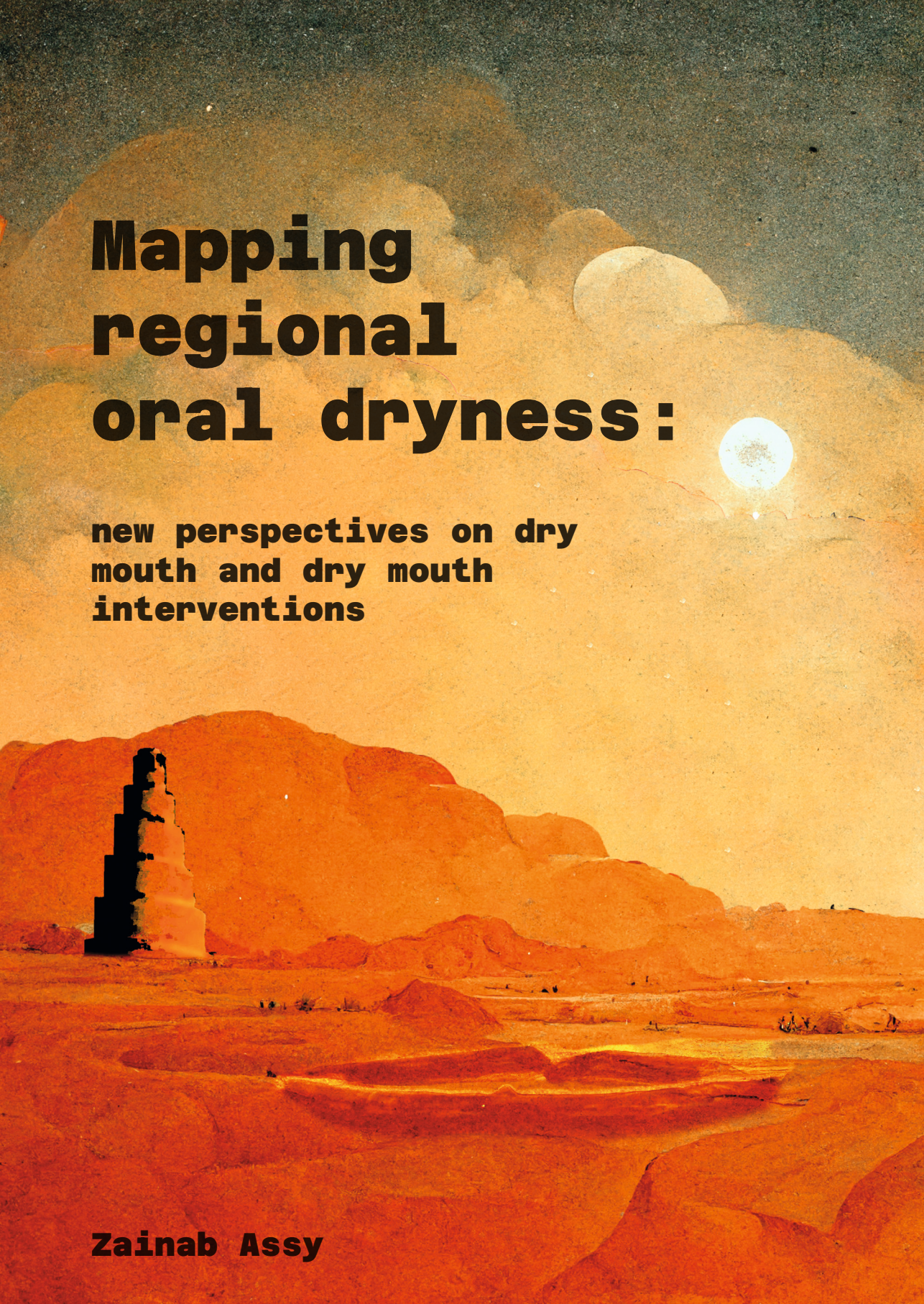
Mapping regional oral dryness:

new perspectives on dry mouth and dry mouth interventions



Zainab Assy

MAPPING REGIONAL ORAL DRYNESS:

NEW PERSPECTIVES ON DRY MOUTH AND DRY MOUTH INTERVENTIONS

Zainab Assy

Mapping regional oral dryness:
new perspectives on dry mouth and dry mouth interventions

The research described in this thesis was conducted at the Academic Centre for Dentistry Amsterdam department Oral Biochemistry, Amsterdam, the Netherlands.
An unrestricted research grant from the Nederlands Tijdschrift voor Tandheelkunde (NTVT, grant number OZB2018.01) was received by Z. Assy to perform all described research.

Printing of this thesis was financially supported by:
Nationale Vereniging Sjögrenpatiënten (NVSP), and
Research Institute of the Academic Centre for Dentistry Amsterdam

DOI: http://doi.org/10.5463/thesis.91

Zainab Assy
Department of Oral Biochemistry

Provided by thesis specialist Ridderprint, ridderprint.nl
Author: Zainab Assy
Printing: Ridderprint
Cover, layout and design: Wessel Bosscher, persoonlijkproefschrift.nl

ISBN:978-94-6458-944-3

VRIJE UNIVERSITEIT

MAPPING REGIONAL ORAL DRYNESS: NEW PERSPECTIVES ON DRY MOUTH AND DRY MOUTH INTERVENTIONS

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor of Philosophy aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. J.J.G. Geurts,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Tandheelkunde
op woensdag 22 maart 2023 om 9.45 uur
in een bijeenkomst van de universiteit,
De Boelelaan 1105

door

Zainab Assy

geboren te Bagdad, Irak

promotoren:	prof.dr. F.J. Bikker dr. H.S. Brand
copromotor:	dr. D.H.J. Jager
promotiecommissie:	prof.dr. M.L. Laine prof.dr. A. Vissink prof.dr. T. Forouzanfar prof.dr. W.M. Thomson prof.dr. B. O'Connell

This thesis is dedicated to
my mum, Alia Hussein

TABLE OF CONTENTS

INTRODUCTION

1

General introduction, aim and outline of the thesis

GENERAL INTRODUCTION

Human saliva is produced by three pairs of major salivary glands and multiple minor salivary glands. The major salivary glands are the parotid, the submandibular, and the sublingual glands. The parotid glands mostly secrete serous saliva. The submandibular glands secrete both mucinous and serous saliva. The sublingual glands and the minor salivary glands secrete only mucinous saliva [1]. The minor glands comprise approximately 600–1000 glands, distributed throughout the mouth except for the anterior part of the palate [2, 3]. Only recently, and surprisingly to many in the salivary and anatomical fields, a report was published in 2020 describing the location of a potential 'new' salivary gland, the 'tubarial gland' [4]. The glands were proposed to be situated close to the torus tubarius, a structure in the human nasopharynx composed of cartilage, which supports the auditory tube. Though, it is questioned whether the tubarial glands are *bona fide* salivary glands and the quest to their identity is currently under investigation [5-7].

The major salivary glands are responsible for 90% of the total volume of saliva, while the minor glands are responsible for <10% of the total secretion. Although the contribution of these minor glands is limited quantitatively, the ingredients in saliva secreted by these glands are essential for the local protection and moistening of the mucosal surface [2]. The salivary flow is regulated by the autonomic nervous system, with the parasympathetic response primarily responsible for stimulating secretion of watery saliva and the sympathetic system involved in salivary protein production [8, 9]. Healthy, unstimulated salivary secretion rates vary between 800 and 1500 mL per day or 0.25-0.35 mL/min, but can increase upon stimulation, *e.g.* by chewing and taste, up to 1.0-3.0 mL/min [1, 10, 11]. The salivary flow rate and salivary composition are dependent upon the type and duration of the stimulus, and the glands that secrete the saliva [8]. Besides, the circadian rhythm affects the salivary secretion rate as well [10, 12]. This circadian rhythm has a high amplitude with a peak in saliva secretion in the late afternoon, while the flow rate is virtually negligible during sleep [10].

Saliva is considered as fundamental for the maintenance of the oral cavity homeostasis [2], due to its multiple functions including moistening and lubrication, microbial homeostasis, wound healing, tooth mineralization, and pH buffering (Figure 1) [10, 11, 13]. Additionally, saliva is involved in digestion and taste perception [10]. Although saliva consists of 99% water, its functions are effectuated by a great variety of compounds including ions, peptides and proteins such as glycoproteins and immunoglobulins (Figure 1) [14].

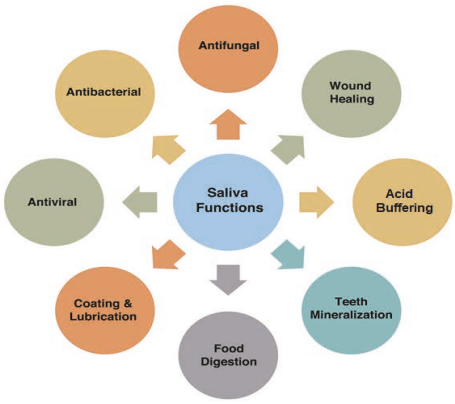


Figure 1: Overview of salivary functions, modified from Vila et al. [13].

Aetiologies of salivary dysfunction

Under various conditions the salivary gland function can be partially or totally impaired, resulting in a quantitative and/or qualitative change in the output of saliva (salivary gland dysfunction) [15]. This can be attributed to various aetiologies. The most common aetiology is the intake of multiple medications (polypharmacy), especially of (combinations of) antidepressants, anxiolytics, opiates, antihypertensives, diuretics and antihistamines [15]. Over 500 medications are known to cause or increase oral dryness as a side-effect [11]. These medications affect the salivary secretory mechanisms in various ways; some have anticholinergic or sympathomimetic actions that affect the neural control of salivary glands. Others have a cytotoxic effect on the salivary glands, or they have a diuretic effect that depletes body fluids, or damage the ion-transport pathways in the acinar cells [16, 17]. It has been suggested that the number of medications administered is more significant in the aetiology of oral dryness than specific types of medication [11].

Also, numerous diseases and medical conditions are associated with salivary gland dysfunction (Table 1) [16, 18]. These conditions can result in *e.g.* dysfunctions in neurotransmitter receptors, destruction of glandular parenchyma, immune dysregulations that may interfere with the secretion process or alterations in fluid composition and electrolytes [16]. One of the disorders with a very high association with salivary dysfunction is Sjögren's syndrome, an autoimmune disease that affects the integrity of exocrine glands, mainly the salivary and lacrimal glands. Also, endocrine disorders (such as diabetes mellitus), neurologic disorders (such as depression), genetic disorders

Table 1: Systemic disorders that may be associated with salivary dysfunction. Based on Saleh et al. [16] and Coke et al. [18]

Rheumatological chronic inflammatory disorders	Sjögren's syndrome
	Rheumatoid arthritis
	Juvenile idiopathic arthritis
	Systemic lupus erythematosus
	Primary biliary cirrhosis
Endocrine disorders	Diabetes mellitus
	Hyperthyroidism
	Hypothyroidism
Neurologic disorders	Depression
	Parkinson's disease
Genetic disorders	Agenesis of salivary glands
	Ectodermic dysplasia
	Prader-Willi syndrome
	Down syndrome
	Familial amyloidotic polyneuropathy
	Gaucher disease
	Papillon-Lefèvre syndrome
	Hereditary hemochromatosis
Metabolic disorders	Dehydration
	Chronic renal failure
	Bulimia
	Anaemia
	Alcohol abuse
Infectious disorders	HIV/AIDS
	HCV infection
	COVID
Others	Fibromyalgia
	Graft-versus-host disease
	Sarcoidosis
	Chronic pancreatitis

(such as agenesis of the salivary glands), metabolic disorders (such as dehydration), infectious disorders (such as HIV and COVID) and other diseases (such as fibromyalgia) are associated with impaired salivary function [16]. In addition, salivary gland dysfunction is common in patients who have received radiotherapy of the head and neck region. In these patients, the development of salivary gland dysfunction depends on the cumulative dose of radiation and the volume of salivary gland tissue included in the field of radiation [15].

Additionally, various other factors can inhibit the secretion of saliva, such as tobacco smoking, using alcohol, the wearing of complete dentures, heavy snoring, and mouth breathing due to functional impairment of the upper airways [11, 19]. Also, periods of acute anxiety and stress can induce transient oral dryness due to predominant activation of the sympathetic stimulation [9].

Finally, during the physiological process of aging unfavorable changes in the number of secretory cells (acini) within the salivary glands may develop which also induce reduced secretion and/or a dry mouth. This problem mainly affects menopausal women and individuals older than 65 years [11]. However, the literature also depicts that these dry-mouth symptoms are not solely explained by the physiological processes of aging, but can also be caused by age-related increase in the number of medications used [11].

Consequences of impaired salivary function

There is hyposalivation when the unstimulated salivary flow rate is <0.1 mL/min or <0.7 mL/min under stimulated conditions [16]. The overall prevalence of hyposalivation in older people is approximately 33% [20]. Persistent and severe hyposalivation commonly results in mucosal changes, an increased caries activity, oral fungal infections and a proclivity towards acute gingivitis [15, 21]. In addition, consequences such as disturbed taste sensation, impaired lubrication, dysphagia, difficulty with chewing, difficulty with speaking, fetor ex ore, fissures and ruptures of the lips are also very common. These consequences may lead to behavioral changes like avoiding certain foods. In turn, changes in dietary intake may result in nutritional deficiencies and atrophy of the masticatory muscles and decreased masticatory ability [15, 19]. Consequently, hyposalivation and its related symptoms and clinical consequences often have negative effects on social functioning, quality of life in general and oral health in particular [15, 22, 23].

Also xerostomia, the subjective sensation of dry mouth that in most cases is present throughout the day [15], is sometimes associated with hyposalivation. The prevalence of xerostomia ranges between 1-65%, dependent on parameters studies such as study sample, gender, age, used medications, and used diagnostic tool [8, 19, 24-26]. In the general population, this prevalence is estimated to be approximately 20%, with increased prevalence in females (up to 30%) and in the elderly (up to 50%) [8, 19, 24]. Discomfort, especially disturbed sleep at night, is the most common symptom associated with xerostomia [11].

Current diagnostic tools for xerostomia and hyposalivation

In order to prevent the negative consequences of xerostomia and hyposalivation a careful and systematic diagnosis of their respective cause(s), symptoms and signs is essential. Also, analysis of glandular function and inspecting the salivary glands is supportive in dry-mouth diagnostics. Currently, several tools are available. Firstly, there are various questionnaires for the assessment of xerostomia. One of the most frequently used questionnaires, and also internationally validated, is the Xerostomia Inventory (XI) developed by Thomson *et al.* [27]. The XI consists of 11-items on a 5-point Likert scale, summated into a single continuous scale score for the severity of xerostomia [28, 29]. Van der Putten *et al.* proposed a shorter version of the Xerostomia Inventory: the Summated Xerostomia Inventory-Dutch. In this questionnaire, five items of the original XI were used, with a 3 point-Likert scale [28, 29]. On the other hand, Fox *et al.* developed a questionnaire with four items about

the severity of dry mouth, which may predict hyposalivation [28]. For this questionnaire a binary scale is used [28, 29]. Eisbruch *et al.* evaluated the grade of xerostomia through a validated scale: subjective grade 1: no disability; grade 2: dryness requiring additional fluids for swallowing; and grade 3: dryness causing dietary alterations, interference with sleep, speaking, or other activities [28, 29]. In turn, Pai *et al.* developed an 8-item visual analog scale (VAS) to assess xerostomia [28, 29]. Finally, Suh *et al.* developed a questionnaire with a combination of a binary scale, categorical scoring scale and VAS [30]. Yet, at the moment there is no clear scientific consensus on the best form of grading xerostomia, mainly due to differences in opinion about the best way to obtain information from the patient [16].

In the past, Navazesh and co-workers developed a clinical scale consisting of four clinical measures; dryness of lips, dryness of buccal mucosa, absence of saliva produced by gland palpation, and total DMFT (decayed, missing, and filled teeth). Together, these four measures could successfully predict the presence or absence of salivary gland hypofunction [31]. More recently, the clinical oral dryness score (CODS) has been designed to objectively quantify clinical signs of reduced salivary secretion [32, 33]. The CODS has been developed to help oral health professionals with the objective and quick determination of salivary gland function in a clinical setting [32, 33]. The CODS is based on clinical and visual inspection of the mouth to inspect for various signs of oral dryness such as the presence of frothy saliva and stickiness of the dental mirror to the tongue or the buccal mucosa [32, 33].

In turn, measurement of the salivary flow rate is the objective tool for hyposalivation. It is relatively easy to perform and requires little time [28]. Salivary flow rates can be determined by various methods, either by collection of unstimulated and stimulated whole saliva or by collection of saliva from specific salivary glands [29]. By draining saliva passively into a pre-weighed cup unstimulated whole saliva can be collected (draining method) [28]. Alternatively, Leal *et al.* suggested the use of pre-weighed cotton rolls for collection of saliva from the orifices of the ducts of the major salivary glands. After a specific time, the cotton rolls are weighed again and saliva flow can be calculated. Also, absorbent strips can be used, which can be placed at various intra-oral locations to determine salivary flow [28]. Other methods to assess the unstimulated whole salivary flow rate include the so called 'spitting method' and the 'suction method' [28]. Stimulated salivary flow rate is determined while the patient chews an unflavored gum base or paraffin wax (1–2 g) for 1 or several minutes. Otherwise, saliva production can be stimulated with a solution

of 2% citric acid applied on the sides of the tongue at intervals of 30 seconds [28].

Despite this wide range of techniques, accurate assessment of dry mouth according to the quantity of saliva might be difficult as salivary quantity and flow rate vary dramatically within and between individuals. Additional biochemical and mechanical measurements could support diagnostic tests for dry mouth. To understand the salivary quality, it is important to investigate both the compositional feature and mechanical properties of saliva, such as adsorption, rheological and tribological properties [30].

Salivary gland function can also be measured by scintigraphy, in which a radionuclide is injected intravenously and subsequently this radionuclide is taken up by the salivary glands and then secreted [8]. Measurement of uptake and secretion into the oral cavity can determine the presence and extent of functional salivary tissue [8]. Additionally, a number of other imaging techniques can help in identifying salivary gland abnormalities, for example magnetic resonance imaging (MRI). MRI can identify solid and cystic masses in the glands. Sialography is a radiographic examination of the salivary glands to visualize the anatomy of ducts, acinar integrity, calcifications, and some tumors [8]. It usually involves the injection of a small amount of contrast medium into the salivary duct of a single gland, followed by routine X-ray projections.

Investigating the medical history of dry-mouth patients contributes to proper diagnosis. A detailed inventory of present symptoms, type and number of xerogenic medications used, the presence of systemic and oral diseases, and previous dry-mouth therapies is important in this process [29]. This information also helps to investigate the potential underlying influence of condition like psychiatric and cardiovascular diseases [29]. The European Medical Risk-Related History questionnaire is a good example of an internationally validated patient-administered questionnaire that is used to retrieve information about the health status of a patient [34, 35].

Furthermore, an extra-oral and intra-oral examination should also be part of the examination of the patient. This should include the inspection and palpation of the salivary glands, expulsion (“milking”) of saliva from the major salivary duct orifices (at rest and after a stimulus), and inspection of the oral mucosa and the dentition [29]. Finally, salivary gland biopsy provides a definitive diagnosis of glandular pathology. A labial minor salivary gland biopsy is more commonly and easily performed than a biopsy of the major salivary glands [8]. Although, recent literature discusses parotid biopsies as an alternative for minor salivary gland biopsies [26, 36]. As the sensitivity and specificity of parotid and labial biopsies for diagnosing Sjögren’s syndrome

are comparable. In addition, parotid gland incision biopsy can overcome most of the disadvantages of labial gland excision biopsy [26, 36].

Taken together for a systematic and detailed investigation of xerostomia, hyposalivation, salivary gland function and gland inspection, the use of a combination of diagnostic tools (both objective and subjective parameters) is recommended.

Dry-mouth interventions

Effective management of dry-mouth complaints is important in order to improve the quality of life of those who seek treatment for their symptoms and to minimize associated oral problems [24]. In some patients it may be possible to manage the problems associated with a dry mouth through optimal management of the underlying condition(s); for example, an adjustment of the medication used or its dosage. In individuals with mild symptoms sucking ice chips, frequent sipping of water, and reducing or avoiding irritants, such as alcohol, caffeine, smoking or hot, spicy foods may provide sufficient relief for their symptoms [24]. When there is some residual salivary function, saliva secretion can be stimulated with the use of topical sialagogues, such as using sugar-free chewing gums and lozenges [37]. Systemic pharmacotherapies with parasympathomimetic activity including pilocarpine and cevimeline could also stimulate the salivary secretion [37, 38]. Other interventions such as acupuncture, salivary neuro-electrostimulation and sialendoscopy have also been used to increase saliva production and, in some cases, might also lessen the associated dry-mouth symptoms [37, 39, 40]. Especially in Sjögren's syndrome patients, sialendoscopy of the major salivary glands showed promising results as it increased the salivation and reduced oral dryness up to at least 60 weeks [40]. However, when the salivary secretion is irreversibly impaired, *e.g.* as a consequence of radiotherapy, then salivary substitutes could be used as dry-mouth interventions. Examples of salivary substitutes are mouth washes, mouth gels and oral sprays [24, 37]. These substitutes help to moisturize the mucosa in absence of saliva [24].

State of the art in dry-mouth diagnostics and dry-mouth interventions

Despite the wide range of questionnaires and clinical tests available, an accurate diagnosis of dry mouth is still challenging, due to the complex aetiology of dry mouth and the various potential underlying mechanisms. Besides, the current diagnostic methods map dry-mouth symptoms in general terms, but they do not adequately reveal the complexity of this problem and they are also not entirely discriminating. To illustrate, the total salivary flow rate

measures only the whole saliva secretion, but this flow rate does not provide information on the extent of the moisturizing effect of saliva. For this reason, hyposalivation and xerostomia are not correlated per se [23, 41]. Moreover, the sensation of a dry mouth is not only related to the reduction in salivary secretion rate, but possibly also to the unequal thickness of the saliva film on both soft and hard oral tissue surfaces [23]. The salivary film thickness could influence the moisturizing effect of saliva, and consequently the surface over which saliva is spread can influence the salivary film thickness and possibly also its moisturizing effect.

An important gap in the scientific literature is a dry-mouth questionnaire which explores the perceived dryness at various intra-oral locations, specifically various mucosal surfaces such as the tongue or palate. And, although, a large number of studies have shown that the salivary film is not equally distributed within the oral cavity [33, 42-49], a questionnaire to determine dryness at specific oral locations is still lacking. For this reason, it is envisaged that a questionnaire to determine dryness at specific oral locations could increase our understanding about the distribution of saliva in the oral cavity and the relation with perceived dryness. Additionally, such a new questionnaire could even contribute to determining the cause of oral dryness in specific patient groups. In addition, as the (size of) surface area plays a role in the distribution of saliva, measurement of the oral surface areas could be an important additional tool during the diagnosis of oral dryness.

Nowadays, various interventions are available to relieve oral dryness. The reasons that affect the use of dry-mouth interventions by patients are not fully understood yet. In previous research it was shown that age, gender and the presence of a dental prothesis could affect the use of dry-mouth interventions in Sjögren's syndrome patients [50]. However, it is still unclear whether the severity of intra-oral dryness could also affect the use of dry-mouth interventions. For this reason, factors that affect the choice of dry-mouth interventions will be further explored.

Aim and outline of the thesis

The overall aim of this thesis was to improve the current, available diagnostic tools for dry mouth by developing a new method for measuring the perceived dryness at specific various intra-oral locations and to investigate the effect of intra-oral surface areas on the distribution of saliva within the oral cavity. Additionally, the use of dry-mouth interventions by various dry-mouth patients will be investigated to understand which factors affect the use of these interventions. In this thesis, eight research chapters are divided into three main themes:

1) perceived intra-oral dryness (**Chapters 2, and 3**);
2) intra-oral surface area and salivary distribution (**Chapters 4, 5 and 6**); and
3) interventions to relieve oral dryness (**Chapters 7, 8 and 9**).

In **Chapter 2**, a novel questionnaire, the Regional Oral Dryness Inventory (RODI) was developed to quantify the severity of dryness at various locations in the mouth. Next, this questionnaire was validated in various groups of dry-mouth patients aiming to differentiate between causes of oral dryness (**Chapter 3)**. As the size of the surface area of the intra-oral regions, the palate, can possibly influence the distribution and average thickness of the salivary film, in **Chapter 4** intra-oral surface areas were quantified using cone-beam computed tomography (CBCT). Besides, potential correlations between intra-oral surface areas and facial anthropomorphic measurements were analyzed which would enable easy and safe chair-side approximation of intra-oral surface areas. In **Chapter 5** the relation between the palate surface area, measured using an intra-oral scanner, and anthropometric measurements of the head and face was validated in living subjects. In **Chapter 6**, palatal surface area measurements were explored in healthy volunteers in combination with measurements of the salivary film and the salivary consistency, especially the concentration of a salivary mucin, at various intra-oral locations.

The aim of **Chapter 7** was to investigate the use of dry-mouth interventions by subgroups of patients with different causes of oral dryness and explored the possible relation of the applied interventions with intra-oral dryness and salivary flow rate. In accordance with this study, **Chapter 8** has a similar aim, but focuses on Sjögren's syndrome patients specifically. The purpose of **Chapter 9** was to explore the preferences of Sjögren's syndrome patients regarding various product characteristics of potential new saliva substitutes, important functions of possible substitutes, objections against certain ingredients, desired flavors for the substitutes, objections against potential side-effects of saliva substitutes, and the preferred method of administration.

REFERENCES

1. Turner MD. Hyposalivation and Xerostomia: Etiology, Complications, and Medical Management. Dent Clin North Am. 2016;60(2):435-43.
2. de Paula F, Teshima THN, Hsieh R, Souza MM, Nico MMS, Lourenco SV. Overview of Human Salivary Glands: Highlights of Morphology and Developing Processes. Anat Rec (Hoboken). 2017;300(7):1180-8.
3. Hamada T, Kawazoe Y, Sekino K, Nagasawa T, Tsuru H. Palatal gland distribution. J Dent Res. 1974;53(4):944.
4. Valstar MH, de Bakker BS, Steenbakkers R, de Jong KH, Smit LA, Klein Nulent TJW, *et al.* The tubarial salivary glands: A potential new organ at risk for radiotherapy. Radiother Oncol. 2021;154:292-8.
5. Nascimento JJC, Ribeiro ECO, Silva-Neto EJ. Letter to the Editor regarding "The tubarial salivary glands: A potential new organ at risk for radiotherapy". Radiother Oncol. 2021;154:323.
6. Bikker FJ, Vissink A. Letter to the editor concerning Valstar *et al.*, [Radiother Oncol 2020 Sep 23;S0167-8140(20)30809-4. doi: 10.1016/j.radonc.2020.09.034]. Radiother Oncol. 2021;154:318.
7. Valstar MH, de Bakker BS, Steenbakkers R, de Jong KH, Smit LA, Klein Nulent TJW, *et al.* The tubarial glands paper: A starting point. A reply to comments. Radiother Oncol. 2021;154:308-11.
8. Napeñas JJ, Brennan MT, Fox PC. Diagnosis and treatment of xerostomia (dry mouth). Odontology. 2009;97(2):76-83.
9. Guggenheimer J, Moore PA. Xerostomia: etiology, recognition and treatment. J Am Dent Assoc. 2003;134(1):61-9; quiz 118-9.
10. Dawes C, Pedersen AM, Villa A, Ekstrom J, Proctor GB, Vissink A, *et al.* The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. Arch Oral Biol. 2015;60(6):863-74.
11. Tanasiewicz M, Hildebrandt T, Obersztyn I. Xerostomia of Various Etiologies: A Review of the Literature. Adv Clin Exp Med. 2016;25(1):199-206.
12. Dawes C. Circadian rhythms in human salivary flow rate and composition. J Physiol. 1972;220(3):529-45.
13. Vila T, Rizk AM, Sultan AS, Jabra-Rizk MA. The power of saliva: Antimicrobial and beyond. PLoS Pathog. 2019;15(11):e1008058.
14. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001;85(2):162-9.
15. Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. J Oral Rehabil. 2018;45(9):730-46.
16. Saleh J, Figueiredo MA, Cherubini K, Salum FG. Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol. 2015;60(2):242-55.
17. Ying Joanna ND, Thomson WM. Dry mouth - An overview. Singapore Dent J. 2015;36:12-7.
18. Coke CJ, Davison B, Fields N, Fletcher J, Rollings J, Roberson L, *et al.* SARS-CoV-2 Infection and Oral Health: Therapeutic Opportunities and Challenges. J Clin Med. 2021;10(1).

19. Millsop JW, Wang EA, Fazel N. Etiology, evaluation, and management of xerostomia. Clin Dermatol. 2017;35(5):468-76.
20. Pina GMS, Mota Carvalho R, Silva BSF, Almeida FT. Prevalence of hyposalivation in older people: A systematic review and meta-analysis. Gerodontology. 2020;37(4):317-31.
21. Porter SR, Scully C, Hegarty AM. An update of the etiology and management of xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97(1):28-46.
22. Roblegg E, Coughran A, Sirjani D. Saliva: An all-rounder of our body. Eur J Pharm Biopharm. 2019;142:133-41.
23. Kho HS. Understanding of xerostomia and strategies for the development of artificial saliva. Chin J Dent Res. 2014;17(2):75-83.
24. Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R. Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev. 2011(12):Cd008934.
25. Quock RL. Xerostomia: current streams of investigation. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(1):53-60.
26. Delli K, Spijkervet FK, Kroese FG, Bootsma H, Vissink A. Xerostomia. Monogr Oral Sci. 2014;24:109-25.
27. Thomson WM, Chalmers JM, Spencer AJ, Williams SM. The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health. 1999;16(1):12-7.
28. Villa A, Connell CL, Abati S. Diagnosis and management of xerostomia and hyposalivation. Ther Clin Risk Manag. 2015;11:45-51.
29. Villa A, Wolff A, Aframian D, Vissink A, Ekström J, Proctor G, *et al.* World Workshop on Oral Medicine VI: a systematic review of medication-induced salivary gland dysfunction: prevalence, diagnosis, and treatment. Clin Oral Investig. 2015;19(7):1563-80.
30. Hu J, Andablo-Reyes E, Mighell A, Pavitt S, Sarkar A. Dry mouth diagnosis and saliva substitutes-A review from a textural perspective. J Texture Stud. 2021;52(2):141-56.
31. Navazesh M, Christensen C, Brightman V. Clinical criteria for the diagnosis of salivary gland hypofunction. J Dent Res. 1992;71(7):1363-9.
32. Jager DHJ, Bots CP, Forouzanfar T, Brand HS. Clinical oral dryness score: evaluation of a new screening method for oral dryness. Odontology. 2018;106(4):439-44.
33. Osailan SM, Pramanik R, Shirlaw P, Proctor GB, Challacombe SJ. Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(5):597-603.
34. Smeets EC, de Jong KJ, Abraham-Inpijn L. Detecting the medically compromised patient in dentistry by means of the medical risk-related history. A survey of 29,424 dental patients in The Netherlands. Prev Med. 1998;27(4):530-5.
35. Abraham-Inpijn L, Russell G, Abraham DA, Backman N, Baum E, Bullon-Fernandez P, *et al.* A patient-administered Medical Risk Related History questionnaire (EMRRH) for use in 10 European countries (multicenter trial). Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(5):597-605.
36. Delli K, Vissink A, Spijkervet FK. Salivary gland biopsy for Sjögren's syndrome. Oral Maxillofac Surg Clin North Am. 2014;26(1):23-33.

37. Al Hamad A, Lodi G, Porter S, Fedele S, Mercadante V. Interventions for dry mouth and hyposalivation in Sjögren's syndrome: A systematic review and meta-analysis. Oral Dis. 2019;25(4):1027-47.
38. Furness S, Bryan G, McMillan R, Worthington HV. Interventions for the management of dry mouth: non-pharmacological interventions. Cochrane Database Syst Rev. 2013(8):Cd009603.
39. Aframian DJ, Baaton S, Mazor S, Nadler C, Keshet N, Haviv Y, *et al.* Improvement of dry mouth following intraductal irrigation of salivary glands. Oral Dis. 2019;25(7):1735-43.
40. Karagozoglu KH, Vissink A, Forouzanfar T, de Visscher J, Maarse F, Brand HS, *et al.* Sialendoscopy increases saliva secretion and reduces xerostomia up to 60 weeks in Sjögren's syndrome patients: a randomized controlled study. Rheumatology (Oxford). 2021;60(3):1353-63.
41. Fox PC, Busch KA, Baum BJ. Subjective reports of xerostomia and objective measures of salivary gland performance. J Am Dent Assoc. 1987;115(4):581-4.
42. Won S, Kho H, Kim Y, Chung S, Lee S. Analysis of residual saliva and minor salivary gland secretions. Arch Oral Biol. 2001;46(7):619-24.
43. DiSabato-Mordarski T, Kleinberg I. Measurement and comparison of the residual saliva on various oral mucosal and dentition surfaces in humans. Arch Oral Biol. 1996;41(7):655-65.
44. Wolff M, Kleinberg I. Oral mucosal wetness in hypo- and normosalivators. Arch Oral Biol. 1998;43(6):455-62.
45. Chaudhury NM, Proctor GB, Karlsson NG, Carpenter GH, Flowers SA. Reduced Mucin-7 (Muc7) Sialylation and Altered Saliva Rheology in Sjögren's Syndrome Associated Oral Dryness. Mol Cell Proteomics. 2016;15(3):1048-59.
46. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH, Proctor GB. Changes in Saliva Rheological Properties and Mucin Glycosylation in Dry Mouth. J Dent Res. 2015;94(12):1660-7.
47. Osailan S, Pramanik R, Shirodaria S, Challacombe SJ, Proctor GB. Investigating the relationship between hyposalivation and mucosal wetness. Oral Dis. 2011;17(1):109-14.
48. Pramanik R, Osailan SM, Challacombe SJ, Urquhart D, Proctor GB. Protein and mucin retention on oral mucosal surfaces in dry mouth patients. Eur J Oral Sci. 2010;118(3):245-53.
49. Challacombe S, Bds P, Bsc P. Clinical Scoring Scales for Assessment of Dry Mouth. 2015. p. 119-32.
50. Brand HS, Bots CP, Veerman ECI. Therapies for xerostomia in Sjögren's disease are age- and gender-dependent. J Dent Res. 2011;90(Special Issue A):1347.

PERCEIVED INTRA-ORAL DRYNESS

2

Regional differences in perceived oral dryness as determined with a newly developed questionnaire, the Regional Oral Dryness Inventory

Z. Assy
D. H. J. Jager
E. Mashhour
F. J. Bikker
H. S. Brand

Clinical Oral Investigations, 2020 Nov; 24(11):4051-4060.

ABSTRACT

Objectives

Several questionnaires, such as the internationally validated and frequently used Xerostomia Inventory (XI), have been developed to quantify the subjective feeling of a dry mouth. These questionnaires quantify the overall perception of dry mouth but lack the possibility to differentiate between various intra-oral regions. In this light, a novel questionnaire, the Regional Oral Dryness Inventory (RODI), which quantifies the severity of dryness at various locations in the mouth, was evaluated.

Material and method

A retrospective case report study was designed. Data were collected from patients who visited the saliva clinic for Special Care Dentistry in Amsterdam. Data, including the saliva secretion rates, RODI scores, the Xerostomia Inventory (XI) score, and Clinical Oral Dryness Score (CODS), were extracted from the electronic health record system Oase Dental.

Results

A total of 337 patients participated in this study with an average age of 54 ± 17 years. The majority of the patients were female (68.5%). The perceived dryness as determined by the RODI was the highest for the posterior palate and the lowest for the floor of the mouth. The highest correlations were found between the corresponding regions in the RODI and regionally related individual items of the XI and CODS.

Conclusion

There is a significant difference in dry-mouth feeling at different intra-oral locations.

Clinical relevance

Regional evaluation of xerostomia with the RODI might improve diagnosis of xerostomia by helping to discriminate between different potential causes of oral dryness in patients and for evaluating the efficacy of mouth-moistening products. The RODI is highly accessible and easy to perform in dental practices during routine clinical assessment.

Keywords

Dry mouth, Xerostomia, Salivary flow rate, Xerostomia Inventory, Clinical Oral Dryness Score

INTRODUCTION

Saliva is a multi-functional fluid which provides mucosal lubrication and moistening, and protection of the teeth and oral mucosa surface, and plays an important role in digestion, protecting oral tissues, swallowing, taste, and speaking [1, 2]. Therefore, an adequate saliva flow is important for the maintenance of oral health [3, 4].

Saliva flow can be impaired due to many factors. A reduction in saliva secretion rate can be the result of xerogenic medications, radiotherapy of the head and neck, or systemic diseases such as Sjögren's syndrome [5–7]. Patients suffering from a reduced salivary flow rate may complain about taste alterations, swallowing difficulties, and a burning sensation in the mouth. Other oral complications include increased risk of ulcerations, caries, gingivitis, periodontitis, and oral Candida spp. infections [8, 9].

A reduced salivary flow rate is known as hyposalivation and can objectively be determined by sialometry. Hyposalivation is defined as a salivary flow rate is < 0.1 mL/min at rest or < 0.7 mL/min upon stimulation [8]. In contrast, the subjective sensation of a dry mouth experienced by the patient is called xerostomia [9, 10], which can only be determined with self-reported questionnaires [11–15]. Over the past decades, several questionnaires have been developed to quantify the overall feeling of a dry mouth [11–15]. For example, the Xerostomia Inventory (XI) is an internationally validated and frequently used questionnaire with 11 items on a 5-point Likert scale to quantify the severity of the xerostomia [11].

The sensation of a dry mouth is not solitarily related to the reduction in salivary secretion rate changes but might also be related to the unequal thickness of the saliva film on both soft and hard oral tissue surfaces [16]. To exemplify, the salivary film that remains in the oral cavity after swallowing is the thickest at the dorsal area of the tongue and the thinnest at the hard palate [17–21].

In addition, differences in salivary composition have also been implicated in the perception of dry mouth [19–21]; the salivary mucin MUC5B retains large amounts of water and contributes to the generation of a hydrophilic gel essential for lubrication of the oral epithelium [22–24]. Moreover, MUC5B is the main component that determines the viscoelasticity of saliva [24]. Local variations in the MUC5B concentration have been reported with higher intensity on the hard palate than other oral surfaces [18].

In light of these local variations [17, 18, 21], the palate may be more frequently related to xerostomia complaints than other areas, *e.g.*, the tongue [19].

So far, xerostomia questionnaires were aimed to quantify the overall feeling of mouth dryness and not the perceived xerostomia at different intra-oral locations. Therefore, the purpose of this study is to evaluate a recently developed questionnaire, Regional Oral Dryness Inventory (RODI), which quantifies the severity of dryness at various locations in the mouth.

MATERIALS AND METHODS

Study design

A retrospective case report study was designed. Data were collected from patients older than 18 years, who visited the saliva clinic for Special Care Dentistry in Amsterdam. These patients were referred to the saliva clinic by dentists, general physicians, and medical specialists between January 2014 and April 2019. All the patients included in this study had saliva-related and/or dry-mouth complaints.

The Ethics Review Committee of the Academic Centre for Dentistry Amsterdam (ACTA) confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study (protocol number 201910). The reporting of this study conforms to the STROBE statement [25].

All the questionnaires and clinical parameters have been collected and interpreted by a single practitioner (DHJJ). A standardized protocol is used for this process, which takes approximately 45 min. All the procedures described in the present study are part of the regular patient care routine in the saliva clinic.

Data collection methods

The relevant data were extracted by one abstractor (EM) from the electronic health record system Oase Dental (VST Software B.V., Haarlem, The Netherlands). Patients were included when most of the relevant data were present in the record of the patient. The extracted data were registered pseudonymized in a Microsoft Excel under a code number so that the data can no longer be traced back to the patients. The following clinical data were retrieved: gender, age, the Xerostomia Inventory (XI) score, Clinical Oral Dryness Score (CODS), scores on the newly developed Regional Oral Dryness Inventory, and the secretion rates of unstimulated whole saliva (UWS), chewing-stimulated whole saliva (CH-SWS), and citric acid–stimulated whole saliva (A-SWS).

Random checks were done after data entry, by two researchers (EM and ZA), to verify correct transfer of data from the medical record to the case reports. This was performed according to the 100-20 rule in which 100% of the data is

checked in 20% of the case reports and 20% of the most important data are checked in 100% of the case reports [26].

Subjective oral dryness

Before a patient visited the saliva clinic, he or she received several questionnaires by mail to fill out at home. These questionnaires included the Xerostomia Inventory (XI) which consists of 11 items on a 5-point Likert scale ranging from 1 = "never" to 5 = "very often." The items are about oral dryness and mouth feel in the patients. Patients indicate in each item how often they suffer from problems with regard to mouth feel and oral dryness. The scores of the 11 items are summed resulting in a total XI score that ranges between 11 (no xerostomia) and 55 (extreme xerostomia) [11].

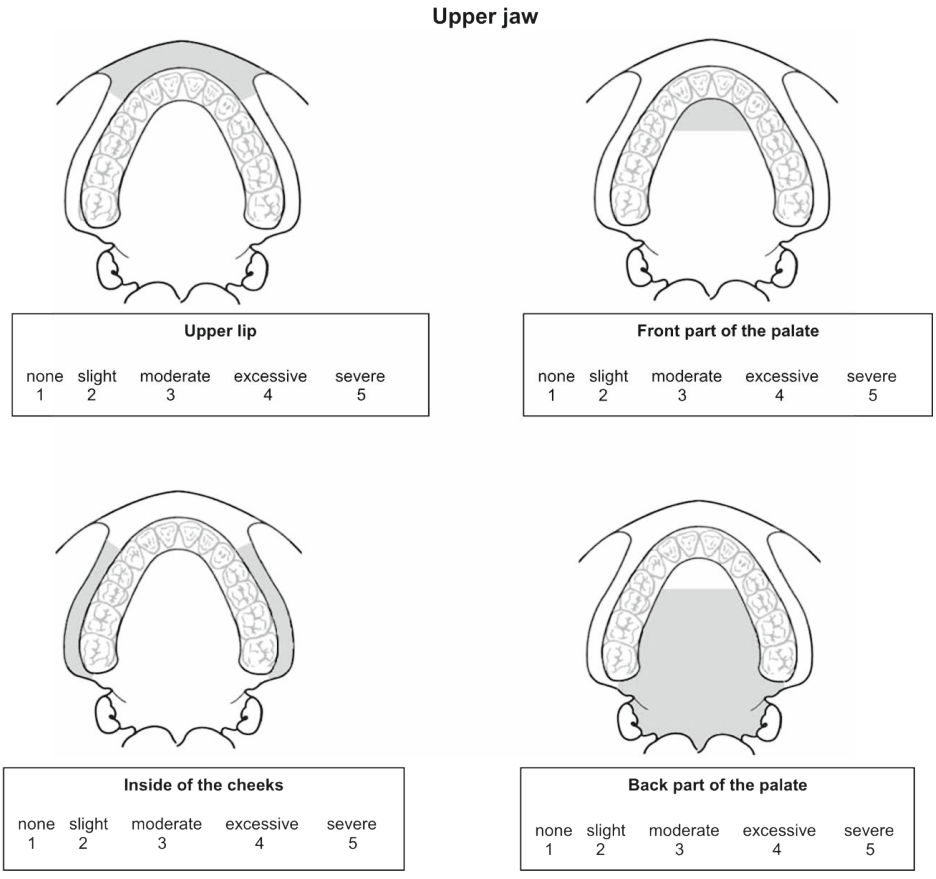
In addition, the patients received a newly developed Regional Oral Dryness Inventory (RODI) (Fig. 1). This questionnaire contains 9 schematic illustrations of different locations in the oral cavity. Four illustrations represent areas in the upper jaw: the upper lip, anterior part of the palate (including the rugae), inside part of the cheeks, and posterior part of the palate (from the rugae up to the end of the soft palate). Four illustrations represent areas in the lower jaw: the lower lip, floor of the mouth, posterior part of the tongue (from vallate papilla up to end of the tongue), and anterior part of the tongue (from tip of the tongue up to vallate papilla). Finally, one illustration represents the pharynx. At each location, the patient can indicate the severity of the perceived oral dryness using a 5-point Likert scale ranging from 1 = "no dryness" to 5 = "severe dryness."

Clinical oral dryness score

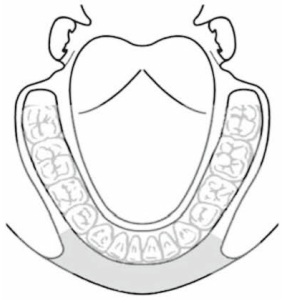
During the visit to the saliva clinic, the Clinical Oral Dryness Score (CODS) was scored for all patients by a single examiner (DHJJ). The CODS was recorded before determining the salivary flow rates and analyzing the xerostomia questionnaires, so the examiner was not aware during the recording of the CODS whether a patient suffered from hyposalivation/ xerostomia or not.

The examiner scored the patient's mouth for the presence or absence of ten features of oral dryness: (1) mirror sticks to buccal mucosa; (2) mirror sticks to tongue; (3) tongue shows loss of papillae; (4) tongue lobulated/ fissured; (5) frothy saliva; (6) no saliva pooling in floor of mouth; (7) glassy appearance of other oral mucosa, especially palate; (8) debris on palate (excluding debris under dentures); (9) altered/smooth gingival architecture; and (10) active or recently restored (last 6 months) cervical caries (> 2 teeth) [27]. A specially designed form with illustrations of dry-mouth features from

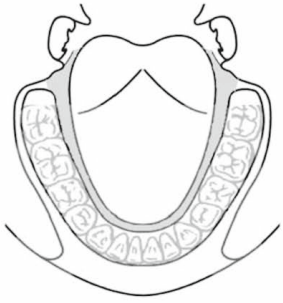
Fig. 1 The Regional Oral Dryness Inventory with the nine intra-oral regions and instructions. Regional Oral Dryness Inventory. The following questions are about dryness perception in the mouth during the last 4 weeks. The illustrations below show four different regions in the upper jaw, four different regions in the lower jaw, and an illustration of the throat. Please indicate the severity of dryness for each of these different locations on a scale from 1 to 5, where 1 = no dryness and 5 = severe dryness. It is advisable to answer spontaneously and not spend too much time considering your answer.



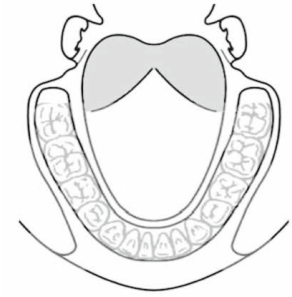
Lower jaw and throat



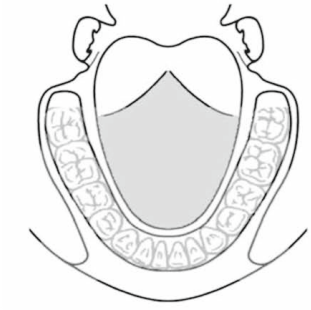
Lower lip				
none	slight	moderate	excessive	severe
1	2	3	4	5



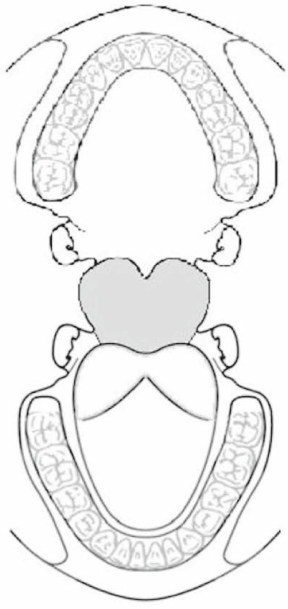
Floor of the mouth				
none	slight	moderate	excessive	severe
1	2	3	4	5



Back part of the tongue				
none	slight	moderate	excessive	severe
1	2	3	4	5



Front part of the tongue				
none	slight	moderate	excessive	severe
1	2	3	4	5



Throat				
none	slight	moderate	excessive	severe
1	2	3	4	5

the original publication was used to score each feature [27]. The scores from the ten features were added together resulting in a total CODS ranging from 0 (no oral dryness) to 10 (extreme oral dryness).

Sialometry

The patients were instructed not to eat, drink, chew gum, brush teeth, use mouthwash, or smoke for at least 1 h before their visit to the saliva clinic. The procedure to determine the saliva secretion rate has been described by Jager and co-workers [28]. At the time of the collection of saliva, patients were placed in a quiet room and asked to sit in an upright position. The UWS was collected by the draining method in a pre-weighed plastic container [29]. To collect unstimulated saliva, patients were asked to immediately collect saliva after an initial swallow. Afterwards, they were asked to expectorate in the container as soon as they collected saliva. During saliva collection, the patients were not allowed to swallow. To collect CH-SWS, patients were asked to chew a 5 × 5-cm sheet of parafilm (Parafilm M, Pechiney, Chicago, IL, USA) with a frequency of approximately 60 chews per minute. The patients were instructed to expectorate the saliva every 30 s into a pre-weighed plastic container during a 5 min period. For stimulation of A-SWS secretion, a citric acid solution (2% w/v) was applied with cotton buds on the lateral borders of the tongue at 30 s intervals [30]. After the collection period was finished, the plastic containers were reweighed, and the collected volume was determined by subtracting the weight of the container prior to collection. The salivary flow was calculated by dividing the collected volume (assuming 1 g of saliva equals 1 mL) by collection time (min). Salivary flow rates were expressed in mL/min [29].

To determine whether patients suffered from hyposalivation, the following cut-off values were used: UWS < 0.10 mL/min, CH-SWS < 0.70 mL/min, and ASWS < 0.70 mL/min [8].

Data analysis

The data were processed in Microsoft Excel and then converted into SPSS, version 25.0 (IBM Corp SPSS Statistics, Armonk, NY, USA) for the statistical analysis. The Shapiro– Wilk test was used to assess the normality of the data. The data were presented as median, and their interquartile range (IQR) as all parameters were not normally distributed. The mean and standard deviation were also reported to clarify relatively small differences.

A Friedman test was conducted for the scores of the RODI and XI scores, followed by a Wilcoxon signed-rank test as post hoc procedure.

Possible relationships among the RODI scores of the nine intra-oral regions, and the relation of the RODI scores with XI scores, UWS, CH-SWS, and A-SWS salivary flow rates were analyzed with a bootstrapped Spearman rank correlation test (1000 × bootstrapping). The Spearman's rho coefficient and bias-corrected accelerated (Bca) 95% confidence interval were extracted. A significance level (α) of 0.01 was chosen for the correlation test.

The Mann-Whitney U test (significance level of $\alpha = 0.05$) was performed to explore a possible relation between a positive CODS score and the associated region in the RODI.

RESULTS

A total of 337 patients participated in this study with an average age of 54 ± 17 years. The majority of the patients were female (68.5%). The RODI scores, XI scores, CODS and UWS, CH-SWS, and A-SWS salivary flow rates were not normally distributed (Shapiro–Wilk test; $p < 0.01$). Table 1 presents the different salivary flow rates of the study sample. Based on the UWS, CH-SWS, and A-SWS flow rates, respectively, 26.9%, 48.6%, and 13.1% of the study sample respectively suffered from hyposalivation.

Regional Oral Dryness Inventory

In Table 2, the median and the corresponding IQR, and mean with standard deviation are shown for each of the nine intraoral regions of the RODI. There was a significant difference in perceived oral dryness between the nine intra-oral regions (Friedman test $p < 0.05$, followed by Wilcoxon signed-rank tests $p < 0.05$). The highest scores were obtained for the posterior palate, while the lowest scores were obtained for the floor of the mouth (Table 2).

The scores of all regions correlated significantly with each other (Table 3) indicating that patients who suffer from severe xerostomia at one location in general also have high levels of xerostomia at other intra-oral locations. The correlation coefficient varied between 0.51 (pharynx with lower lip) and 0.82 (lower lip and upper lip). Four different regions have a correlation coefficient ≥ 0.75: the lower lip and upper lip, the posterior palate and posterior tongue, the anterior tongue and posterior tongue, and the floor of the mouth and inside the cheeks. The correlations of the scores between these four regions can be considered strong, whereas the other regions have a moderate correlation according to the standards described by Mukaka and co-workers and Akoglu and co-workers [31, 32].

Table 1 The unstimulated whole saliva (UWS), chewing-stimulated whole saliva (CH-SWS), and acid-stimulated whole saliva secretion rates of the study sample. Data are expressed as the median with the corresponding interquartile range (IQR), and mean with standard deviation (SD).

	Median	**IQR**	**Mean**	**SD**	**N**
UWS (mL/min)	0.18	0.08–0.34	0.27	0.33	264
CH-SWS (mL/min)	0.70	0.34–1.18	0.89	0.84	313
A-SWS (mL/min)	1.80	1.05–2.78	2.00	1.23	321

Table 2 Perceived oral dryness in nine different intra-oral regions as determined with the Regional Oral Dryness Inventory (RODI) in patients visiting a saliva clinic. Data are presented as median with corresponding interquartile range (IQR) and mean with standard deviation (SD).

	Median	**IQR**	**Mean**	**SD**	**N**
Upper lip	3.0	2.0–4.0	2.80	1.26	303
Anterior palate	3.0	1.0–4.0	2.82	1.40	302
Inside cheeks[a,b]	3.0	1.0–4.0	2.68	1.34	302
Posterior palate[a,b,c]	3.0	2.0–4.0	3.09	1.35	302
Lower lip[d]	3.0	2.0–4.0	2.70	1.26	299
Floor of the mouth[a,b,c,d,e]	2.0	1.0–4.0	2.54	1.34	297
Posterior tongue[a,b,c,e,f]	3.0	2.0–4.0	3.03	1.32	297
Anterior tongue[a,c,d,e,f]	3.0	2.0–4.0	2.94	1.40	297
Pharynxa,b,c,d,e,f	3.0	2.0–4.0	2.96	1.36	297

Wilcoxon signed-rank tests: [a] $p < 0.05$ vs upper lip, [b] $p < 0.05$ vs anterior palate, [c] $p < 0.05$ vs inside cheeks, [d] $p < 0.05$ vs posterior palate, [e] $p < 0.05$ vs lower lip, [f] $p < 0.05$ vs floor of mouth, [g] $p < 0.05$ vs posterior tongue, and [h] $p < 0.05$ vs anterior tongue

Table 3 Correlation of the nine regions of the Regional Oral Dryness Inventory, r: Spearman's rho correlation coefficient (BCa 95% confidence interval).

	Upper lip	Anterior palate	Inside Cheeks	Posterior palate	Lower lip	Floor of the mouth	Posterior tongue	Anterior tongue	Pharynx
Upper lip		r 0.69 (0.61–0.76)*	r 0.65 (0.56–0.72)*	r 0.56 (0.46–0.65)*	r 0.82 (0.76–0.86)*	r 0.61 (0.51–0.70)*	r 0.54 (0.44–0.63)*	r 0.57 (0.47–0.66)*	r 0.54 (0.44–0.63)*
Anterior palate			r 0.72 (0.65–0.78)*	r 0.73 (0.66–0.79)*	r 0.65 (0.57–0.72)*	r 0.70 (0.62–0.76)*	r 0.66 (0.58–0.75)*	r 0.67 (0.58–0.75)*	r 0.58 (0.49–0.67)*
Inside cheeks				r 0.65 (0.56–0.73)*	r 0.65 (0.56–0.72)*	r 0.75 (0.68–0.82)*	r 0.65 (0.56–0.74)*	r 0.64 (0.54–0.72)*	r 0.61 (0.51–0.70)*
Posterior palate					r 0.56 (0.47–0.64)*	r 0.69 (0.61–0.76)*	r 0.79 (0.73–0.85)*	r 0.65 (0.57–0.73)*	r 0.69 (0.61–0.76)*
Lower lip						r 0.67 (0.60–0.74)*	r 0.55 (0.45–0.63)*	r 0.63 (0.54–0.71)*	r 0.51 (0.41–0.59)*
Floor of mouth							r 0.73 (0.66–0.79)*	r 0.72 (0.66–0.78)*	r 0.65 (0.56–0.73)*
Posterior tongue								r 0.75 (0.67–0.81)*	r 0.71 (0.63–0.78)*
Anterior tongue									r 0.57 (0.47–0.65)*
Pharynx									

** = $p < 0.01$*

The RODI scores at the nine intra-oral regions showed weak to non-significant negative correlations with the UWS, CH-SWS, and A-SWS with Spearman's rho correlation coefficient ranging between - 0.27 and - 0.13.

Xerostomia Inventory

Table 4 shows that the median of the 11 items of the XI ranged between 2.0 and 4.0. There was a significant difference in perceived oral dryness and mouth feel between the 11 items of the XI (Friedman test $p < 0.05$, followed by Wilcoxon signed-rank tests $p < 0.05$). The XI item 4 (my mouth feels dry) had the highest scores and items XI 1 (sip liquids to swallow food) and XI 7 (I have difficulty swallowing food) had the lowest scores. The scores on the nine areas of the RODI correlate significantly with all items of the XI (data not shown) (presented in Appendix 1, for review purposes only). The highest correlation coefficient was observed between XI item 4 (mouth feel dry) and the dryness of the anterior tongue ($r = 0.70$). XI items related to extra-oral regions have poor correlations with RODI scores (correlation coefficient varying between 0.21 and 0.49) according to the standards described by Mukaka and co-workers and Akoglu and co-workers, for example, items 8 (skin of face), 9 (eyes), and 11 (nose) [31, 32]. In contrast, scores on XI items related to intra-oral locations show a stronger correlation with and the associated region of the RODI. Mainly XI item 7 (difficulty swallowing certain food) and XI item 10 (lips feel dry) have the highest correlation with the local dryness of respectively the pharynx ($r = 0.56$) and both upper and lower lip ($r = 0.63$ and 0.62).

Clinical Oral Dryness Score

The median CODS of 319 persons is 4.0 with IQR of 2.0–5.0 (mean = 3.57, SD = 1.82).

Table 5 presents how frequently each item of the CODS was scored. In the overall sample, item 1 (the mirror sticks to the cheek; 78.9%) was most frequently scored, and item 8 (debris on the palate; 2.5%) the least. The presence of CODS item 1 (mirror sticks to buccal mucosa) was associated with a significant difference in dry-mouth feeling inside the cheeks (Mann-Whitney $U = 4897$, $p = 0.009$, $r = - 0.16$). CODS item 2 (mirror sticks to tongue) and CODS item 4 (tongue lobulated/fissured) were associated with higher dryness of the regions anterior and posterior tongue (CODS 2 respectively for anterior and posterior tongue; $U = 6960$, $p = 0.000$, $r = - 0.26$ and $U = 7520$, $p = 0.000$, $r = - 0.21$) (CODS 4 respectively anterior and posterior tongue; $U = 5424$, $p = 0.000$, $r = - 0.22$ and $U = 6208$, $p = 0.023$, $r = - 0.14$). CODS item 6 (no saliva pooling in floor of mouth) corresponds with a higher dry-mouth feeling of the floor of the

Table 4 The scores of the 11 Xerostomia Inventory items (XI), presented as median with the corresponding interquartile range (IQR), and the mean with standard deviation (SD). N is the total numbers of participants.

	Median	IQR	Mean	SD	N
XI 1 (sip liquids to swallow food)	2.0	1.0–4.0	2.61	1.59	336
XI 2 (mouth dry when eating a meal) [a]	3.0	1.0–4.0	2.93	1.46	329
XI 3 (get up night to drink) [a,b]	3.0	2.0–5.0	3.19	1.49	336
XI 4 (my mouth feels dry) [a,b,c]	4.0	3.0–5.0	3.84	1.30	334
XI 5 (difficulty eating dry foods) [a,d]	3.0	1.0–5.0	3.03	1.59	336
XI 6 (suck sweets to relieve dry mouth) [b,c,d,e]	2.0	1.0–4.0	2.69	1.64	336
XI 7 (difficulty swallowing certain foods) [b,c,d,e]	2.0	1.0–4.0	2.55	1.52	337
XI 8 (the skin of my face feels dry) [a,c,d,e,g]	3.0	1.0–4.0	2.80	1.47	334
XI 9 (my eyes feel dry) [a,d,f,g,h]	3.0	1.0–5.0	3.05	1.58	337
XI 10 (my lips feel dry) [a,b,c,d,e,f,g,h,i]	4.0	3.0–5.0	3.63	1.34	337
XI 11 (the inside of my nose feels dry) [a,c,d,f,g,j]	3.0	1.0–4.0	2.91	1.54	335
XI total	33.0	22.5–43.0	32.94	11.88	337

Wilcoxon signed-rank tests: [a] $p < 0.05$ vs XI 1, [b] $p < 0.05$ vs XI 2, [c] $p < 0.05$ vs XI 3, [d] $p < 0.05$ vs XI 4, [e] $p < 0.05$ vs XI 5, [f] $p < 0.05$ vs XI 6, [g] $p < 0.05$ vs XI 7, [h] $p < 0.05$ vs XI 8, [i] $p < 0.05$ vs XI 9, [j] $p < 0.05$ vs XI 10

Table 5 Percentage of how frequently each item of the Clinical Oral Dryness Score (CODS) was identified (N = 319).

	CODS % yes
CODS 1 (mirror sticks to buccal mucosa)	78.9%
CODS 2 (mirror sticks to tongue)	48.7%
CODS 3 (tongue lobulated/fissured)	19.2%
CODS 4 (tongue shows loss of papillae)	24.8%
CODS 5 (frothy saliva)	61.8%
CODS 6 (no saliva pooling in floor of mouth)	19.2%
CODS 7 (glassy appearance of other oral mucosa especially palate)	47.4%
CODS 8 (debris on palate)	2.5%
CODS 9 (altered/smooth gingival architecture)	21.6%
CODS 10 (active or recently restored cervical caries)	36.4%

mouth (U = 4466, p = 0.006, r = - 0.16). CODS item 7 (glassy appearance of oral mucosa especially palate) was associated with more severe oral dryness of the anterior and posterior palate (U = 7058, p = 0.000, r = - 0.27 and U = 6541, p = 0.000, r = - 0.31 respectively anterior and posterior palate). There were no significant relations between CODS item 3 (tongue shows loss of papillae) and item 8 (debris on palate and perceived oral dryness of the corresponding anatomical regions).

All the reported significant associations can be considered robust to distributional violations as the bootstrapped 95% confidence interval did not exceed 0.

DISCUSSION

The results of this study demonstrated intra-oral differences in perceived mouth dryness between different locations in the mouth by using the RODI, a recently developed xerostomia questionnaire. The perceived dryness was considered the highest for the posterior palate and the lowest for the floor of the mouth. The highest correlations were found between regions in the RODI and corresponding related individual items of the XI and CODS.

As described in the introduction, the saliva film on intraoral tissue has local differences. The saliva film is thinnest at the anterior hard palate (~ 10 µm), while the saliva film at the anterior dorsal area of the tongue is much thicker (~ 54 µm) [18]. This pattern of different saliva film thickness at various intra-oral locations has been confirmed by other studies, where the palate is considered most dry, and tongue and floor of the mouth are considered as most wet, which explains the high MUC5 concentration on the palate [17, 19–21].

Several factors make the hard palate more susceptible to oral dryness than other intra-oral locations: paucity of (hard) palatal glands, gravity, and evaporation [1, 19, 33]. Gravity forces part of the whole saliva to pool in the floor of the mouth between swallowing episodes. As a consequence, the palate can be insufficiently moistened, especially in case of hyposalivation [20]. Furthermore, the palate is more prone to saliva evaporation, especially during speaking and breathing; and during speech air passes more or less continuously from the lungs over the mucosa of the palate [19]. The advantage of the tongue is that it is located near the opening from Wharton's ducts [17, 19, 20]. Here, saliva from the many minor glands in this region and the nasopalatine glands as well as the secretions of the submandibular and sublingual glands is collected [20]. This pattern of saliva thickness on the various mucosal sites

does not only apply to healthy subjects but is also applicable for dry-mouth patients [18, 20, 21].

The current study found intra-oral differences in perceived mouth dryness, in line with previous research finding different saliva film thickness at different intra-oral locations. This study found that the posterior palate was experienced as most dry, whereas other studies indicated that the anterior hard palate had the thinnest saliva coating [17, 18, 21]. The latter region is comparable with the anterior palate in this study. A possible explanation for this difference could be that patients find it hard to distinguish between two directly adjacent regions: the anterior part (up to the rugae) and posterior part (from the rugae to the end of the soft palate) of the palate and the posterior palate and the pharynx. In both cases, these regions have higher correlations than those of non-adjacent regions.

Another study reported the whole hard palate as having the thinnest saliva film without making a distinction between the anterior and posterior part [19]. Our results are in line with this study, as the schematic illustration of the posterior palate in the RODI is a combination of the hard palate and soft palate, which partly resembles the area studied by DiSabato-Mordarski and co-workers. Wolff and co-workers concluded that mostly hyposalivation patients had lower saliva film thickness at the posterior palate about 5-mm palatal to the second molars [20]. This could indicate that these patients could experience more dryness at the soft palate which is a part of the posterior palate in the present study.

In our study, the floor of the mouth was the wettest of all intra-oral regions. This finding is in line with previous studies [19, 20]. Another study also showed that the CODS item, no saliva pooling in the floor of the mouth, was only scored positively in the most severe hyposalivation patients [28]. However, three other studies only indicated the dorsal surface of the tongue as most wet [17, 18, 21]. These differences can be explained by the fact that these studies only measured the saliva thickness at the tongue and did not investigate the floor of the mouth.

The salivary flow rates had only negligible correlations with the perceived oral dryness at the nine regions. This supports the hypothesis that flow rates and severity of xerostomia do not have to be correlated [16, 23, 34]. Pai and co-workers explored self-reported dryness at four locations (lips, mouth, tongue, and throat) with a Visual Analogue Scale (VAS). They also found that the VAS scores showed little or no significant correlations with salivary flow rates [35].

Although the XI has been developed to quantify the overall feeling of mouth dryness, it contains some items referring to the dryness at different parts of the

body, for example the lips, the eyes, the skin, and the inside parts of the nose. As expected, XI items on extra-oral regions had poor correlations with regions of the RODI, whereas XI items related to dryness of the lips and difficulty in swallowing correlated higher with respectively upper and lower lip and pharynx of the RODI compared with all other regions. The regionally related CODS items also had a significant association with related regions in the RODI.

This study has some limitations. The patients who participated in this study are patients referred to a specialized saliva clinic. These patients suffer from saliva-related complaints and might be more concerned about their oral dryness than average patients suffering from dry mouth. Therefore, the results of this study could not be generalised to healthy subjects and other patients with dry-mouth complaints yet, and further studies with the RODI in other groups of patients seem warranted.

These subsequent studies could also explore different groups of patients, grouped according to the etiological factors for oral dryness. It is feasible that patients with oral dryness due to irradiation of the head and/or neck region might have another pattern of intra-oral dryness than patients suffering from Sjögren's disease or medication-induced hyposalivation.

MAIN CONCLUSIONS

The present study suggests that there is a significant difference in dry-mouth feeling among different intra-oral locations, with the highest perceived oral dryness for the posterior palate and the lowest for the floor of the mouth. Introduction of the RODI might help to discriminate among different potential causes of oral dryness in patients and for evaluating the efficacy of mouth-moistening products.

REFERENCES

1. Holmberg KV, Hoffman MP (2014) Anatomy, biogenesis and regeneration of salivary glands. Monogr Oral Sci 24:1–13
2. de Paula F, Teshima THN, Hsieh R, Souza MM, Nico MMS, Lourenco SV (2017) Overview of human salivary glands: highlights of morphology and developing processes. Anat Rec (Hoboken) 300:1180–1188
3. Humphrey SP, Williamson RT (2001) A review of saliva: normal composition, flow, and function. J Prosthet Dent 85:162–169
4. Dawes C, Pedersen AM, Villa A, Ekström J, Proctor GB, Vissink A, Aframian D, McGowan R, Aliko A, Narayana N, Sia YW, Joshi RK, Jensen SB, Kerr AR, Wolff A (2015) The functions of human saliva: a review sponsored by the world workshop on oral medicine VI. Arch Oral Biol 60:863–874
5. Tanasiewicz M, Hildebrandt T, Obersztyn I (2016) Xerostomia of various etiologies: a review of the literature. Adv Clin Exp Med 25: 199–206
6. Porter SR, Scully C, Hegarty AM (2004) An update of the etiology and management of xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endodont 97:28–46
7. Ying Joanna ND, Thomson WM (2015) Dry mouth - an overview. Singap Dent J 36:12–17
8. Saleh J, Figueiredo MA, Cherubini K, Salum FG (2015) Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol 60:242–255
9. Niklander S, Veas L, Barrera C, Fuentes F, Chiappini G, Marshall M (2017) Risk factors, hyposalivation and impact of xerostomia on oral health-related quality of life. Braz Oral Res 31:e14
10. Plemons JM, Al-Hashimi I, Marek CL (2014) Managing xerostomia and salivary gland hypofunction: executive summary of a report from the American Dental Association Council on Scientific Affairs. J Am Dent Assoc 145:867–873
11. Thomson WM, Chalmers JM, Spencer AJ, Williams SM (1999) The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health 16:12–17
12. Villa A, Connell CL, Abati S (2015) Diagnosis and management of xerostomia and hyposalivation. Ther Clin Risk Manag 11:45–51
13. Eisbruch A, Kim HM, Terrell JE, Marsh LH, Dawson LA, Ship JA (2001) Xerostomia and its predictors following parotid-sparing irradiation of head-and-neck cancer. Int J Radiat Oncol Biol Phys 50: 695–704
14. Thomson WM, van der Putten GJ, de Baat C et al (2011) Shortening the xerostomia inventory. Oral Surg Oral Med Oral Pathl Oral Radiol Endodont 112:322–327
15. van der Putten GJ, Brand HS, Schols JM, de Baat C (2011) The diagnostic suitability of a xerostomia questionnaire and the association between xerostomia, hyposalivation and medication use in a group of nursing home residents. Clin Oral Invest 15:185–192
16. Kho HS (2014) Understanding of xerostomia and strategies for the development of artificial saliva. Chin J Dent Res 17:75–83
17. Won S, Kho H, Kim Y, Chung S, Lee S (2001) Analysis of residual saliva and minor salivary gland secretions. Arch Oral Biol 46:619– 624

18. Pramanik R, Osailan SM, Challacombe SJ, Urquhart D, Proctor GB (2010) Protein and mucin retention on oral mucosal surfaces in dry mouth patients. Eur J Oral Sci 118:245–253
19. DiSabato-Mordarski T, Kleinberg I (1996) Measurement and comparison of the residual saliva on various oral mucosal and dentition surfaces in humans. Arch Oral Biol 41:655–665
20. Wolff M, Kleinberg I (1998) Oral mucosal wetness in hypo- and normosalivators. Arch Oral Biol 43:455–462
21. Lee SK, Lee SW, Chung SC, Kim YK, Kho HS (2002) Analysis of residual saliva and minor salivary gland secretions in patients with dry mouth. Arch Oral Biol 47:637–641
22. Alliende C, Kwon YJ, Brito M, Molina C, Aguilera S, Pérez P, Leyton L, Quest AF, Mandel U, Veerman E, Espinosa M, Clausen H, Leyton C, Romo R, González MJ (2008) Reduced sulfation of muc5b is linked to xerostomia in patients with Sjogren syndrome. Ann Rheum Dis 67:1480–1487
23. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH, Proctor GB (2015) Changes in saliva rheological properties and mucin glycosylation in dry mouth. J Dent Res 94:1660–1667
24. de Vries SAG, Tan CXW, Bouma G, Forouzanfar T, Brand HS, de Boer NK (2018) Salivary function and oral health problems in Crohn's disease patients. Inflamm Bowel Dis 24:1361–1367
25. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Int J Surg 12:1495– 1499
26. Jansen AC, van Aalst-Cohen ES, Hutten BA, Buller HR, Kastelein JJ, Prins MH (2005) Guidelines were developed for data collection from medical records for use in retrospective analyses. J Clin Epidemiol 58:269–274
27. Osailan SM, Pramanik R, Shirlaw P, Proctor GB, Challacombe SJ (2012) Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. Oral Surg Oral Med Oral Pathol Oral Radiol 114:597–603
28. Jager DHJ, Bots CP, Forouzanfar T, Brand HS (2018) Clinical oral dryness score: evaluation of a new screening method for oral dryness. Odontology 106:439–444
29. Navazesh M, Kumar SK. Measuring salivary flow: challenges and opportunities (2008). J Am Dent Assoc 139(Suppl:35s–40s)
30. Kalk WW, Vissink A, Spijkervet FK, Bootsma H, Kallenberg CG, Nieuw Amerongen AV (2001) Sialometry and sialochemistry: diagnostic tools for Sjogren's syndrome. Ann Rheum Dis 60:1110– 1116
31. Akoglu H (2018) User's guide to correlation coefficients. Turk J Emerg Med 18:91–93
32. Mukaka MM (2012) Statistics corner: a guide to appropriate use of correlation coefficient in medical research. Malawi Med J 24:69–71
33. Kessler AT, Bhatt AA (2018) Review of the major and minor salivary glands, part 1: anatomy, infectious, and inflammatory processes. J Clin Imaging Sci 8:47
34. Fox PC, Busch KA, Baum BJ (1987) Subjective reports of xerostomia and objective measures of salivary gland performance. J Am Dent Assoc 115:581–584
35. Pai S, Ghezzi EM, Ship JA (2001) Development of a visual analogue scale questionnaire for subjective assessment of salivary dysfunction. Oral Surg Oral Med Oral Pathol Oral Radiol Endodont 91: 311–316.

3

Differences in perceived intra-oral dryness in various dry-mouth patients as determined using the Regional Oral Dryness Inventory

Z. Assy
C. P. Bots
H. Z. Arisoy
S. S. Gülveren
F. J. Bikker
H. S. Brand

Clinical Oral Investigations, 2021 Jun; 25(6):4031–4043.

ABSTRACT

Objectives

Recently, it was shown that the Regional Oral Dryness Inventory (RODI) could determine differences in dry-mouth perception at different intra-oral locations. The main aim of this study was to determine whether the RODI might help to discriminate between various causes of oral dryness in dry-mouth patients. The second aim was to ascertain whether the RODI could become an additional diagnostic tool in dry-mouth patients.

Materials and methods

Data were collected retrospectively from patients who visited a specialized saliva clinic. Salivary flow rates, Xerostomia Inventory scores, and RODI scores were extracted from the medical records. Patients were stratified into subgroups according to their health status.

Result

Five hundred twenty-eight patients participated in this study (mean age of 59.6 ± 16.0 years; 68.4% female). Specific patient groups differed with regard to the region of the mouth they experienced as the most and least dry. The posterior palate was the area perceived as most dry by controls and Sjögren patients. In patients using limited or multiple medications, it was the anterior tongue. RODI scores also differed significantly among dry-mouth patient groups: whereas controls and patients using limited medication had the lowest RODI scores and experienced less intra-oral dryness, Sjögren patients had the highest RODI scores.

Conclusion

Our use of the RODI questionnaire showed that perceived intra-oral dryness differed between the various dry-mouth patients.

Clinical relevance

The RODI can be a valuable clinical diagnostic tool in dry-mouth diagnostics, in which it can be used to discriminate between the various causes of oral dryness in patients.

Keywords:

Dry mouth, Xerostomia, Salivary flowrate, Salivary pH, Xerostomia Inventory

INTRODUCTION

Saliva plays a crucial role in the preservation and maintenance of oral health due to its multiple functions, which include buffering capacity, lubrication, moistening, microbial homeostasis, and wound healing [1–4]. The consequences when salivary flow is impaired are therefore multidimensional, transcending oral health. For example, hyposalivation increases the risk of dental caries, gingivitis, and periodontitis. In addition, patients with impaired salivary flow can experience dry mouth, oral discomfort and pain, difficulty in speaking, taste alterations, and difficulty in swallowing [1, 2, 5]. Altogether, the effects of hyposalivation can have physical, emotional, and social impacts, thereby negatively affecting the quality of life, and particularly oral health [5, 6].

Dry-mouth symptoms can be caused by the use of xerogenic medications or multiple medications, but also by radiotherapy of the head and neck region, systemic diseases such as Sjögren's syndrome, and chronic stress [1, 5, 7, 8]. Obviously, dry-mouth symptoms may also be induced by a combination of factors [5]. For example, multiple medication usage is common in patients with Sjögren's syndrome. These etiologic factors produce dry-mouth symptoms through a variety of mechanisms. For example, dry-mouth–inducing medications have anticholinergic or sympathomimetic actions that affect the neural control of salivary glands, have a cytotoxic effect on the salivary glands, have a diuretic effect that depletes fluids, or damage the ion-transport pathways in the acinar cells. Irradiation of tumour sites in the head and neck region can also damage the salivary glands, leading to complete dysfunction of acini. On the other hand, Sjögren's syndrome induces progressive immune-mediated self-destruction of the salivary glands and lacrimal glands [1, 5]. Several mechanisms thus lead to impaired salivary function, and, as a consequence, hyposalivation and xerostomia, *i.e.,* perceived oral dryness.

As hyposalivation and xerostomia are not correlated per se [9, 10], any diagnosis of dry mouth should include objective parameters such as total salivary flow and subjective parameters such as total perceived oral dryness. However, due to the complex etiology of dry mouth and the various mechanisms underlying them, these parameters do not seem entirely discriminative. Diagnosis is difficult, especially for early-stage Sjögren's patients who lack specific clinical manifestations and biomarkers [11]. As the median delay between the onset of Sjögren's syndrome and diagnosis is 4 years (range 0– 28 years) [12], these current diagnostic tools are not sufficient for a more advanced dry-mouth diagnosis.

Recently, it was shown that a new questionnaire, the Regional Oral Dryness Inventory (RODI), could be used to determine differences in dry-mouth perception at different locations in the mouth [13]. The study in question concluded that the dry-mouth feeling differed significantly among intra-oral locations, with the highest perceived oral dryness in the posterior palate and the lowest in the floor of the mouth. It was speculated that, clinically, the RODI might help to discriminate between different potential causes of oral dryness in patients. It was thus hypothesized that patients with oral dryness caused by irradiation of the head and/or neck region might have a different distribution of intra-oral dryness than those with Sjögren's disease or medication-induced dry mouth [13].

The main aim of this study was therefore to determine whether the RODI might help to discriminate between causes of oral dryness in dry-mouth patients. To contribute to the study of dry-mouth diagnostics, the second aim was to ascertain whether the RODI might become an additional diagnostic tool in dry-mouth patients.

MATERIALS AND METHODS

Study design

Data for this retrospective case report study were collected from patients at the saliva clinic of the Dutch Institute for Salivary Research in Bunschoten, the Netherlands. They had been referred to this clinic by their dentists, general physicians, and medical specialists between October 2012 and April 2019. All patients had hyposalivation, xerostomia, hypersalivation, or other saliva-related problems. The study was approved by the Ethics Review Committee at the Academic Centre for Dentistry Amsterdam (ACTA, protocol number 201951). The reporting of this study conforms to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement [14]. All data, questionnaires, and clinical variables were collected and interpreted by a single practitioner (CB) according to the standard operating procedures of the regular patient-care routine, which generally took approximately 25 min.

Data collection methods

The relevant data were extracted from the medical record by two abstractors (HZA and SSG). The following clinical data were retrieved: age, sex, health status, number of medications used, Xerostomia Inventory (XI) scores, Regional Oral Dryness Inventory scores, salivary flow rate and salivary pH of unstimulated

whole saliva (UWS), chewing-stimulated whole saliva (CH-SWS), and citric acid-stimulated whole saliva (A-SWS). The extracted data were pseudonymized so they could no longer be traced back to the patients.

Because some questionnaires or salivary variables were incomplete, the total number (N) for some of the collected data differs. After data entry, one researcher (ZA) verified that data transfer for all records was correct.

Study variables

Questionnaires

When they visited the saliva clinic, all patients returned the prefilled questionnaires, including the Xerostomia Inventory (XI), the Regional Oral Dryness Inventory (RODI), and the European Medical Risk-Related History questionnaire. The XI consists of 11 items on a 5-point Likert scale ranging from 1 = "Never" to 5 = "Very often." The items concern patients' oral dryness and mouth feel. Per item, patients indicate how often they experience problems regarding mouth feel and oral dryness. The scores of the 11 items are summed to produce a total XI score that ranges between 11 (no xerostomia) and 55 (extreme xerostomia) [15].

The RODI questionnaire contains nine schematic illustrations of different locations in the oral cavity [13]. In our study, we used a slightly modified version with eight regions, excluding the throat. Four illustrations show areas in the upper jaw: the upper lip, the posterior part of the palate (from the rugae up to the end of the soft palate), the anterior part of the palate (including the rugae), and the inside part of the cheeks. The other four illustrations represent areas in the lower jaw: the lower lip, the anterior part of the tongue (from the tip of the tongue up to the vallate papilla), the posterior part of the tongue (from the vallate papilla up to end of the tongue), and the floor of the mouth. At each location, the patient uses a 5-point Likert scale ranging from 1 = "No dryness" to 5 = "severe dryness" to indicate the severity of the oral dryness they perceive [13].

The European Medical Risk-Related History questionnaire is an internationally validated patient-administered questionnaire that is used to map a patient's current health status [16, 17]. On the basis of their health status, patients were allocated to the following groups: controls, patients using limited medication, patients using multiple medications, irradiated patients, irradiated patients using multiple medications, Sjögren syndrome patients, and Sjögren patients using multiple medications (Table 1 for further details).

Only prescription medications that were used on a structural basis were counted. We scored different types of medication, but not their dosages.

We did not score the following types of medication and self-medication: oily crèmes, Vaseline-like ointments, over-the-counter drugs, vitamins (even if they had been prescribed by a physician), nutritional supplements, homeopathic remedies, and medications or products to relieve dry mouth or dry eye (such as artificial tears or dry eye gel/ointment, pilocarpine tablets or eye drops, artificial saliva, and mouth moistening gels or sprays). On the other hand, the following products were viewed as medication: corticosteroids or other anti-inflammatory crème/ointments and eye drops or eye gels with corticosteroid or other anti-inflammatory medicaments. But if a patient indicated clearly that he or she used over-the-counter anti-inflammatory drugs such as paracetamol or ibuprofen daily, these, too, were considered as medication.

Table 1 Dry-mouth patient groups in this study listed on the basis of their health status. The abbreviation used per patient group is listed, together with the group's health status.

Patient groups	Abbreviation used in this study	Health status
Controls	Controls	None of the conditions listed below (*i.e.*, radiation head and/or neck and Sjögren syndrome). Used no prescription medication
Patients using limited medication	Low Med patients	None of the conditions listed below. Used < 4 different prescription medications
Patients using multiple medications	High Med patients	None of the conditions listed below. Used ≥ 4 different prescription medications
Irradiated patients	RTX patients	Radiation of the head and/or neck area. Used < 4 different prescription medications
Irradiated patients using multiple medications	RTX + High Med patients	Radiation of the head and/or neck area. Used ≥ 4 different prescription medications
Sjögren syndrome patients	SS patients	Sjögren syndrome. Used < 4 different prescription medications
Sjögren patients using multiple medications	SS + High Med patients	Sjögren syndrome. Use ≥ 4 different prescription medications

Sialometry and salivary pH

The patients were instructed not to eat, drink, chew gum, brush teeth, use mouthwash, or smoke at least 1 h before their visit to the saliva clinic. The salivary flow rate was determined as described in the following references [18, 19]. At the time of saliva collection, patients were placed in a quiet room and asked to sit in an upright position. The UWS was collected by the draining method in a pre-weighed plastic container [19]. Patients were asked to collect unstimulated saliva immediately after an initial swallow, by expectorating into the container as soon as they had collected the saliva in their mouth. During saliva collection, patients were not allowed to swallow. To collect CH-SWS, they were asked to chew a 5 × 5-cm sheet of parafilm (Parafilm M, Pechiney, Chicago, IL, USA) at a frequency of approximately 60 chews per minute and to expectorate into a pre-weighed plastic container every 30 s. To stimulate A-SWS secretion, a citric acid solution (2% w/v) was applied with cotton buds to the lateral borders of the tongue at 30 s intervals [20]. When the collection period had finished, the plastic containers were reweighted, and the collected volume was determined by subtracting the weight of the container before collection. Salivary flow was calculated by dividing the volume collected (assuming 1 g of saliva equals 1 mL) by the collection time (min). Salivary flow rates were expressed in mL/min [19]. To limit circadian variations, all patients were randomly assigned a time slot between 8:00 and 12:00 A.M. [21].

To determine whether patients suffered from hyposalivation, the following cut-off values were used: UWS < 0.10 mL/min, CH-SWS < 0.70 mL/min, and ASWS < 0.70 mL/min [1].

The pH of saliva was measured immediately after saliva collection, within 5 min to minimize loss of CO2 to the atmosphere. The saliva pH was measured with pH paper (Merck KGaA, Darmstadt, Germany).

Data analysis

The data were processed in Microsoft Excel and then converted into SPSS, version 25.0 (IBM Corp SPSS statistics, Armonk, NY, USA) for the statistical analysis. The Shapiro– Wilk test was used to assess the normality of the data. As not all variables were normally distributed, the data were presented as medians with their interquartile range (IQR). To clarify relatively small differences, the mean and standard deviation (SD) were also reported.

A Friedman test was conducted for the RODI scores of the total study sample, followed by a Wilcoxon signed-rank test as a post hoc procedure.

The Kruskal-Wallis test was used to compare the different patient characteristics and RODI scores for all the various patient groups, followed by the Mann-Whitney U test as a post-hoc procedure.

The possible association between the RODI scores and the total XI scores was analyzed with a bootstrapped Spearman's rank correlation test (1000 × bootstrapping). The Spearman's rho coefficient and Bias-corrected accelerated (Bca) 95% confidence interval were extracted. The effect size of the correlation coefficient was interpreted as a negligible (r = 0.1–0.2), fair (r = 0.3–0.5), moderate (r = 0.6–0.7), or very strong (r = 0.8–0.9) correlation [22]. All significance levels (α) were set at 0.05.

RESULTS

Total study sample

A total of 528 health records were available. The mean age of participants in this study was 59.6± 16.0 years (N=522; the age of 6 participants was not documented). A majority of the patients were female (68.4%) (N=525; the gender of 3 participants was not documented). The RODI scores, XI scores, UWS, CHSWS, A-SWS salivary flow rates, and salivary pH were not normally distributed (Shapiro–Wilk test; $p<0.01$). Table 2 presents the various total XI scores, salivary flow rates, and salivary pH of the study sample. The flow rates suggested that the following proportions of the study sample were considered to have hyposalivation: UWS (33.4%), CH-SWS (55.2%), and A-SWS (29.6%). The mean number of medications used was 3 ±4, with a median of 2 and IQR of 0–4 (N =518; the number of medications was not listed for 10 participants).

Regional Oral Dryness Inventory for the total study sample

Table 3 shows the medians, corresponding IQRs, and means with standard deviations for each of the eight intra-oral regions of the RODI. Perceived oral dryness in the overall sample differed significantly among the eight intra-oral regions (Friedman test $p<0.05$, followed by Wilcoxon signed-rank test). The highest scores were found for the posterior part of the palate, and the lowest for the inside cheeks (Table 3).

Table 2 Patient characteristics for the total study sample. The total N differs because some data were missing for some patients. The total XI scores, the unstimulated whole saliva (UWS), chewing-stimulated whole saliva (CH-SWS), acid-stimulated whole saliva (A-SWS) flow rate (mL/min), and salivary pH of the study sample. Data are expressed as the median with the corresponding interquartile range (IQR) and as a mean with standard deviation (SD).

Saliva		**Mean ± SD**	**Median ± IQR**	**Number of subjects**
UWS	Flow rate (mL/min)	0.21 ± 0.21	0.16 ± 0.07–0.30	434
	pH	6.38 ± 0.56	6.50 ± 6.10–7.00	416
CH-SWS	Flow rate (mL/min)	0.76 ± 0.62	0.60 ± 0.30–1.10	446
	pH	6.75 ± 0.58	7.00 ± 6.50–7.00	444
A-SWS	Flow rate (mL/min)	1.28 ± 0.92	1.11 ± 0.57–1.80	450
	pH	4.91 ± 1.04	4.60 ± 4.00–5.50	450
XI total score		31.8 ± 11.4	32.0 ± 23.0–40.0	507

Table 3 Perceived oral dryness in eight different intra-oral regions as determined with the Regional Oral Dryness Inventory (RODI) in the total study sample. Data are presented as median with corresponding interquartile range (IQR) and as a mean with standard deviation (SD).

	Mean ± SD	**Median ± IQR**	**Total number of subjects for each intra-oral region**
Upper lip	2.84 ± 1.28	3.00 ± 2.00–4.00	449
Posterior part of palate[a]	3.04 ± 1.30	3.00 ± 2.00–4.00	456
Anterior part of palate[b]	2.88 ± 1.31	3.00 ± 2.00–4.00	444
Inside cheeks[a,b,c]	2.51 ± 1.32	2.00 ± 1.00–4.00	447
Lower lip[b,d]	2.84 ± 1.30	3.00 ± 2.00–4.00	448
Anterior part of tongue[a,d,e]	2.96 ± 1.33	3.00 ± 2.00–4.00	445
Posterior part of tongue[d,e]	2.99 ± 1.37	3.00 ± 2.00–4.00	452
Floor of the mouth[a,b,c,e,f,g]	2.58 ± 1.35	3.00 ± 1.00–4.00	445

a Wilcoxon signed-rank tests: $p < 0.05$ vs. upper lip
b Wilcoxon signed-rank tests: $p < 0.05$ vs. posterior palate
c Wilcoxon signed-rank tests: $p < 0.05$ vs. anterior palate
d Wilcoxon signed-rank tests: $p < 0.05$ vs. inside cheeks
e Wilcoxon signed-rank tests: $p < 0.05$ vs. lower lip
f Wilcoxon signed-rank tests: $p < 0.05$ vs. anterior part of the tongue
g Wilcoxon signed-rank tests: $p < 0.05$ vs. posterior part of the tongue

Various dry-mouth patient groups

The European Medical Risk-Related History questionnaire was completed by 517 patients in the total study sample. On the basis of their health status, we distinguished seven different groups of patients (Table 1). All patient groups were included in the statistical comparisons, except for the RTX + High Med group, due to its small number of patients (N = 6).

Table 4 shows the different patient characteristics for all six patient groups. Low Med patients were the largest group, and RTX patients were the smallest. High Med patients had the highest mean age, while controls had the lowest.

There were significantly higher percentages of women in the SS and SS + High Med patient groups than in the other four patient groups (Table 4).

The number of medications used also differed significantly among the six patient groups; High Med and SS + High Med patients used the highest number of medications. All other patient groups used between zero and two medications (Table 4).

Controls had significantly lower total XI scores than all other groups, indicating that the overall dry-mouth feeling they experienced was less. On the other hand, SS and SS + High Med patients had the highest XI scores, indicating that their dry-mouth feeling was significantly more severe than that of controls, Low Med patients, and High Med patients (Table 4).

With regard to the salivary flow rates, there was a trend whereby controls and Low Med patients had the highest salivary flow rates for UWS, CH-SWS, and A-SWS, while SS and SS + High Med patients had the lowest (Table 4). The difference among the patient groups with the highest and lowest flow rate was significant for UWS, CH-SWS, and A-SWS. Only the pH of A-SWS differed significantly from that in the various patient groups, being significantly higher in controls and Low Med patients than in SS and SS + High Med patients.

Overall, these results indicate that controls and Low Med patients had the highest salivary flow rates and pH. These groups also experienced less overall dry-mouth feeling as measured with the XI. On the other hand, SS and SS + High Med patients had the lowest salivary flow rates and pH, indicating that their salivary glands produced less saliva and that their salivary pH was lower. Further, these patients had the highest XI scores, indicating that their overall dry-mouth feeling was more severe.

Table 4: Patients were divided into six different patient groups based on their health status. Several patient characteristics for the six groups are shown; age, distribution of women and men in each group, the total number of medications used, the total XI-scores, salivary flow rates (in mL/min), and the salivary pH of UWS, CH-SWS, and A-SWS. The age is presented as mean with standard deviation (SD.) For the number of medications used, the total XI-scores, salivary flow rates, and salivary pH the median with corresponding interquartile range (IQR) is shown. The distribution of women and men is given in percentages. N indicates the total subjects for each patient group.

Patient groups (N=517)	**N**	**Age: mean±SD.***	**% of women: men***	**Number of medication: median±IQR ***	**Total XI-scores: median±IQR***	**UWS: median±IQR***	**pH UWS: median±IQR**	**CH-SWS: median±IQR***	**pH CH-SWS: median±IQR**	**A-SWS: median±IQR***	**pH A-SWS: median±IQR***
Controls	136	50.6±17.7	64.2: 35.8	-	27.0±19.0-34.0	0.22±0.07-0.36	6.50±6.10-7.00	0.76±0.44-1.33	7.00±6.70-7.00	1.62±1.01-2.20	4.70±4.40-5.80
Low Med patients	157	*60.7±14.8[a]*	68.2: 31.8	*2±1-2[a]*	*30.0±22.0-37.0[a]*	0.17±0.07-0.32	6.50±6.10-7.00	0.72±0.39-1.18	7.00±6.50-7.00	*1.17±0.71-1.80[a]*	4.70±4.00-6.10
High Med patients	140	*65.9±13.1[a,b]*	65.7: 34.3	*6±4-9[a,b]*	*33.0±24.0-40.0[a,b]*	*0.11±0.04-0.30[a,b]*	6.5±5.80-6.90	*0.57±0.27-1.08[a]*	7.00-6.10-7.00	*0.95±0.44-1.50[a,b]*	*4.40±4.00-5.00[a,b]*
RTX patients	10	58.7±17.9	40.0: 60.0	*1±0-1[a,b,c]*	*37.5±31.0-43.8[a,b]*	*0.13±0.05-0.23*	6.10±5.65-6.90	0.45±0.29-0.76	7.00±6.40-7.00	*0.52±0.24-0.95[a,b]*	4.55±4.15-5.08
SS patients	46	*61.7±14.0[a]*	*84.8: 15.2[a,b,c,d]*	*2±-2[a,b,c,d]*	*44.0±37.0-49.8[a,b,c]*	*0.08±0.03-0.16[a,b]*	6.50±6.00-6.80	*0.30±0.05-0.61[a,b,c]*	6.90±6.10-7.00	*0.50±0.23-1.15[a,b,c]*	*4.40±4.00-5.00[a,b]*
SS + High Med patients	22	*62.1±9.1[a]*	*95.5: 4.5[a,b,c,d]*	*7±5-9[a,b,d,e]*	*46.0±37.5-49.5[a,b,c,d]*	*0.06±0.03-w0.16[a,b]*	6.50±5.50-7.00	*0.28±0.14-0.56[a,b,c]*	7.00±6.50-7.00	*0.48±0.23-1.35[a,b]*	*4.40±4.00-4.70[a,b]*

** Indicates significant differences between the six patient groups, Kruskal Wallis test p<0.01.*
Mann-Whitney U test: [a] p<0.05 vs controls, [b] p<0.05 vs Low Med patients, [c] p<0.05 vs High Med patients, [d] p<0.05 vs RTX patients, and [e] p<0.05 vs SS patients.

Regional Oral Dryness Inventory for the various dry-mouth patient groups
Tables 5 and 6 show the perceived oral dryness in eight different intra-oral regions as determined with RODI for the six patient groups. In these patient groups, all eight intra-oral regions differed significantly (Kruskal Wallis test, $p < 0.01$).

The first function of Tables 5 and 6 is to provide an overview of the regions that each of the six patient groups experienced as the most dry and least dry. While the most dry in controls and SS patients was the posterior palate, in Low Med and High Med patients, it was the anterior tongue. The region that was experienced as least dry also differed between groups. In Low Med, High Med, and SS patients, it was the inside cheeks; in controls, it was the floor of the mouth. In RTX and SS + High Med patients, there were no significant differences among the intra-oral regions.

The second function of Tables 5 and 6 is to present the RODI scores for all intra-oral regions for the upper jaw (Table 5) and lower jaw (Table 6). SS and SS + High Med patients had the highest RODI scores for all these regions, while controls and Low Med patients had the lowest. The difference among the patient groups with the highest and lowest RODI scores was significant for all eight intra-oral regions (Mann-Whitney U test, $p < 0.05$). This result indicates that SS and SS + High Med patients experienced more severe intra-oral dryness than controls and Low Med patients.

High Med patients experienced significantly more severe intra-oral dryness than controls and Low Med patients, as shown by the higher RODI scores for all eight regions. On the other hand, High Med and SS patients differed only significantly with regard to the RODI scores of the posterior palate, indicating that SS patients experienced more severe dryness of the posterior palate than High Med patients (Table 5).

The RODI scores highlighted significant differences between High Med and SS + High Med patients for several regions. Higher scores showed that SS + High Med patients experienced more severe dryness in the inside cheeks, posterior tongue, and floor of the mouth than High Med patients did.

RTX patients had a significantly higher RODI score than controls and Low Med patients only for the inside cheeks. The RODI scores of RTX and SS + High Med patients differed significantly for the floor of the mouth, RTX patients having lower RODI scores than SS + High Med patients. This means that RTX patients experienced the floor of the mouth as less dry than SS + High Med patients.

As Tables 5 and 6 also show, SS and SS + High Med patients did not differ significantly, indicating that no clear distinction could be made between these two groups on the basis of their RODI scores.

Together, these results provide important insights into perceived intra-oral dryness in the various dry-mouth patient groups, which differed with regard to the regions they experienced as the most and least dry. Their RODI scores also differed significantly for the various intra-oral regions. The lowest RODI scores indicated that controls and Low Med patients experienced less intra-oral dryness and the highest RODI scores that SS and SS + High Med patients experienced more.

Table 5: Perceived oral dryness in four different intra-oral regions of the upper jaw as determined with the Regional Oral Dryness Inventory (RODI) in six different patient groups. Data are presented as median with corresponding interquartile range (IQR) and as a mean with standard deviation (SD.). N indicates the total number of subjects per intra-oral region.

Patient groups	Upper lip: mean±SD. (N)*	Upper lip: median±IQR	Anterior palate: mean±SD. (N)*	Anterior palate: median±IQR	Posterior palate: mean±SD. (N)*	Posterior palate: median±IQR	Inside cheeks: mean±SD. (N)*	Inside cheeks: median±IQR
Controls (N=136)	2.40±1.31 (N=113)	2.00±1.00-3.00	2.34±1.25 (N=114)	2.00±1.00-3.00	2.64±1.23 (N=115)	3.00±1.00-4.00	2.05±1.25 (N=113)	1.00±1.00-3.00
Low Med patients (N=157)	2.68±1.21 (N=134)	3.00±2.00-4.00	*2.74±1.26 (N=131)[a]*	3.00±2.00-4.00	2.81±1.32 (N=135)	3.00±1.00-4.00	2.28±1.30 (N=130)	2.00±1.00-3.00
High Med patients (N=140)	*3.08±1.28 (N=121)[a,b]*	3.00±2.00-4.00	*3.23±1.35 (N=119)[a,b]*	4.00±2.00-4.00	*3.23±1.32 (N=122)[a,b]*	4.00±2.00-4.00	*2.78±1.34 (N=121)[a,b]*	3.00±2.00-4.00
RTX patients (N=10)	3.00±1.05 (N=10)	3.00±2.00-4.00	3.00±1.00 (N=9)	3.00±2.50-4.00	3.30±1.42 (N=10)	4.00±1.75-4.00	*3.30±0.82 (N=10)[a,b]*	3.50±2.75-4.00
SS patients (N=46)	*3.40±0.98 (N=40)[a,b]*	3.00±3.00-4.00	*3.33±1.14 (N=40)[a,b]*	4.00±3.00-4.00	*3.79±0.90 (N=42)[a,b,c]*	4.00±3.00-4.00	*2.98±1.07 (N=40)[a,b]*	3.00±2.00-4.00
SS + High Med patients (N=22)	*3.50±1.04 (N=18)[a,b]*	4.00±2.75-4.00	*3.72±0.75 (N=18)[a,b]*	4.00±3.00-4.00	*3.84±0.50 (N=19)[a,b]*	4.00±4.00-4.00	*3.42±1.07 (N=19)[a,b,c]*	4.00±3.00-4.00

** Indicates significant differences between the six patient groups; Kruskal Wallis test p<0.01.*
Mann-Whitney U test: [a] p<0.05 vs. controls, [b] p<0.05 vs. Low Med patients, [c] p<0.05 vs. High Med patients, [d] p<0.05 vs. RTX patients, and [e] p<0.05 vs. SS patients.

Table 6: Perceived oral dryness in four different intra-oral regions of the lower jaw as determined with the Regional Oral Dryness Inventory (RODI) in six different patient groups. Data are presented as median with corresponding interquartile range (IQR) and as a mean with standard deviation (SD.). N indicates the total number of subjects per intra-oral region.

Patient groups	Lower lip: mean±SD. (N)*	Lower lip: median±IQR	Anterior tongue: mean±SD. (N)*	Anterior tongue: median±IQR	Posterior tongue: mean±SD. (N)*	Posterior tongue: median±IQR	Floor mouth: mean±SD. (N)*	Floor mouth: median±IQR
Controls (N=136)	2.38±1.27 (N=111)	2.00±1.00-3.00	2.48±1.33 (N=112)	2.50±1.00-4.00	2.57±1.34 (N=113)	3.00±1.00-4.00	2.01±1.20 (N=112)	2.00±1.00-3.00
Low Med patients (N=157)	*2.70±1.28 (N=135)[a]*	3.00-1.00-4.00	*2.83±1.29 (N=132)[a]*	3.00±2.00-4.00	2.79±1.34 N=134)	3.00±1.00-4.00	*2.42±1.31 (N=132)[a]*	2.00±1.00-4.00
High Med patients (N=140)	*3.05±1.27 (N=120)[a,b]*	3.00±2.00-4.00	*3.25±1.29 (N=118)[a,b]*	3.00±2.75-4.00	*3.24±1.30 (N=120)[a,b]*	3.00±2.00-4.00	*2.91±1.35 (N=118)[a,b]*	3.00±2.00-4.00
RTX patients (N=10)	3.10±1.10 (N=10)	3.50±2.00-4.00	3.00±1.41 (N=10)	4.00±1.00-4.00	3.10±1.37 (N=10)	3.50±1.75-4.00	2.70±1.16 (N=10)	3.00±1.75-4.00
SS patients (N=46)	*3.40±1.03 (N=40)[a,b]*	3.00±3.00-4.00	*3.41±1.14 (N=41)[a,b]*	4.00±3.00-4.00	*3.43±1.21 (N=42)[a,b]*	4.00±3.00-4.00	*3.07±1.27 (N=41)[a,b]*	3.00±2.00-4.00
SS + High Med patients (N=22)	*3.53±1.12 (N=19)[a,b]*	4.00±3.00-4.00	*3.72±1.02 (N=18)[a,b]*	4.00±3.00-4.00	*4.00±0.67 (N=19)[a,b,c]*	4.00±4.00-4.00	*3.68±0.95 (N=19)[a,b,c,d]*	4.00±3.00-4.00

** Indicates significant differences between the six patient groups, Kruskal Wallis test p<0.01.*
Mann-Whitney U test: [a] p<0.05 vs. controls, [b] p<0.05 vs. Low Med patients, [c] p<0.05 vs. High Med patients, [d] p<0.05 vs. RTX patients, and [e] p<0.05 vs. SS patients.

Relationship between the Regional Oral Dryness Inventory and the Xerostomia Inventory in various dry-mouth patient groups

Table 7 presents the Spearman's correlation between the intra-oral region scores of the RODI and the total XI scores for the six patient groups.

The XI scores of controls, Low Med patients, and High Med patients correlated significantly with all eight intra-oral regions (Spearman's rank test, $p < 0.01$). The correlation coefficients of these three patient groups ranged between 0.43 and 0.66 and can be viewed as representing fair to moderate correlations.

The XI scores of RTX patients correlated significantly with only three regions: the anterior palate and the anterior and posterior tongue (Spearman's rank test $p < 0.05$). These regions had a moderate to very strong correlation with the total XI scores (correlation coefficients between 0.69 and 0.78).

For SS patients, all regions except for the upper lip correlated significantly with total XI scores. The correlation coefficients of these regions ranged between 0.34 and 0.68. As for SS + High Med patients, only the following four regions correlated significantly with the total XI scores: the upper lip, the lower lip, the inside cheeks, and the floor of the mouth. Their correlation coefficients ranged between 0.57 and 0.63, which can be viewed as representing fair to moderate correlation.

Taken together, these results suggest that the correlations between the total XI scores of controls, Low Med, and High Med patients and all eight intra-oral regions of the RODI can be considered as fair to moderate. On the other hand, RTX, SS, and SS + High Med patients had only a small number of intra-oral regions that correlated significantly with the total XI scores. However, these correlations were stronger than the correlations of controls, Low Med, and High Med patients.

Table 7: Correlations between the perceived oral dryness in eight different intra-oral regions and the total XI-scores for the six different patient groups. The Spearman's rho correlation coefficient is shown with the Bias-corrected accelerated (BCa) 95% confidence interval.

Patient groups	Upper lip	Anterior palate	Posterior palate	Inside cheeks	Lower lip	Anterior tongue	Posterior tongue	Floor mouth
XI total for controls	0.57 (0.43-0.69)**	0.66 (0.53-0.77)**	0.66 (0.54-0.75)**	0.54 (0.38-0.68)**	0.51 (0.36-0.64)**	0.61 (0.48-0.71)**	0.61 (0.48-0.70)**	0.49 (0.33-0.63)**
XI total for Low Med patients	0.43 (0.25-0.58)**	0.43 (0.29-0.57)**	0.47 (0.29-0.62)**	0.52 (0.36-0.67)**	0.47 (0.30-0.62)**	0.58 (0.46-0.68)**	0.56 (0.42-0.68)**	0.48 (0.30-0.65)**
XI total for High Med patients	0.56 (0.39-0.70**	0.64 (0.52-0.75)**	0.62 (0.46-0.75)**	0.61 (0.46-0.73)**	0.51 (0.34-0.66)**	0.52 (0.34-0.67)**	0.59 (0.44-0.72)**	0.56 (0.41-0.70)**
XI total for RTX patients	NS	0.69 (0.12-0.98)*	NS	NS	NS	0.70 (0.00-1.00)*	0.78 (0.11-1.00)*	NS
XI total for SS patients	NS	0.48 (0.14-0.76)**	0.66 (0.46-0.82)**	0.48 (0.15-0.74)**	0.34 (-0.01-0.62)*	0.68 (0.47-0.83)**	0.58 (0.30-0.79)**	0.56 (0.24-0.81)**
XI total for SS + High Med patients	0.59 (0.14-0.88)*	NS	NS	0.57 (0.04-0.90)*	0.57 (0.07-0.87)*	NS	NS	0.63 (0.07-0.88)**

** indicates that that the correlation is significant at level 0.05.*
*** indicates that that the correlation is significant at level 0.01.*
NS = not significant.

DISCUSSION

The results of this study, in which we explored the RODI questionnaire in specific subgroups of dry-mouth patient groups, show that the regions of perceived intra-oral dryness differed between the groups. Controls and Low Med patients had the lowest RODI scores and experienced less intra-oral dryness than the other groups of patients. On the other hand, SS and SS + High Med patients had the highest RODI scores, meaning that they experienced more intra-oral dryness.

The RODI scores of our sample revealed that the posterior palate was experienced as the most dry, while the inside cheeks were experienced as the least dry. This result is consistent with the findings of a previous study in which patients also indicated that the posterior palate was the most dry [13].

Several factors make the palate more susceptible to oral dryness than other intra-oral locations: gravity, evaporation, and the paucity of palatal glands [23–25]. For the region that was experienced as the least dry, perceived dryness did not differ significantly between the inside cheeks and the floor of the mouth (Table 3). Both regions include orifices of the major salivary glands [23]. Because of their proximity to the orifices of the salivary glands, the saliva film in these regions is probably more moisturizing than the saliva film on the palate [24, 26–28]. For this reason, all patients experienced the inside cheeks and the floor of the mouth as less dry. This finding is comparable with that in the previous study, which found that patients experienced the floor of the mouth as the least dry [13].

Our results showed that the controls and SS patients experienced the posterior palate as the driest. Notably, they show that SS patients had significantly higher RODI scores (median score 4.00) for the posterior palate than controls did (median score 3.00). This can be explained by the fact that except for the palatal salivary flow rate [29, 30], the UWS flow rate in SS patients is lower [20, 29–34]. Indeed, the number of patients with xerostomia was higher in SS patients [29, 30, 32]. A plausible explanation is that the subjective feeling of xerostomia is strongly related to the UWS flow. In controls—who had sufficient UWS—the palatal glands contributed little to the dry-mouth feeling [28]. This suggestion is further supported by Wang and co-workers, who did not find a significant correlation between summated XI scores and minor salivary-gland flow rates [35]. This is consistent with the fact that under healthy conditions, the saliva secreted by the minor salivary glands' accounts for less than 10% of whole saliva [36]. Additionally, SS patients have other saliva-related characteristics that induce dry mouth: an altered sialochemical composition, such as higher

concentrations of sodium, chloride, and phosphate [20]; a higher protein concentration on the palate [37]; a significantly reduced saliva film on the hard palate; a reduced spinnbarkeit of UWS; and an altered glycosylation of salivary mucins [38]. In conclusion, a drier mouth could be induced in SS patients when altered rheological properties of saliva, reduced mucosal hydration (due to a reduced saliva film), and altered glycosylation combine to cause functional loss of the salivary coating and the lubricating properties of saliva [38].

Low Med and High Med patients experienced the anterior tongue as the most dry. Other studies reported that the thickness of saliva film on the anterior tongue was significantly less in dry-mouth patients—including those with medication-induced hypofunction—than in healthy controls [28, 31, 37, 39]. The saliva-film thickness on the anterior tongue was approximately half of that in controls. In some dry-mouth patients who could not secrete unstimulated saliva, it was even less than that [28]. This finding was confirmed by another study that indicated that oral mucosal wetness varied with the resting salivary flow rate; the lower the flow rate, the thinner the salivary film [27]. Thus, xerostomia was apparent when the salivary flow rate was half of its normal value [9, 40, 41].

Reduction of the salivary flow rate and thereby a reduced salivary film thickness on the anterior tongue might therefore explain why Low Med and High Med patients experienced the anterior tongue as the most dry. Besides, the threshold for perceiving dryness is about ≤ 10 µm—the same as that seen in the study of Lee and co-workers [28]. The significantly lower salivary flow rates in High Med patients than in controls (Table 4) may have induced a very low saliva-film thickness on the anterior tongue below this threshold, thereby causing dryness of the tongue.

Some of the controls in our study had a low salivary flow rate and at times even had hyposalivation of UWS and CHSWS (Table 4). Explanations for this may lie in these participants' age and the possibility that participants had systemic disorders other than Sjögren's syndrome that were associated with salivary dysfunction. The salivary flow rate in older people, even those not using systemic drugs, was significantly lower, especially in non-medicated women in the 45–54 age groups [42]. This finding corresponds with the mean age in our control group (50.6 ± 17.7 years), in which most participants were female (64.2%). Other systemic conditions such as endocrine disorders (diabetes mellitus), neurological disorders (Parkinson's disease), and metabolic disorders (dehydration) have also been associated with a lower salivary flow rate [1].

Within our study sample, the SS and SS + High Med patients had the lowest salivary flow rates and a reduced pH of A-SWS: proof of hypofunction of the

salivary glands. As one would expect, these patient groups also had the highest XI scores and RODI scores for all intra-oral regions. The severe mouth dryness (both overall dry-mouth experience and intraoral dryness) they experienced may have been due to the reduced flow rate, but also to altered rheological properties of saliva, and altered glycosylation of mucins.

The RODI questionnaire nonetheless seemed capable of differentiating between dry-mouth patient groups. For example, SS patients could easily be differentiated from controls, Low Med, and High Med patients, as Low Med and High Med patients experienced the anterior tongue as the most dry, while SS patients experienced the posterior palate as the most dry. On the other hand, SS patients had more severe dryness of the posterior palate than controls. These differences in intra-oral dryness can be diagnosed only using the RODI questionnaire and not the XI, as the latter is used only to diagnose the overall dry-mouth experience. For this reason, the RODI questionnaire may be a valuable tool in dry-mouth diagnostics.

It is interesting to note that there were no significant differences between RODI scores in RTX patients. Even when these scores were compared with those of other patient groups, few regions showed intra-oral differences. These results might be related to a lack of statistical power, as the RTX group only comprised 10 subjects. However, RTX patients are not usually difficult to identify, because they can indicate whether they have been treated with radiotherapy of the head and neck region. Most patients will also have been referred to their dentist before and after radiotherapy [43, 44].

With regard to the association between the RODI score and the total XI scores in various dry-mouth patients, the correlations in the RTX, SS, and SS + High Med patient groups were stronger than the other patient groups. The correlations for these patients were especially strong for the floor of the mouth and for the anterior and posterior tongue (Table 7). These indicate that patients with a very dry mouth overall (higher XI scores) will also experience more severe oral dryness on the floor of the mouth, and on the anterior and posterior tongue (higher RODI scores for these regions). A previous study that used the Clinical Oral Dryness Score (CODS), a clinical tool to semi-quantitatively assess oral dryness, also found that the CODS items “No saliva pooling in the floor of mouth” and “Tongue fissured” scored higher in the hyposalivation group [18]. This idea was supported by Osailan and co-workers, who reported that the clinical features of oral dryness that are included in the CODS—such as fissured or depapillated tongue, and lack of saliva pooling in the floor of the mouth—are recognized signs of chronic hyposalivation [31]. Other clinical features of their study, such as a mirror sticking to the tongue, a lack of saliva

pooling in the floor of the mouth, and a tongue showing loss of papillae, can be associated with a moderate but significant reduction in mucosal wetness [31]. The combination of their findings with ours confirms that an important role in dry-mouth perception may be played by two regions: the floor of the mouth and the anterior and posterior tongue. Potentially, the RODI questionnaire would thus play a useful role in early dry-mouth screening, when a patient could be asked specifically about dryness of the floor of the mouth, and of the anterior and posterior tongue. If high RODI scores (score ≥ 3) are obtained for these regions, further dry-mouth diagnostics may be implemented.

A possible limitation of the current study is that the patients included were allocated to the various dry-mouth patient groups on the basis of their self-reported answers to the European Medical Risk-Related History questionnaire [16, 17]. A patient's health status was thus dependent on his or her reportage. In most cases, there was no confirmation by a physician or a pharmacist either that the patient had Sjögren's syndrome, or had been irradiated in the head and/or neck region, or about the number of prescription medications that were used. While this information was sometimes confirmed in the referral letter or a medication overview provided by a pharmacist, it was not always available for all patients. The data of this study therefore need to be interpreted with caution. However, the European Medical Risk-Related History questionnaire has a high validity. In previous studies that compared the results of this questionnaire with those of a verbal history taken by a physician experienced in pre-assessment control, sensitivity ranged between 88% and 92%, and specificity was 98–99% [45, 46].

Another possible limitation of the current study is the bias that may have resulted from our collection of saliva at the beginning of a working day, when the unstimulated flow rate changes most rapidly [21]. However, as all patients had been randomly assigned to time slots between 8:00 and 12:00, this potential bias was evenly distributed over the total study sample.

MAIN CONCLUSIONS

The present study shows that the RODI questionnaire was able to identify differences between perceived intra-oral dryness in various dry-mouth patient groups. Dry-mouth patients differed with regard to the regions they experienced as the most and least dry. Controls and SS patients experienced the posterior palate as the most dry, and Low Med and High Med patients the anterior tongue. The RODI scores for the various intra-oral regions differed significantly among dry-mouth patients. SS and SS + High Med patients had the highest RODI scores for all intra-oral regions, while controls and Low Med patients had the lowest. These findings suggest that the RODI questionnaire might be a useful additional diagnostic tool for dry-mouth diagnostics, as it may be used to discriminate between potential causes of oral dryness in patients. With the help of this questionnaire, SS patients could be easily differentiated from controls, Low Med, and High Med patients.

The RODI might play an important role in early dry-mouth diagnostics as the floor of the mouth, and the anterior and posterior tongue of the RODI may play important roles in dry-mouth perception.

REFERENCES

1. Saleh J, Figueiredo MA, Cherubini K, Salum FG (2015) Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol 60(2):242–255. https://doi.org/10.1016/j.archoralbio.2014.10.004
2. Roblegg E, Coughran A, Sirjani D (2019) Saliva: an all-rounder of our body. Eur J Pharm Biopharm 142:133–141. https://doi.org/10.1016/j.ejpb.2019.06.016
3. Dawes C, Pedersen AM, Villa A, Ekström J, Proctor GB, Vissink A, Aframian D, McGowan R, Aliko A, Narayana N, Sia YW, Joshi RK, Jensen SB, Kerr AR, Wolff A (2015) The functions of human saliva: a review sponsored by the world workshop on oral medicine VI. Arch Oral Biol 60(6):863–874. https://doi.org/10.1016/j.archoralbio.2015.03.004
4. Humphrey SP, Williamson RT (2001) A review of saliva: normal composition, flow, and function. J Prosthet Dent 85(2):162–169. https://doi.org/10.1067/mpr.2001.113778
5. Ying Joanna ND, Thomson WM (2015) Dry mouth - an overview. Singap Dent J 36:12–17. https://doi.org/10.1016/j.sdj.2014.12.001
6. Niklander S, Veas L, Barrera C, Fuentes F, Chiappini G, Marshall M (2017) Risk factors, hyposalivation and impact of xerostomia on oral health-related quality of life. Brazilian oral research 31:e14. https://doi.org/10.1590/1807-3107BOR-2017.vol31.0014
7. Porter SR, Scully C, Hegarty AM (2004) An update of the etiology and management of xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 97(1):28–46. https://doi.org/10.1016/j.tripleo.2003.07.010
8. Tanasiewicz M, Hildebrandt T, Obersztyn I (2016) Xerostomia of various etiologies: a review of the literature Adv Clin Exp Med 25 (1):199-206. doi:https://doi.org/10.17219/acem/29375
9. Kho HS (2014) Understanding of xerostomia and strategies for the development of artificial saliva. Chin J Dent Res 17(2):75–83
10. Fox PC, Busch KA, Baum BJ (1987) Subjective reports of xerostomia and objective measures of salivary gland performance. J Am Dent Assoc 115(4):581–584. https://doi.org/10.1016/s0002-8177(87)54012-0
11. Jin Y, Li J, Chen J, Shao M, Zhang R, Liang Y, Zhang X, Zhang X, Zhang Q, Li F, Cheng Y, Sun X, He J, Li Z (2019) Tissue-specific autoantibodies improve diagnosis of primary Sjögren's syndrome in the early stage and indicate localized salivary injury. J Immunol Res 2019:3642937–3642938. https://doi.org/10.1155/2019/3642937
12. Douglas L (2018) Facilitating timely diagnosis of Sjögren's syndrome. BDJ Team 5(2):18026. https://doi.org/10.1038/bdjteam.2018.26
13. Assy Z, Jager DHJ, Mashhour E, Bikker FJ, Brand HS (2020) Regional differences in perceived oral dryness as determined with a newly developed questionnaire, the Regional Oral Dryness Inventory. Clin Oral Investig 24:4051–4060. https://doi.org/10.1007/s00784-020-03276-7
14. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Int J Surg 12(12): 1495–1499. https://doi.org/10.1016/j.ijsu.2014.07.013
15. Thomson WM, Chalmers JM, Spencer AJ, Williams SM (1999) The xerostomia inventory: a multi-item approach to measuring dry mouth. Community Dent Health 16(1):12–17

16. Smeets EC, de Jong KJ, Abraham-Inpijn L (1998) Detecting the medically compromised patient in dentistry by means of the medical risk-related history. A survey of 29,424 dental patients in the Netherlands. Prev Med 27(4):530–535. https://doi.org/10.1006/pmed.1998.0285
17. Abraham-Inpijn L, Russell G, Abraham DA, Backman N, Baum E, Bullon-Fernandez P, Declerck D, Fricain JC, Georgelin M, Karlsson KO, Lamey PJ, Link-Tsatsouli I, Rigo O (2008) A patient administered Medical Risk Related History questionnaire (EMRRH) for use in 10 European countries (multicenter trial). Oral Surg Oral Med Oral Pathol Oral Radiol Endod 105(5):597–605. https://doi.org/10.1016/j.tripleo.2007.09.032
18. Jager DHJ, Bots CP, Forouzanfar T, Brand HS (2018) Clinical oral dryness score: evaluation of a new screening method for oral dryness. Odontology 106(4):439–444. https://doi.org/10.1007/s10266-018-0339-4
19. Navazesh M, Kumar SK (2008) Measuring salivary flow: challenges and opportunities J Am Dent Assoc 139 Suppl:35s-40s. doi:https://doi.org/10.14219/jada.archive.2008.0353
20. Kalk WW, Vissink A, Spijkervet FK, Bootsma H, Kallenberg CG, Nieuw Amerongen AV (2001) Sialometry and sialochemistry: diagnostic tools for Sjögren's syndrome. Ann Rheum Dis 60(12): 1110–1116. https://doi.org/10.1136/ard.60.12.1110
21. Dawes C (1972) Circadian rhythms in human salivary flow rate and composition. J Physiol 220(3):529–545. https://doi.org/10.1113/jphysiol.1972.sp009721
22. Akoglu H (2018) User's guide to correlation coefficients. Turkish journal of emergency medicine 18(3):91–93. https://doi.org/10.1016/j.tjem.2018.08.001
23. Holmberg KV, Hoffman MP (2014) Anatomy, biogenesis and regeneration of salivary glands. Monogr Oral Sci 24:1–13. https://doi.org/10.1159/000358776
24. DiSabato-Mordarski T, Kleinberg I (1996) Measurement and comparison of the residual saliva on various oral mucosal and dentition surfaces in humans. Arch Oral Biol 41(7):655–665. https://doi.org/10.1016/s0003-9969(96)00055-6
25. Kessler AT, Bhatt AA (2018) Review of the major and minor salivary glands, part 1: anatomy, infectious, and inflammatory processes. J Clin Imaging Sci 8:47. https://doi.org/10.4103/jcis.JCIS_45_18
26. Won S, Kho H, Kim Y, Chung S, Lee S (2001) Analysis of residual saliva and minor salivary gland secretions. Arch Oral Biol 46(7): 619–624. https://doi.org/10.1016/s0003-9969(01)00018-8
27. Wolff M, Kleinberg I (1998) Oral mucosal wetness in hypo- and normosalivators. Arch Oral Biol 43(6):455–462. https://doi.org/10.1016/s0003-9969(98)00022-3
28. Lee SK, Lee SW, Chung SC, Kim YK, Kho HS (2002) Analysis of residual saliva and minor salivary gland secretions in patients with dry mouth. Arch Oral Biol 47(9):637–641. https://doi.org/10.1016/s0003-9969(02)00053-5
29. Marton K, Boros I, Fejerdy P, Madlena M (2004) Evaluation of unstimulated flow rates of whole and palatal saliva in healthy patients wearing complete dentures and in patients with Sjögren's syndrome. J Prosthet Dent 91(6):577–581. https://doi.org/10.1016/j.prosdent.2004.03.031
30. Marton K, Boros I, Varga G, Zelles T, Fejerdy P, Zeher M, Nagy G (2006) Evaluation of palatal saliva flow rate and oral manifestations in patients with Sjögren's syndrome. Oral Dis 12(5):480–486. https://doi.org/10.1111/j.1601-0825.2005.01224.x

31. Osailan SM, Pramanik R, Shirlaw P, Proctor GB, Challacombe SJ (2012) Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. Oral Surg Oral Med Oral Pathol Oral Radiol 114(5):597–603. https://doi.org/10.1016/j.oooo.2012.05.009

32. Ergun S, Cekici A, Topcuoglu N, Migliari DA, Kulekci G, Tanyeri H, Isik G (2010) Oral status and Candida colonization in patients with Sjögren's syndrome. Med Oral Patol Oral Cir Bucal 15(2): e310–e315. https://doi.org/10.4317/medoral.15.e310

33. Rusthen S, Young A, Herlofson BB, Aqrawi LA, Rykke M, Hove LH, Palm O, Jensen JL, Singh PB (2017) Oral disorders, saliva secretion, and oral health-related quality of life in patients with primary Sjögren's syndrome. Eur J Oral Sci 125(4):265–271. https://doi.org/10.1111/eos.12358

34. Culp DJ, Stewart C, Wallet SM (2019) Oral epithelial membrane associated mucins and transcriptional changes with Sjögren's syndrome. Oral Dis 25(5):1325–1334. https://doi.org/10.1111/odi.13098

35. Wang Z, Li W, Hong X, Su JZ, Hua H, Peng X, Lv L, Yu GY (2016) Minor salivary glands function is decreased in hyposalivation-related diseases. Arch Oral Biol 69:63–70. https://doi.org/10.1016/j.archoralbio.2016.05.012

36. Dawes C, Wood CM (1973) The contribution of oral minor mucous gland secretions to the volume of whole saliva in man. Arch Oral Biol 18(3):337–342. https://doi.org/10.1016/0003-9969(73)90156-8

37. Pramanik R, Osailan SM, Challacombe SJ, Urquhart D, Proctor GB (2010) Protein and mucin retention on oral mucosal surfaces in dry mouth patients. Eur J Oral Sci 118(3):245–253. https://doi.org/10.1111/j.1600-0722.2010.00728.x

38. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH, Proctor GB (2015) Changes in saliva rheological properties and mucin glycosylation in dry mouth. J Dent Res 94(12):1660–1667. https://doi.org/10.1177/0022034515609070

39. Osailan S, Pramanik R, Shirodaria S, Challacombe SJ, Proctor GB (2011) Investigating the relationship between hyposalivation and mucosal wetness. Oral Dis 17(1):109–114. https://doi.org/10.1111/j.1601-0825.2010.01715.x

40. Lofgren CD, Wickstrom C, Sonesson M, Lagunas PT, Christersson C (2012) A systematic review of methods to diagnose oral dryness and salivary gland function. BMC Oral Health 12:29. https://doi.org/10.1186/1472-6831-12-29

41. Moerman RV, Bootsma H, Kroese FG, Vissink A (2013) Sjögren's syndrome in older patients: aetiology, diagnosis and management. Drugs Aging 30(3):137–153. https://doi.org/10.1007/s40266-013-0050-7

42. Yeh CK, Johnson DA, Dodds MW (1998) Impact of aging on human salivary gland function: a community-based study. Aging (Milano) 10(5):421–428. https://doi.org/10.1007/bf03339889

43. Cohen EE, LaMonte SJ, Erb NL, Beckman KL, Sadeghi N, Hutcheson KA, Stubblefield MD, Abbott DM, Fisher PS, Stein KD, Lyman GH, Pratt-Chapman ML (2016) American Cancer Society head and neck cancer survivorship care guideline. CA Cancer J Clin 66(3):203–239. https://doi.org/10.3322/caac.21343

44. Margalit DN, Losi SM, Tishler RB, Schoenfeld JD, Ann Fugazzotto J, Stephens J, Cebulski AL, Hammerstrand EL, Ma L, Lopes HM, Haddad RI, Treister NS, Frustino JL (2015) Ensuring head and neck oncology patients receive recommended pre-treatment dental evaluations. J Oncol Pract 11(2):151–154. https://doi.org/10.1200/jop.2014.000414

45. de Jong KJ, Abraham-Inpijn L, Vinckier F, Declerck D (1997) The validity of a medical risk-related history for dental patients in Belgium. Int Dent J 47(1):16–20. https://doi.org/10.1111/j.1875-595x.1997.tb00672.x

46. de Jong KJ, Borgmeijer-Hoelen A, Abraham-Inpijn L (1991) Validity of a risk-related patient-administered medical questionnaire for dental patients. Oral Surg Oral Med Oral Pathol 72(5):527–533. https://doi.org/10.1016/0030-4220(91)90488-x

INTRA-ORAL SURFACE AREA AND SALIVARY DISTRIBUTION

4

Determination of intra-oral surface areas by cone-beam computed tomography analysis and their relation with anthropometric measurements of the head

Z. Assy
C. Klop
H. S. Brand
R. C. Hoogeveen
J. H. Koolstra
F. J. Bikker

Surgical and Radiologic Anatomy, 2020 Sep;42(9):1063-1071.

ABSTRACT

Purpose Determination of intra-oral surface areas might contribute to our understanding of the physiology of the oral cavity and oral diseases. In previous studies, the intra-oral surface area was determined using a laborious and technically challenging method. Our aim was to develop an easy and non-invasive method to determine the intra-oral surface areas.

Methods In this study, we used cone-beam computed tomography (CBCT) and digital analysis in 20 human cadavers to determine various intra-oral surface areas, based on digital segmentation. Next, we explored whether there was a relationship between various intra-oral surface areas and anthropometric measurements of the head using Pearson correlation coefficient.

Results Using CBCT and digital analysis, it was possible to determine various intra-oral surface areas. On average, the total intra-oral surface area was 173 ± 19 cm^2. Moderate, statistical significant correlations were observed between (1) the length of the head and the palatal surface area, as well as (2) the depth of the head and the surface area of the tongue. These correlations suggest the feasibility of estimating intra-oral surface areas without relying on CBCT imaging.

Conclusions This study presents a technique for measuring the intra-oral surface areas by CBCT imaging in combination with digital analysis. The results of this study suggest that anthropometric measurements of the head might be used to estimate the surface areas of the palate and tongue.

Keywords Tongue, Palate, Anthropometry, Cone-Beam Computed Tomography

INTRODUCTION

Knowledge of the integrity and anatomy of the intra-oral surface areas, including the oral mucosa, contributes to a better understanding of the physiology of the mouth and the oral health [23]. In addition, knowledge of the intra-oral anatomy and surface areas is important for therapeutic purposes [23], for example in orthodontic treatment and maxillofacial surgery.

Under healthy conditions, the intra-oral surfaces are covered by a salivary film, which moistens the oral cavity [3, 24]. In this light, the size of the intra-oral surface area has previously been measured to determine the distribution and average thickness of the salivary film covering the teeth and oral mucosa [8, 19, 29]. For this reason, dental impressions were made of all structures (including hard and soft tissue) inside the oral cavity. Then, from these impressions, stone models were produced and covered with aluminium foil. Subsequently, this foil was weighed, and surface areas were deduced [8, 19, 29]. This foil technique has been proven to be reproducible [8, 19, 29]. However, the adaptation of the foil onto the models without stretching appeared to be technically challenging, as stretching would possibly lead to thinning of the foil and subsequent underestimation of the surface [8]. Another reported challenge was the difficulty to manually extend the foil completely into all interdental spaces, the labial and buccal vestibular mucosa.

Aiming to provide an alternative method, we performed a study to quantify the intra-oral surface areas using cone-beam computed tomography (CBCT) in combination with digital analysis. This method was inspired by previous studies, in which CBCT was used for soft tissue analysis including determination of the void volume of the oral cavity [5, 11, 18, 28]. CBCT involves the use of ionizing radiation, rendering this approach unsuitable for routine medical care. This led to the concept to investigate correlations between facial anthropomorphic measurements and intra-oral surface areas. It was, therefore, hypothesized that a relation between anthropomorphic measurements and intra-oral surfaces would potentially enable easy approximation of the intra-oral surface area in a chair-side medical setting, without exposure to radiation. For ethical reasons, we explored this hypothesis on cadavers.

MATERIALS AND METHODS

Cadavers

In total, 23 human cadaver heads were provided by the Anatomical-Embryological Laboratory of the University of Amsterdam. All cadavers were testamentary donations of volunteers to this department. The use of the material was in accordance with the Dutch Law (Wet Medisch-wetenschappelijk Onderzoek met Mensen, WMO) and the study was approved by the ethical committee of Academic Centre for Dentistry Amsterdam (ACTA, protocol number 2017011).

Arterial embalming has been used to fixate whole body cadavers [6, 12, 25]. A chemical preservative based on formaldehyde was injected through the femoral artery with slight pressure to prevent deformation of the blood vessels in the head. Afterwards, the head was dissected and preserved in a mixed solution of 16.7% glycerol, 8.3% ethanol, and 0.21% phenol.

The cadaver heads all had a complete oral cavity, with the mandibula, the maxilla, the palate, soft tissues and some teeth present. As metallic restoration materials cause scattering on CBCT images, and hence reduce soft-tissue visualization by loss of contrast resolution and image artifacts [2], all metallic materials were removed prior to CBCT scanning.

Cadavers previously dissected in the intra-oral region or cadavers in which mouth opening was impossible were excluded. In this way, three of the 23 cadaver heads were excluded from this study. In the case of seven cadavers, no information about their sex and age was available. The mean age at death of the remaining 13 cadavers was 83 years (range 70–96 years) with a female–male ratio of 8:5. Prior to analysis, the cadavers were removed from the fixation liquid and air dried in a fume cupboard. Additionally, the oral cavity was dried using cotton rolls (PURE, Akzenta International Sa., Chiasso, Switzerland).

Analysis of anthropometric measurements

The distance between anthropometric landmarks was measured by two independent measurements using an anatomical sliding calliper (resolution 0.5 mm) which conforms to other studies (Table 1 and Fig. 1) [7, 9, 10, 17, 26].

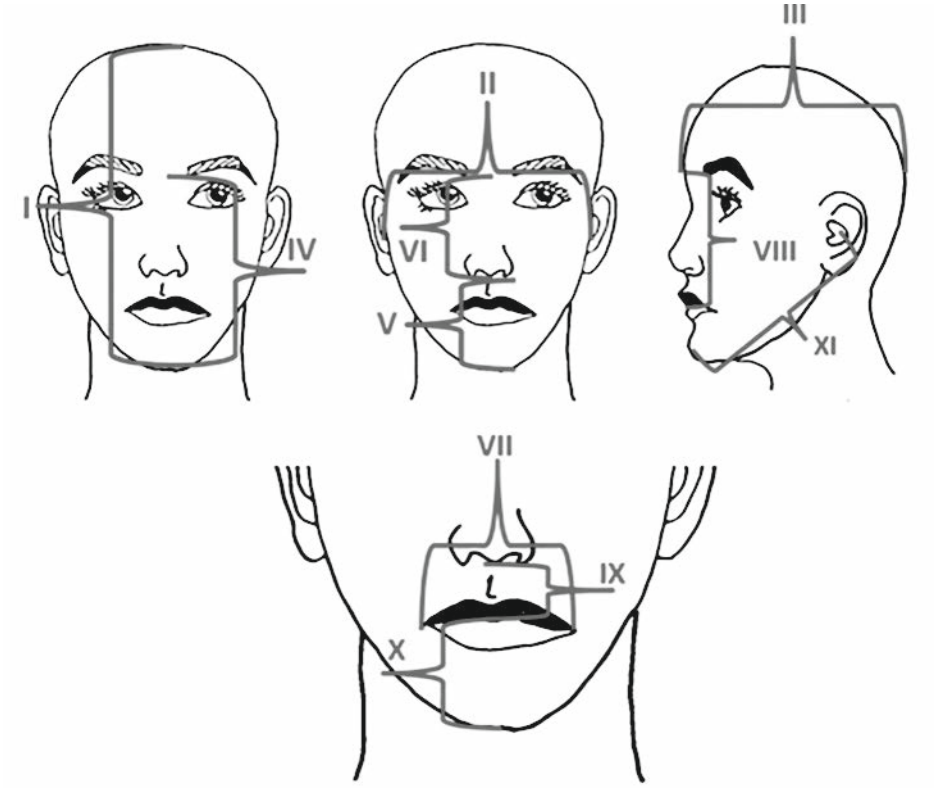


Fig. 1 Schematic illustration of human head with all the anthropometric measurements used in this study. Each number indicates a different proportion, Table 1 for the exact definitions; length of the head (I), width of the head (II), depth of the head (III), face height (IV), lower face height (V), nose height (VI), width of the mouth (VII), upper face height (VIII), upper lip height (IX), mandible height (X) and tragus–gnathion distance (XI).

Table 1 Definitions of anthropometric measurements in the present study.

Anthropometric measurements	Anthropometric landmark	Illustrated in Fig. 1 as
Length of the head	Vertex–gnathion	I
Width of the head	Straight line distance as measured with sliding calliper between the right external auditory meatus and left external auditory meatus	II
Depth of the head	Straight line distance as measured with a sliding calliper between back of the head and glabella	III
Face height	Glabella–gnathion	IV
Lower face height	Subnasale–gnathion	V
Nose height	Glabella–subnasale	VI
Width of the mouth	Right chelion–left chelion	VII
Upper face height	Glabella–upper lip	VIII
Upper lip height	Subnasale–upper lip	IX
Mandible height	Gnathion–lower lip	X
Mandibular length	Straight line distance as measured with a sliding calliper between the tragus and gnathion	XI
Palatal width	Straight line distance from the upper right first molar (16) to the left first molar (26), if one or both teeth were extracted then the distance from the alveolar ridges of the estimated location of the first molars was used	Not shown

Removal of metallic restoration materials and preparation of the cadavers for CBCT

After conducting anthropometric measurements, atraumatic extraction of teeth restored with metallic materials was performed to prevent scattering in the CBTC scan. The remaining teeth were all-natural teeth with an average of 8.7 teeth in total (SD: 6.2) and a mean number of 5.4 teeth (SD: 3.3) in the lower jaw and 3.3 teeth (SD: 3.8) in the upper jaw. Following extraction, the wet cotton rolls were removed and replaced by six styrofoam bars of approximately 5 × 1 × 1 cm, as styrofoam is undetectable by CBCT and does not absorb fluid, in contrast to the cotton rolls. The styrofoam bars were placed in the oral cavity to separate the cheeks and tongue from the oral mucosa and oral gingiva at the following locations: one between the cheek and the lower teeth on the left and right sides, one at both sides between the cheek and the upper teeth and another one was placed between the tongue and teeth at both sides. In some cases where the tongue contacted the palate, additional styrofoam bars were placed between the tongue and the palate. To separate the lips from the frontal teeth, a lip retractor (Henry Schein Dental, Melville, NY, USA) was used [5, 18, 28]. The lip contractor facilitated the insertion of styrofoam bars into the oral cavity but did not influence tissue stretching as the cadavers were preserved in a fixative, which had solidified the tissues.

CBCT scanning

CBCT scans were acquired using a NewTom 5G CBCT scanner (QR systems, Verona, Italy) at 110 kV, 4 mA, 0.3 mm voxel size and exposure time of 3.6 s. Each cadaver was covered with a plastic bag and placed inside the scanner as described in the users' guide. The selected field of view was 12 cm × 8 cm. After selecting patient scan protocol, a regular scan (scanning time 18 s) with a boosted dose was initiated. The scans were saved as Digital Imaging and Communications in Medicine (DICOM) files.

CBCT analysis

The DICOM files were reconstructed using Matlab R2019a (Mathworks, Natick, MA, USA). Reconstruction involved segmentation at - 300 Hounsfield units (HU) for soft tissue and 350 HU for bone, filtering with a small smoothing kernel, a morphological closing and conversion to stereolithography (STL) format. Morphological closing is an operation on binary images to remove small gaps while preserving the overall shape and size. In our case, this operation was used to fill small air bubbles in the cadaveric tissue. Subsequently, the STL objects were analyzed in Meshmixer (Autodesk, San Rafael, CA, USA) independently by two researchers (ZA and CK). This analysis involved manual separation of the intra-oral cavity into four regions (Fig. 2): (I) the hard palate, bounded anteriorly and laterally by the maxillary alveolar ridge and posteriorly by the bony pterygoid hamuli. The bony reconstruction was used to determine the positions of the pterygoid hamulus, (II) the tongue, bordered anteriorly and laterally by the mandibular alveolar ridge. Posteriorly, the tongue was limited to the alveolar ridge on the sides and medially to the top view projection of the bony pterygoid hamulus, (III) the hard tissue region was defined as the total of all crowns in situ and dental alveoli of extracted teeth, and (IV) the remaining soft tissue was classified as mucosa, anteriorly limited by the crease of the lip retractor.

After segmentation, the surface areas (in cm^2) of the four separate regions were determined in Meshmixer.

Fig. 2 The four different regions segmented in this study from two different views. **a** The palatal surface area is shown in blue color. **b** The tongue surface area is shown in pink color. **c** The hard tissue surface area is shown in green color. **d** The mucosal surface area is shown whereby the palatal, tongue and hard tissue surface areas are made invisible.

Statistical analysis

For statistical analysis, SPSS version 25.0 (IBM Corp SPSS statistics, Armonk, NY, USA) was used. The intraclass correlation coefficient (ICC) was used to determine the degree of agreement between the anthropometric measurements and the oral surface areas. A two-way mixed, absolute agreement, average-measures ICC was calculated for the anthropometric measurements. To measure the agreements between the different researchers for the oral surface areas, a two-way random, absolute agreement, average measures ICC was used [14, 21]. The reliability index is indicative of poor (values less than 0.5), moderate (between 0.5 and 0.75), good (between 0.75 and 0.9) and excellent (greater than 0.90) reliability [20].

The mean of the two anthropometric measurements and the mean of different intra-oral surface areas were used for further analysis. The relationship between anthropometric measurements and the intra-oral surface area was analyzed by a Pearson correlation coefficient. The size of the correlation coefficient was interpreted as negligible (r = 0.1–0.2), fair (r = 0.3–0.5), moderate (r = 0.6–0.7) or very strong (r = 0.8–0.9) correlation [1]. An ANOVA one-way test was performed to check for significant differences between females and males. All significance levels (P) were set at 0.05.

RESULTS

Intra-oral surface areas

Using CBCT and digital analysis, it was possible to determine the intra-oral surface area. The median of the ICC for the intra-oral surface areas was 0.95 (Table 2). The resulting ICC for the different areas was good or excellent. The mean and standard deviation for the intra-oral surface areas, determined by CBCT and digital analysis, were calculated for the total cadavers, and females and males separately (Table 2). The mean intra-oral surface area of all the included cadavers was 173.3 ± 19.3 cm^2. ANOVA testing found no significant differences in mean surface areas of the four different regions and the total surface area between females and males.

Anthropometric measurements

The ICC for the anthropometric measurements is presented in Table 3. The median of the ICC for the anthropometric measurements was 0.91. The resulting ICC was in the good or excellent range except for two measurements. The

length of the head and the mandible height were in the moderate range, indicating less agreement between the first and second measurements.

The anthropometric measurements for all the cadavers are also shown in Table 3. The mean and standard deviation for the different anthropometric measurements are presented for all cadavers, female and male cadavers. The results of seven cadavers were not reported separately because their gender was unknown.

Most of the anthropometric measurements showed signifcant diferences between females and males (Table 3). For all measurements, males showed higher values compared to females, except for the palatal width, which was significantly larger in females compared to males.

Table 2 The mean and standard deviation of the intra-oral surface area (in cm^2) for the cadavers, stratified according to gender.

Surface area in c m^2	Total (N = 20)	Female (N = 8)	Male (N = 5)	P-value difference female vs male	ICC
Palate	20.0 ± 2.88	20.0 ± 1.78	19.4 ± 4.05	0.748	0.77
Tongue	35.2 ± 5.16	35.0 ± 3.26	34.0 ± 3.67	0.633	0.90
Hard tissue	21.5 ± 11.06	26.4 ± 10.32	15.6 ± 9.76	0.087	0.95
Mucosa	96.6 ± 12.10	94.8 ± 14.55	96.9 ± 12.6	0.792	0.95
Total area	173.3 ± 19.3	176.1 ± 18.6	165.9 ± 18.2	0.353	0.99

N indicates the number of cadavers. The P-value of the ANOVA one-way test is shown. The ICC indicates the degree of agreements between the different researchers for the oral surface areas. For 7 cadavers the gender was unknown, for this reason they are not included in the ANOVA comparison.

Table 3 The mean and the standard deviation of anthropometric measurements (in cm) for the cadavers, stratified according to gender.

Anthropometric measurements in cm (ref Fig. 1)	Total (N = 20)	Female (N = 8)	Male (N = 5)	P-value difference female vs male	ICC
Length of head (I)	22.8 ± 0.99 (*N* = 12)	22.3 ± 1.10 (*N* = 5)	23.4 ± 0.95 (*N* = 3)	0.195	0.64
Width of head (II)	15.8 ± 0.89	15.6 ± 0.56	16.6 ± 1.29	0.076	0.96
Depth of head (III)	18.8 ± 0.75 (*N* = 18)	18.5 ± 0.70 (*N* = 8)	19.0 ± 0.48 (*N* = 3)	0.380	0.95
Face height (IV)	14.1 ± 1.04	13.8 ± 0.62	15.1 ± 1.09	0.023	0.96
Lower face height (V)	8.3 ± 0.81	8.2 ± 0.51	8.7 ± 1.16	0.303	0.91
Nose height (VI)	6.1 ± 0.53	5.8 ± 0.44	6.6 ± 0.56	0.018	0.83
Width of mouth (VII)	5.6 ± 0.52	5.7 ± 0.38	5.8 ± 0.51	0.534	0.90
Upper face height (VIII)	8.1 ± 0.65	7.8 ± 0.58	8.7 ± 0.58	0.015	0.91
Upper lip height (IX)	2.2 ± 0.31	2.1 ± 0.25	2.5 ± 0.33	0.021	0.92
Mandible height (X)	4.7 ± 0.61	4.4 ± 0.36	5.2 ± 0.64	0.011	0.66
Mandibular length (XI)	14.7 ± 0.81	14.4 ± 0.47	15.8 ± 0.56	0.001	0.88
Palatal width	4.3 ± 0.31	4.4 ± 0.23	3.9 ± 0.24	0.006	0.96

N indicates the number of cadavers. The P-value of ANOVA one-way test is shown. The ICC indicates the degree of agreement between the two independent anthropometric measurements. For 7 cadavers the gender was unknown, for this reason they are not included in the ANOVA comparison.
Different N as in some cases this anthropometric measurements could not be performed.

Relation between intra-oral surface areas and anthropometric measurements

A moderate positive correlation was found between the surface of the palate and the length of the head, Pearson's r(12) = 0.59, P = 0.045. Also, the surface of the tongue and the depth of the head were positively correlated, Pearson's r(18) = 0.50, P = 0.036. The Pearson correlation analysis did not reveal significant relations between anthropometric measurements and the total intra-oral surface area (P value varying from 0.097 for the palatal width and 0.995 for the upper face height). Also, no significant correlation was found for the surface area of the hard tissue and the mucosa with the anthropometric measurements.

DISCUSSION

Using CBCT and digital analysis, it was possible to determine the intra-oral surface area. The good and excellent ICCs for the various intra-oral surface areas indicated that this technique is reliable. After the analysis of 20 available cadaver heads, it was found that the average total intra-oral surface was 173 ± 19 cm^2. In addition, moderate significant correlations between the length of the head and the palatal surface area and between the depth of the head and tongue surface area were observed.

The current study is not the first study to investigate the relationship between extra-oral and intra-oral measurements. Inoue and co-workers found significant correlations between the body profile (especially weight and Body Mass Index) and the salivary gland size [16]. This indicates the possibility to estimate the size of the oral structures by determining extra-oral measurements. In contrast to our study, they found a stronger correlation. A possible reason for this fact could be that they included more subjects (50 young adults vs. 20 cadavers). Another possibility is that some of the cadaver heads included in the current study were incomplete. As a consequence of missing part of the skull (N = 8 cadavers), the ICC of the length of the head was moderate. So, the number of included cadavers and the incompleteness of the cadaver heads could have influenced the strength of the correlation between anthropometric measurements and the intra-oral surface area.

The mucosal surface area was found to be 152 ± 16 cm^2. In comparison, the mucosal surface area found by Naumova and co-workers, who included cadavers of elderly individuals (age 65–75 years), was 197 ± 24 cm^2 [23]. A possible explanation for this difference might be that, in contrast to our method, Naumova and co-workers used the aluminium foil technique where the outlines of the foils were digitized into AutoCAD [23]. Additionally, they used a profilometer to investigate the dorsal side of the tongue, which measures the tongue surface at high resolution on microscopic level [23]. The dorsal surface of the tongue is covered with lingual papillae which give the tongue an irregular surface texture. As a consequence, the use of this technique may have led to the determination of apparent larger surface areas than those found in the present study using CBCT.

Other investigators also used the foil technique to determine the surface area in different regions of the mouth including the teeth. Two studies determining the oral surface areas in infants found that the average total surface area ranged between 118 ± 8 and 143 ± 15 cm^2, which obviously is smaller than the surface area in the cadavers of the elderly subjects in the

current study (173 ± 19 cm^2) [19, 29]. This age-related increase of the surface area is partly due to the growth of the face and partly to the development of the dentition [15, 27, 29]. Adolescents showed an average intra-oral surface area of 167 ± 13 cm^2, which is comparable to the findings of the present study [19].

Collins and Dawes also calculated the surface area for twenty living adults using the foil technique [8]. The mean surface area in their study was found to be 215 ± 13 cm^2, which is larger than the surface area found in the present study, *i.e.* 173 ± 19 cm^2. This difference could be attributed to the contribution of the teeth surface area to the total area. Collins and Dawes included subjects having an average of 28 teeth, whereas the cadavers in this study had an average of 8.7 teeth. For this reason, the surface area of the teeth in the study of Collins and Dawes (45 ± 5 cm^2) is approximately twice the surface area of all the hard tissue measured in the present study (22 ± 11 cm^2).

In accordance with the present study, Collins and Dawes found comparable surface areas for the mucosa and the palate [8]. The mean surface areas of the total mucosa and palate in their study were 96 and 20 cm^2, respectively, which is comparable to the present study. However, the surface area of the tongue differed from our study as Collins and Dawes found a surface area of 52 cm^2 compared to 35 cm^2 in the present study. Possibly, these differences may be caused by the incomplete measurement of the posterior tongue surface and variation in mouth opening of the cadavers. In some cases, the posterior tongue was not completely separated from the palate with the concomitant risk of missing data on the CBCT scan. Due to limited access to the oral cavity, it was not possible to verify whether the posterior part of the tongue was completely separated from the palate. Additionally, in the current study the length of the tongue was determined by a line on the dorsum of the tongue, corresponding to the bony pterygoid hamuli. However, the cadavers varied in mouth opening, which seemed to introduce variation in the length of the tongue.

Consistent with the present study, Collins and Dawes found no significant gender differences in the surface areas for any of the intra-oral regions [8]. The current study revealed a significant difference in some face proportions between females and males. This finding is broadly supported by the work of other studies describing the association between gender and anthropometric orofacial measures, mentioning larger measures for males than females [13, 22, 30].

This study has also some potential limitations. It has to be taken into account that the upper part of the palate was imaged incompletely in some

cadavers due to a limited field of view. The missing data were reconstructed in Meshmixer by flat filling the defect; for this purpose, the "Inspector" analysis tool of Meshmixer was used. Given this fact, the surface area of the palate can be considered a calculated approximation for some cadavers. However, on average, the palatal surface area found in the present study (20.0 cm^2) was in accordance with two other studies (ranging between 18.0 and 20.1 cm^2) [8, 19].

Besides, it has to be noted that soft tissues of living persons are more flexible than those of embalmed cadavers. In line, several articles mention that embalming procedures following Thiel's method (main component boric acid) or Imperial College London soft-preservation (main component 80% phenol) give better flexibility and tissue quality than other methods [4, 6, 12, 25]. The embalming technique in the current study might have led to the solidification of the soft tissues.

CONCLUSION

The current study presents a reproducible technique for the determination of intra-oral surface areas using CBCT and digital analysis. In addition, this study indicates that moderate, but statistically significant, correlations exist between (1) the length of the head and the palatal surface area, as well as (2) the depth of the head and the surface area of the tongue. Based on these findings, we postulate that it would be possible to estimate individual intra-oral surface areas in situ by measuring facial features.

REFERENCES

1. Akoglu H (2018) User's guide to correlation coefficients. Turk J Emerg Med 18:91–93. https ://doi.org/10.1016/j. tjem.2018.08.001
2. Alexeev T, Kavanagh B, Miften M, Altunbas C (2018) Two-dimensional antiscatter grid: a novel scatter rejection device for cone-beam computed tomography. Med Phys 45:529–534. https://doi.org/10.1002/mp.12724
3. Amerongen AV, Veerman EC (2002) Saliva–the defender of the oral cavity. Oral Dis 8:12–22. https ://doi.org/10.1034/j.1601-0825.2002.1o816 .x
4. Balta JY, Twomey M, Moloney F, Duggan O, Murphy KP, O'Connor OJ, Cronin M, Cryan JF, Maher MM, O'Mahony SM (2019) A comparison of embalming fluids on the structures and properties of tissue in human cadavers. Anat Histol Embryol 48:64–73. https://doi.org/10.1111/ahe.12412
5. Barriviera M, Duarte WR, Januario AL, Faber J, Bezerra AC (2009) A new method to assess and measure palatal masticatory mucosa by cone-beam computerized tomography. J Clin Periodontol 36:564–568. https ://doi.org/10.1111/j.1600051X.2009.01422 .x
6. Brenner E (2014) Human body preservation—old and new techniques. J Anat 224:316–344. https: //doi.org/10.1111/joa.12160
7. Budai M, Farkas LG, Tompson B, Katic M, Forrest CR (2003) Relation between anthropometric and cephalometric measurements and proportions of the face of healthy young white adult men and women. J Craniofac Surg 14:154–161. https ://doi. org/10.1097/000016 65-200303000-00004 (discussion 162–153)
8. Collins LM, Dawes C (1987) The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. J Dent Res 66:1300–1302. https ://doi. org/10.1177/00220 34587 06600 80201
9. de Almeida NH, Michel-Crosato E, de Paiva LA, Biazevic MG (2013) Facial soft tissue thickness in the Brazilian population: new reference data and anatomical landmarks. Forensic Sci Int 231:404.e401–404.e407. https: //doi.org/10.1016/j.forsciint.2013.05.024
10. DeCarlo D, Metaxas D, Stone M (1998) An anthropometric face model using variational techniques. Paper presented at the proceedings of the 25th annual conference on computer graphics and interactive techniques—SIGGRAPH 98:67–74. https://doi.org/10.1145/28081 4.28082 3
11. Ding X, Suzuki S, Shiga M, Ohbayashi N, Kurabayashi T, Moriyama K (2018) Evaluation of tongue volume and oral cavity capacity using cone-beam computed tomography. Odontology 106:266–273. https://doi.org/10.1007/s1026 6-017-0335-0
12. Eisma R, Lamb C, Soames RW (2013) From formalin to Thiel embalming: what changes? One anatomy department's experiences. Clin Anat 26:564–571. https: //doi.org/10.1002/ca.22222
13. Farkas LG, Katic MJ, Forrest CR (2007) Comparison of craniofacial measurements of young adult African-American and North American white males and females. Ann Plast Surg 59:692–698. https: //doi.org/10.1097/01.sap.0000258954. 5506 8 .b4
14. Hallgren KA (2012) Computing inter-rater reliability for observational data: an overview and tutorial. Tutor Quant Methods Psychol 8:23–34

15. Hellman M (1932) An introduction to growth of the human face from infancy to adulthood. Int J Orthodontia Oral Surg Radiogr 18:777–798. https ://doi.org/10.1016/ S0099 -6963(32)90114 -8

16. Inoue H, Ono K, Masuda W, Morimoto Y, Tanaka T, Yokota M, Inenaga K (2006) Gender difference in unstimulated whole saliva flow rate and salivary gland sizes. Arch Oral Biol 51:1055–1060. https ://doi.org/10.1016/j.archo ralbi o.2006.06.010

17. Jagadish Chandra H, Ravi MS, Sharma SM, Rajendra Prasad B (2012) Standards of facial esthetics: an anthropometric study. J Maxillofac Oral Surg 11:384–389. https ://doi.org/10.1007/ s1266 3-012-0355-9

18. Januario AL, Barriviera M, Duarte WR (2008) Soft tissue conebeam computed tomography: a novel method for the measurement of gingival tissue and the dimensions of the dentogingival unit. J Esthet Restor Dent 20:366–373. https ://doi. org/10.111 1/j.1708-8240.2008.00210 .x (discussion 374)

19. Kerr WJ, Kelly J, Geddes DA (1991) The areas of various surfaces in the human mouth from nine years to adulthood. J Dent Res 70:1528–1530. https ://doi.org/10.1177/00220 34591 0700121001

20. Koo TK, Li MY (2016) A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med 15:155–163. https ://doi.org/10.1016/j. jcm.2016.02.012

21. McGraw K, Wong SP (1996) Forming inferences about some intraclass correlation coefficients. Psychol Methods 1:30–46. https ://doi.org/10.1037/1082-989X.1.1.30

22. Nascimento WV, Cassiani Rde A, Dantas RO (2013) Effect of gender, height and race on orofacial measurements. Codas 25:149–153

23. Naumova EA, Dierkes T, Sprang J, Arnold WH (2013) The oral mucosal surface and blood vessels. Head Face Med 9:8. https: // doi.org/10.1186/1746-160x-9-8

24. Naumova EA, Kuehnl P, Hertenstein P, Markovic L, Jordan RA, Gaengler P, Arnold WH (2012) Fluoride bioavailability in saliva and plaque. BMC Oral Health 12:3. https :// doi.org/10.1186/1472-6831-12-3

25. Ottone N, Vargas CA, Fuentes R, Sol M (2016) Walter Thiel's embalming method. review of solutions and applications in different fields of biomedical research. Int J Morphol 34:1442– 1454. https ://doi.org/10.4067/S0717 -95022 01600 04000 44

26. Porter JP, Olson KL (2001) Anthropometric facial analysis of the African American woman. Arch Facial Plast Surg 3:191–197

27. Sillman JH (1965) Some aspects of individual dental development: longitudinal study from birth to 25 years. Am J Orthod 51:1–25. https ://doi.org/10.1016/0002-9416(65)90068 -0

28. Silva JNN, Andrade PF, Sotto-Maior BS, Souza Picorelli Assis NM, Pires Carvalho AC, Devito KL (2017) Influence of lip retraction on the cone beam computed tomography assessment of bone and gingival tissues of the anterior maxilla. Oral Surg Oral Med Oral Pathol Oral Radiol 123:714–720. https ://doi. org/10.1016/j.oooo.2017.02.005

29. Watanabe S, Dawes C (1990) Salivary flow rates and salivary film thickness in five-year-old children. J Dent Res 69:1150–1153. https ://doi.org/10.1177/00220 34590 06900 50601

30. Zhuang Z, Landsittel D, Benson S, Roberge R, Shaffer R (2010) Facial anthropometric differences among gender, ethnicity, and age groups. Ann Occup Hyg 54:391–402. https://doi.org/10.1093/annhy g/meq00 7

5

Correlations of palatal surface area with anthropometric dimensions of head and face

Z. Assy
D.H.J. Jager
H. S. Brand
F. J. Bikker

Surgical and Radiologic Anatomy, 2022 Sep;44(9):1261-1267.

ABSTRACT

Purpose

Saliva distribution over the palatal surface plays an important role in the perception of dry mouth. It is envisaged that non-invasive estimation of the palatal surface area by anthropometric measurements of head and face can be useful in the assessment of oral dryness. For this purpose, the relationship between the palatal surface area and anthropometric measurements of the head and face was investigated.

Methods

The palatal surface was measured in 51 healthy volunteers using an intra-oral scanner. The distances between anthropometric landmarks of the head and face were determined using an anatomical sliding calliper. Correlations between the palatal surface area and the anthropometric landmarks were investigated.

Results

The median palatal surface area for the total study sample was found to be 2120.6 mm^2. Virtually all anthropometric measurements showed significant differences between females and males. Various head and face measurements had a significant correlation with the palatal surface area. However, these correlations disappeared when the participants were stratified based on their sex, with the exception of mandibular length and palatal width in females.

Conclusion

The surface area of the palate correlates with nearly all anthropometric measurements of the head and face included in this study, yet the clinical applicability seems limited to females.

Keywords

Anthropometric measurements, Palatal surface area, Head and face dimensions, TRIOS 3 scanner.

INTRODUCTION

Human saliva is predominantly produced by three pairs of major glands known as the parotid, submandibular, and sublingual glands. These glands are responsible for the production of 90% of the volume of saliva [8]. Each of the glands excretes saliva with a unique consistency into the oral cavity via various salivary ducts [14]. The opening of these salivary ducts is located in various intra-oral locations, such as the buccal mucosa for the parotid glands and the floor of the mouth for the sublingual, and submandibular glands [14].

After secretion, saliva is distributed over the various intra-oral surfaces, especially during chewing and swallowing [16, 29]. Several studies have explored the thickness of the salivary film covering the teeth and oral mucosa at various intra-oral locations [6, 15, 28]. The salivary film thickness at the anterior part of the palate seems to be relatively thin compared to other intra-oral surfaces [4, 5, 10, 18, 22, 23, 25, 29, 30]. In addition, in patients suffering from hyposalivation, a lower salivary film thickness at the anterior palate than in healthy controls was observed [4, 5, 10, 18, 22, 23, 25, 29, 30].

Next to *e.g.* the salivary volume, the size of the surface area of the intra-oral regions relates to the salivary film thickness. To investigate the surface area of the oral cavity, previous studies used the so-called foil technique; stone models of dental impressions were prepared and covered with aluminium foil. Subsequently, this foil was weighed to deduce the surface area [6, 15, 28]. Despite the fact that this foil technique has been proven to be reproducible [6, 15, 28], some drawbacks were noted as well; adaptation of the foil onto the models without stretching appeared challenging. Besides, it was difficult to fold the foil completely into interdental spaces, and around the labial and buccal vestibular mucosa [6]. Therefore, in a recent study, an alternative strategy was explored using cone-beam computed tomography (CBCT) in combination with digital analysis [3]. Though, in contrast to the studies which used the foil technique, the CBCT analysis was performed on cadavers [3]. It was found that CBCT analysis had good reliability for measuring various intra-oral surface areas such as the palate, tongue, mucosa, and hard tissues. The studies using the foil technique and the CBCT analysis showed almost identical results for the palatal surface area (20.1±1.9 vs. 20.0±2.9 cm^2) [3, 6]. In the cadaver study, the sizes of several intra-oral surface areas, including the palatal surface area, were related to facial anthropomorphic measurements [3]. Moderate, yet statistically significant correlations were observed between the palatal surface area and the length of the head, as well as the surface area of the tongue and the depth of the head [3].

However, it was postulated that the study was limited by the fact that soft tissues of the cadavers were solidified by their embalmment in a formaldehyde solution, which would lead to a suboptimal approximation of the surface areas [3]. For this reason, in the current study we included living participants and we also applied an intra-oral scanner, which projects a light on intra-oral surfaces to be scanned. Then, images captured by imaging sensors are processed by scanning software to produce triangulated point clouds that enable a virtual 3D surface model to be created [7]. A recent study revealed promising results using this scanner, especially for the documentation of palatal soft tissue in terms of shape, colour, and curvature [9]. Therefore, this study was designed to validate the relationship between the palate surface area, measured using an intra-oral scanner, and anthropometric measurements of the head and face in living participants. A relation between the anthropomorphic measurements and the palatal intra-oral surfaces would enable easy estimation of the palatal intra-oral surface area in a chair-side medical setting. Determination of the palatal surface area might be relevant for clinicians investigating the oral cavity, such as dentists and oral maxillofacial surgeons.

MATERIAL AND METHODS

Participants

The study was approved by the Ethics Review Committee at the Academic Centre for Dentistry Amsterdam (ACTA; 202065). Volunteers were recruited at ACTA through posters. Eligibility criteria required volunteers to be 18 years or older. Informed written consent was obtained from all volunteers. Data analysis of volunteers was completely anonymously, only age and sex were registered. The reporting of this study conforms to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement [27].

A priori sample size calculation was performed using G*Power software, version 3.1.9.4 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany); the correlation coefficient of previous study was used 0.59 [3], an α of 0.05, and a power of 80%, 20 participants were needed in each group. Because sex differences effect anthropometric of orofacial measures minimally 40 participants were needed with almost equal numbers of females and males [11, 21, 32].

Measuring the palatal surface area

In order to measure the palatal surface area, an intra-oral scan of the upper jaw including the palate (the whole hard palate and part of the soft palate) was taken with the TRIOS 3 scanner (3Shape, version 21.3.5, Copenhagen, Denmark). The scanning protocol of the manufacturer was followed when scanning the intra-oral upper jaw area. Scans were digitally saved as Polygon File Format (PLY) files. Subsequently, each PLY object was analyzed twice in Meshmixer (Autodesk, San Rafael, CA, USA) by one researcher (ZA). This analysis involved manual separation of the palate by using the vibrating line including visible fovea palatine as a cut-off for the length of the palate. Besides, all palatal mucosa including the gingiva around the upper teeth was included in the palatal surface (Figure 1). After segmentation, the palatal surface areas (in mm^2) were determined.

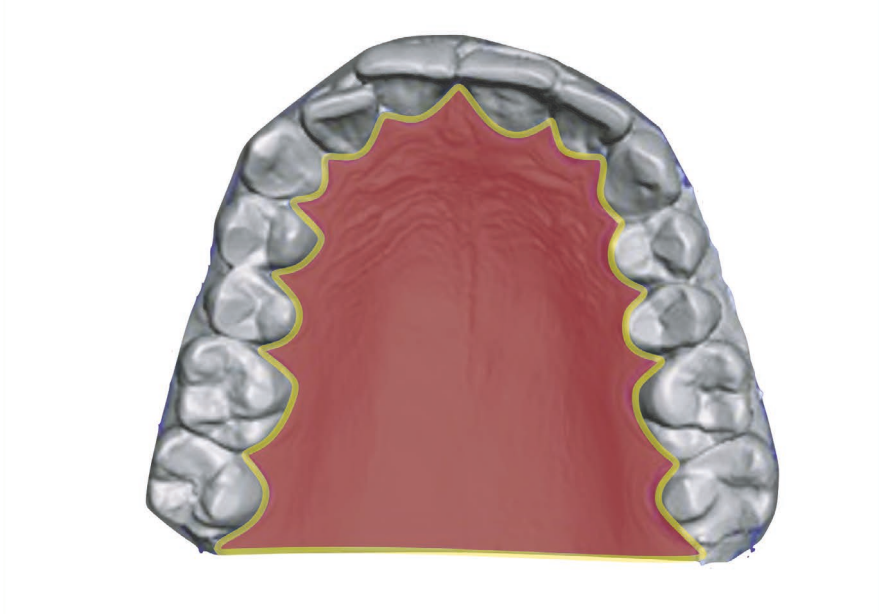


Figure 1: Schematic illustration of a typical example of palatal segmentation (in pink). The yellow line indicates the border of segmentation used for the palatal surface area.

Anthropometric measurements

Anthropometric measurements of the head and face were performed as described previously, using the same anthropometric landmarks (Table 1) [3]. The distance between anthropometric landmarks was determined twice using an anatomical sliding caliper with electronic display showing distance in millimeters (mm). The two measurements were carried out by one researcher (ZA) on the same day.

Statistical analysis

The data were processed in an electronic clinical data-management platform (CastorEdc, Castor, Amsterdam, the Netherlands) and then converted into SPSS, version 27.0 (IBM Corp SPSS Statistics, Armonk, NY, USA) for the statistical analysis. The Shapiro–Wilk test was used to assess the normality of the data. The data were presented as medians and their interquartile range (IQR), as most of the parameters were not normally distributed.

The intraclass correlation coefficient (ICC) was used to determine the degree of agreement between two palatal surface area measurements and between the two anthropometric measurements of the head and face. A two-way mixed, absolute agreement, average-measures ICC was calculated for these measurements [13, 19]. The ICC is indicative of poor (values less than 0.5), moderate (between 0.5 and 0.75), good (between 0.75 and 0.9) and excellent (greater than 0.90) reliability [17].

The mean of the two palatal surface area measurements and the various anthropometric measurements of the head and face was used for further analysis.

Female-male differences for the palatal surface area measurement and the anthropometric measurements of head and face were explored with the Mann-Whitney U test.

The possible relations between the palatal surface area and anthropometric measurements were analyzed with a bootstrapped Spearman rank correlation test (1000 × bootstrapping). The Spearman's rho coefficient and bias-corrected accelerated (Bca) 95% confidence interval were extracted. Furthermore, the participants were stratified based on their sex. The size of the correlation coefficient was interpreted as poor (r = 0.1–0.2), fair (r = 0.3–0.5), moderate (r = 0.6–0.7) or very strong (r = 0.8–0.9) correlation [1]. All significance levels (P) were set at 0.05.

Table 1. Definitions of anthropometric measurements in the present study.

Anthropometric measurements	Anthropometric landmark
Length of the head	Vertex - gnathion
Width of the head	Straight line distance as measured with sliding caliper between the right external auditory meatus and left external auditory meatus
Depth of the head	Straight line distance as measured with a sliding caliper between back of the head and glabella
Face height	Glabella - gnathion
Lower face height	Subnasale - gnathion
Nose height	Glabella - subnasale
Width of the mouth	Right chelion - left chelion
Upper face height	Glabella - lowest border of the upper lip
Upper lip height	Subnasale - lowest border of the upper lip
Mandible height	Gnathion - highest border lower lip
Mandibular length	Straight line distance as measured with a sliding caliper between the tragus and gnathion
Palatal width	Straight line distance from the central fissure of the upper right first molar (16) to the central fissure of the left first molar (26), if one or both teeth were extracted then the distance from the alveolar ridges of the estimated location of the first molars was used.

RESULTS

Fifty-one volunteers signed up for this study; 23 were female and 28 were male (45.1% : 54.9%). The average age was 42.6 ± 14.8 years (range 20-71 years). The average age of female and male participants did not differ significantly (Mann-Whitney U test $p>0.05$).

The palatal surface area and anthropometric measurements

The palatal surface area as well as the anthropometric measurements of the head and face are reported for the total study sample as well as the female and male participants separately (Table 2). The median palatal surface area for the total study sample was 2120.6 ± 2232.0-1976.3 mm^2. The ICC for the palatal surface area measurements and the anthropometric measurements varied between 0.85 and 0.99, which was in the good or excellent range.

Almost all anthropometric measurements showed significant differences between females and males (Mann-Whitney U test $p<0.05$), where male participants showed higher values than females (Table 2). No sex-related differences were observed for the width of the mouth and palatal width. There was also no significant difference for the palatal surface area between females and males (Mann-Whitney U test $p>0.05$).

Relation between the palatal surface area and anthropometric measurements of the head and face

For the total study sample, a significant correlation was found between the palatal surface area and the length of the head, the width of the head, the face height, the nose height, the upper face height, the upper lip height, the mandibular length and the palatal width (Table 3). The correlation coefficients for these correlations ranged between 0.29 and 0.37, which indicate poor to fair correlations. These positive correlations indicate that larger dimensions of the head and face are associated with a larger palatal surface area. When the volunteers were stratified based on sex, the female palatal surface area correlated positively with the mandibular length (0.46) and the palatal width (0.56) (Table 3). These correlations could be considered as fair.

Table 2: The median and interquartile range (IQR) of anthropometric measurements (in mm) and the palatal surface area (mm^2) for the total study sample and stratified according to sex. N indicates the number of participants in each group. The p-value of Mann-Whitney U test is shown. The ICC indicates the degree of agreement between the two independent measurements.

Anthropometric measurements (mm)	**ICC**	**Total (N=51) Median ± IQR**	**Female (N=23 Median ± IQR**	**Male (N=28) Median ± IQR**	**P-value female vs. male**
Length of head	0.95	241.2 ± 230.1-245.6	230.1 ± 224.4-239.8	244.8 ± 240.1-249.6	<0.001
Width of head	0.98	147.3 ± 141.9-154.3	141.9 ± 140.0-145.7	153.0 ± 147.1-158.2	<0.001
Depth of head	0.99	198.4 ± 193.4-203.4	194.1 ± 189.9-198.3	201.3 ± 197.8-207.4	0.002
Face height	0.97	124.8 ± 115.0-129.7	115.0 ± 107.6-122.6	128.4 ± 124.9-131.6	<0.001
Upper face height	0.98	79.7 ± 76.5-84.6	78.4 ± 75.5-80.1	83.1 ± 78.7-86.1	0.003
Lower face height	0.94	62.1 ± 57.0-68.1	57.2 ± 53.2-63.1	67.4 ± 60.3-69.4	0.001
Nose height	0.99	58.6 ± 54.6-61.5	57.6 ± 53.0-59.0	60.0 ± 56.2-63.1	0.016
Width of mouth	0.98	48.8 ± 45.4-53.2	48.3 ± 45.4-50.7	49.8 ± 45.7-54.0	0.219
Upper lip height	0.99	20.8 ± 19.6-23.9	20.2 ± 17.8-22.0	22.6 ± 20.6-25.2	0.004
Mandible height	0.98	41.5 ± 35.0-44.7	36.2 ± 32.7-41.7	43.6 ± 39.5-45.1	<0.001
Mandibular length	0.85	144.2 ± 141.4-149.3	141.5 ± 133.2-144.7	144.3 ± 143.6-151.9	<0.001
Palatal width	0.97	42.8 ± 40.0-45.7	42.4 ± 39.5-45.7	42.8 ± 41.3-46.0	0.374
Surface area (mm2)					
Palatal	0.96	2120.6 ± 1976.3-2232.0	2087.5 ± 1881.9-2184.2	2165.3 ± 2023.0-2257.3	0.069

Table 3: The correlations between the palatal surface area and anthropometric measurements for the total study sample and stratified according to sex. N indicates the number of participants in each group. Data are expressed as the Spearman's rho coefficient and bias-corrected accelerated (Bca) 95% confidence interval.

	Correlation coefficient with the palatal surface area		
Anthropometric measurements	**Total study sample (N=51)**	**Female participants (N=23)**	**Male participants (N=28)**
Length of head	0.30 (-0.01-0.58)*	NS (p=0.13)	NS (p=0.99)
Width of head	0.35 (0.06-0.61)*	NS (p=0.09)	NS (p=0.78)
Depth of head	NS (p=0.27)	NS (p=0.16)	NS (p=0.44)
Face height	0.36 (0.14-0.56)**	NS (p=0.60)	NS (p=0.13)
Lower face height	NS (p=0.19)	NS (p=0.53)	NS (p=0.46)
Nose height	0.31 (0.02-0.55)*	NS (p=0.20)	NS (p=0.24)
Width of mouth	NS (p=0.17)	NS (p=0.08)	NS (p=0.96)
Upper face height	0.36 (0.10-0.58)**	NS (p=0.18)	NS (p=0.14)
Upper lip height	0.31 (0.02-0.56)*	NS (p=0.20)	NS (p=0.49)
Mandible height	NS (p=0.37)	NS (p=0.77)	NS (p=0.22)
Mandibular length	0.29 (0.00-0.53)*	0.56 (0.20-0.78)**	NS (p=0.37)
Palatal width	0.37 (0.10-0.63)**	0.46 (0.06-0.76)*	NS (p=0.08)

NS=Not Significant, (p-value of Spearman Rho correlation)
**Spearman Rho correlation coefficient p-value <0.05*
*** Spearman Rho correlation coefficient p-value <0.01*

DISCUSSION

This study aimed to assess the possible relationship between the dimensions of the palatal surface area and anthropometric measurements of the head and face in living subjects. An intra-oral scanner was used to determine the palatal surface area. The excellent ICC for the palatal surface areas indicated the high reproducibility of the intra-oral scanner technique. Various head and face measurements had a significant correlation with the palatal surface area. When stratified on sex, significant correlations with the female palatal surface were found with the mandibular length and palatal width.

The adult palatal surface area found was 2120.6 mm^2, which was comparable to findings from other studies with a mean of 1990-2010 mm^2 [3, 6, 15]. In those studies, the palatal surface areas were determined using foil impressions taken from stone models [6, 15], while another study used CBCT imaging and digital analysis [3]. Apparently, all methods used so far reveal comparable and representative results as the reported palatal surface areas are in the same range. In addition, the technique presented in the current study, using an intra-oral scanner, adds up to this line of methods as it had a very good reproducibility with an excellent ICC. Moreover, the intra-oral scanner has the beneficial effect that it does not use ionizing radiation and its technique is easy, safe and less laborious.

The palatal surface area in the current study did not differ between the two sexes. This finding is consistent with results of two other studies [3, 6], while another study revealed that male participants had a significantly larger palatal surface areas than females [15]. This latter study, however, included females with a mean age 16.88.02± years and males of 20.713.4± years old [15]. These participants were considerable younger than the volunteers in the current study with a mean age of 42.614.8± years. In this light, it has to be noted that maturation of female facial structures starts at an earlier age than in males [24]. For this reason, in younger groups, there is a significant sex difference in palatal surface area. That could explain why the study by Kerr *et al.* found significant differences for the palatal surface area measurements [15]. However, when investigating older participants, such as the current study, these differences for the palatal surface area were not apparent anymore.
In the current study, head and face proportions differed significantly between females and males. This finding is broadly supported by the work of other studies describing the effects of sex on anthropometric orofacial measures, mentioning larger measures for males then in females [11, 21, 32]. In our previous study, investigating cadavers with CBCT, comparable anthropometric

differences between two sexes were observed [3]. In the cadaver study, the length of the head did not differ significantly for the two sexes, while in the current study there was a significant sex difference for the length of the head. This result could be explained by the lower number of cadavers used in the CBCT study (female N=8, and male N=5) [3] than the larger number of living subjects in the current study (female N=23, and male N=28).

In the current study, various anthropometric measurements had a significant correlation with the palatal surface area. This is in contrast with the CBCT study with human cadavers where only a statistically significant correlation between the length of the head and palatal surface area was observed. There are several possible explanations for this result; firstly, the previous study included cadavers with possibly solidified soft tissues. Secondly, the number of included subjects might also influence this observation; the cadaver study had a possibly limited statistical power due to the limited number of cadavers used (N = 12). Although in the current study more significant correlations were found between palatal surface area and facial anthropometric measurements, most of these correlations are poor or fair (± 0.3). Finally, sex differences have influenced these correlations, as males had significant larger head and face proportions then females. For this reason, most significant correlations disappeared after stratifying the subjects based on their sex, especially for males. And so, females had a significant correlation between palatal surface with the mandibular length and the palatal width. Possibly the face type of females attributed to this significant correlation. It could be that that this relates to the fact that the face type of females is different than males; for females the most common face type is mesoprosop (medium-broad face) or euryprosop (short and wide), while for male it is the leptoprosop (long and narrow) and hyperleptoprosop [2, 31].

Previous studies measured not only the palatal surface, but also palatal volume. This palatal volume can contribute to explore the timing of surgery and surgical protocols [12, 20, 26]. In addition, the palatal volume measurements can help to evaluate changes induced by treatment modalities such as rapid palatal expansion and in the orthopaedic treatment of cleft palate cases, and to evaluate changes in orthodontic treatment [12, 20, 26]. Therefore, future studies exploring the relation between the palatal volume and anthropometric measurements are also warranted.

CONCLUSION

An optical scanner was successfully used to determine the palatal surface area, as the ICC for the palatal surface area was in excellent range. Various head and face proportions had a significant correlation with the palatal surface area. When stratified on sex, significant correlations with the female palatal surface were found with the mandibular length and palatal width.

REFERENCES

1. Akoglu H (2018) User's guide to correlation coefficients. Turk J Emerg Med 18: 91-93.
2. Arslan SG, Genç C, Odabaş B & Kama JD (2008) Comparison of facial proportions and anthropometric norms among Turkish young adults with different face types. Aesthetic Plast Surg 32: 234-242.
3. Assy Z, Klop C, Brand HS, Hoogeveen RC, Koolstra JH & Bikker FJ (2020) Determination of intra-oral surface areas by cone-beam computed tomography analysis and their relation with anthrometric measurements of the head. Surg Radiol Anat 42: 1063-1071.
4. Chaudhury NM, Proctor GB, Karlsson NG, Carpenter GH & Flowers SA (2016) Reduced mucin-7 (Muc7) sialylation and altered saliva rheology in Sjögren's syndrome associated oral dryness. Mol Cell Proteomics 15: 1048-1059.
5. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH & Proctor GB (2015) Changes in saliva rheological properties and mucin glycosylation in dry mouth. J Dent Res 94: 1660-1667.
6. Collins LM & Dawes C (1987) The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. J Dent Res 66: 1300-1302.
7. Daly S, Seong J, Parkinson C, Newcombe R, Claydon N & West N (2021) A proof of concept study to confirm the suitability of an intra oral scanner to record oral images for the non-invasive assessment of gingival inflammation. J Dent 105: 103579.
8. De Paula F, Teshima THN, Hsieh R, Souza MM, Nico MMS & Lourenco SV (2017) Overview of human salivary glands: highlights of morphology and developing processes. Anat Rec (Hoboken) 300: 1180-1188.
9. Deferm JT, Schreurs R, Baan F, Bruggink R, Merkx MaW, Xi T, Bergé SJ & Maal TJJ (2018) Validation of 3D documentation of palatal soft tissue shape, color, and irregularity with intraoral scanning. Clin Oral Investig 22: 1303-1309.
10. Disabato-Mordarski T & Kleinberg I (1996) Measurement and comparison of the residual saliva on various oral mucosal and dentition surfaces in humans. Arch Oral Biol 41: 655-665.
11. Farkas LG, Katic MJ & Forrest CR (2007) Comparison of craniofacial measurements of young adult African-American and North American white males and females. Ann Plast Surg 59: 692-698.
12. Gracco A, Malaguti A, Lombardo L, Mazzoli A & Raffaeli R (2010) Palatal volume following rapid maxillary expansion in mixed dentition. Angle Orthod 80: 153-159.
13. Hallgren KA (2012) Computing inter-rater reliability for observational data: an overview and tutorial. Tutor Quant Methods Psychol 8: 23-34.
14. Holmberg KV & Hoffman MP (2014) Anatomy, biogenesis and regeneration of salivary glands. Monogr Oral Sci 24: 1-13.
15. Kerr WJ, Kelly J & Geddes DA (1991) The areas of various surfaces in the human mouth from nine years to adulthood. J Dent Res 70: 1528-1530.
16. Kho HS (2014) Understanding of xerostomia and strategies for the development of artificial saliva. Chin J Dent Res 17: 75-83.

17. Koo TK & Li MY (2016) A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med 15: 155-163.
18. Lee SK, Lee SW, Chung SC, Kim YK & Kho HS (2002) Analysis of residual saliva and minor salivary gland secretions in patients with dry mouth. Arch Oral Biol 47: 637-641.
19. Mcgraw K & Wong SP (1996) Forming inferences about some intraclass correlation coefficients. Psychol Methods 1: 30-46.
20. Monga N, Kharbanda OP, Balachandran R & Neelapu BC (2020) Palatal volume estimation in operated unilateral and bilateral cleft lip and palate subjects using digital study models. Orthod Craniofac Res 23: 284-290.
21. Nascimento WV, Cassiani Rde A & Dantas RO (2013) Effect of gender, height and race on orofacial measurements. Codas 25: 149-153.
22. Osailan S, Pramanik R, Shirodaria S, Challacombe SJ & Proctor GB (2011) Investigating the relationship between hyposalivation and mucosal wetness. Oral Dis 17: 109-114.
23. Osailan SM, Pramanik R, Shirlaw P, Proctor GB & Challacombe SJ (2012) Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. Oral Surg Oral Med Oral Pathol Oral Radiol 114: 597-603.
24. Oxilia G, Menghi Sartorio JC, Bortolini E, Zampirolo G, Papini A, Boggioni M, Martini S, Marciani F, Arrighi S, Figus C, Marciani G, Romandini M, Silvestrini S, Pedrosi ME, Mori T, Riga A, Kullmer O, Sarig R, Fiorenza L, Giganti M, Sorrentino R, Belcastro MG, Cecchi JM & Benazzi S (2021) Exploring directional and fluctuating asymmetry in the human palate during growth. Am J Phys Anthropol 175: 847-864.
25. Pramanik R, Osailan SM, Challacombe SJ, Urquhart D & Proctor GB (2010) Protein and mucin retention on oral mucosal surfaces in dry mouth patients. Eur J Oral Sci 118: 245-253.
26. Shahen S, Carrino G, Carrino R, Abdelsalam R, Flores-Mir C & Perillo L (2018) Palatal volume and area assessment on digital casts generated from cone-beam computed tomography scans. Angle Orthod 88: 397-402.
27. Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC & Vandenbroucke JP (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Int J Surg 12: 1495-1499.
28. Watanabe S & Dawes C (1990) Salivary flow rates and salivary film thickness in five-year-old children. J Dent Res 69: 1150-1153.
29. Wolff M & Kleinberg I (1998) Oral mucosal wetness in hypo- and normosalivators. Arch Oral Biol 43: 455-462.
30. Won S, Kho H, Kim Y, Chung S & Lee S (2001) Analysis of residual saliva and minor salivary gland secretions. Arch Oral Biol 46: 619-624.
31. Zacharopoulos GV, Manios A, Kau CH, Velagrakis G, Tzanakakis GN & De Bree E (2016) Anthropometric analysis of the face. J Craniofac Surg 27: e71-75.
32. Zhuang Z, Landsittel D, Benson S, Roberge R & Shaffer R (2010) Facial anthropometric differences among gender, ethnicity, and age groups. Ann Occup Hyg 54: 391-402.

6

Salivary film thickness and MUC5B levels at various intra-oral surfaces

Z. Assy
D.H.J. Jager
H. S. Brand
F. J. Bikker

Clinical Oral Investigations, 2023 Feb;27(2):859-869.

ABSTRACT

Objectives

In this study we investigated the salivary film thickness and the MUC5B levels at various intra-oral locations in healthy volunteers, with a focus on the palate. Besides, measurements of the palatal surface area were included to explore the possible relationships between the palatal surface area and the palatal salivary film and MUC5B levels.

Materials and methods

The salivary film thickness was determined using filter strips, which were pressed to the mucosal surfaces of five different intra-oral locations; conductance was then analysed using a Periotron. After elution of the strips, the MUC5B levels at various intra-oral locations were determined using ELISA. The palatal surface area was measured using an intra-oral scanner. The surface area was subsequently calculated using software.

Results

The anterior tongue had the thickest salivary film and also the highest levels of MUC5B, while the anterior palate had the thinnest salivary film and lowest MUC5B levels. There was no association between the palatal surface area and the salivary film thickness of the palate.

Conclusion

The salivary film and MUC5B levels are unequally distributed over the intra-oral regions of the soft tissues. The lack of association between the palatal surface area and the salivary film thickness indicates that a larger surface area is not associated with a relative thinner palatal salivary film.

Clinical relevance

The results of current study increase our understanding of saliva distribution in the oral cavity and could be used as reference values for future studies.

Keywords

Salivary film thickness, Salivary secretions, MUC5B level, Palatal surface area, Sialopapers.

INTRODUCTION

The salivary glands produce saliva which contains a wide range of proteins and ions [1]. After secretion, and facilitated by swallowing, saliva is spread over the hard and soft tissues in the oral cavity as a thin film [2, 3]. A major compound of this salivary film is MUC5B, a large glycoprotein with a wide variety of hydrophilic carbohydrate side chains [4]. MUC5B plays a crucial role in saliva's water retaining properties, such as moistening, visco-elasticity and lubrication [3, 5]. As a consequence, an impaired flow rate, *i.e.* hyposalivation, leads to lower availability of both water and salivary proteins and to the insufficient replenishing of the intra-oral salivary film [6]. Subsequently, this leads to impaired mucosal moistening and clinical problems, such as difficulties with speech and swallowing, pain and xerostomia [7, 8].

It was recently shown that the severity of xerostomia differed at different intra-oral locations [9, 10]. In particular, it was found that the perceived oral dryness was most profound for the (posterior) palate. Hypothetically, this could be related to an impaired salivary film and lower MUC5B content, especially at the palate.

In the past, multiple studies have investigated the salivary film thickness including the total protein concentration at various mucosal surfaces [11–14]. These studies found that the total protein concentration displayed a wide variation depending on its location [12–14]. The protein concentration showed a negative correlation with the salivary film thickness, indicating that thinner salivary films were related to higher protein concentrations [12–14]. These findings reveal that the protein levels in the film are mainly influenced by the film volume, but did not provide detailed insights into the protein composition at various intra-oral locations. Determination of MUC5B levels in the salivary film could help to increase our understanding of salivary distribution in the oral cavity.

To understand the distribution of saliva over various surfaces, it is also important to measure the intra-oral surface areas. The dimensions of the intra-oral surface areas have previously been analysed in order to determine the distribution and average thickness of the salivary film covering the teeth and oral mucosa [15–17]. Especially the palate plays a major role in xerostomia because the salivary film thickness at the anterior part of the palate is relatively thin compared to other intra-oral surfaces [2, 11–14, 18–21]. Besides, the central part of the anterior palate is devoid of minor salivary glands [22]. Therefore, in order to increase our understanding of the distribution of the salivary film, measurements of the palatal surface area were included in the current study.

It is envisaged that these measurements could serve as a reference for future studies, *e.g.* on salivary film integrity related to various oral diseases.

Therefore, the present study aimed to determine the salivary film thickness and the MUC5B levels at various intra-oral locations in healthy volunteers. Furthermore, we included measurements of the palatal surface area to explore the possible relationships between the palatal surface area and the palatal salivary film thickness and MUC5B levels. We hypothesised that healthy individuals with comparable salivary flow rates, but differences in palatal surface area, will have a different distribution of the salivary film and/or the MUC5B levels; individuals with a larger palatal surface area would have a thinner salivary film at the palate and also less availability of MUC5B.

MATERIALS AND METHODS

Participants

The study was approved by the Ethics Review Committee at the Academic Center for Dentistry Amsterdam (ACTA; 202065). Volunteers were recruited at ACTA through posters. Eligibility criteria required volunteers to be 18 years or older, preferably without having the tendency to gag. Informed written consent was obtained from all volunteers. No personal data of volunteers were recorded, with the exception of age and sex. Volunteers using polypharmacy (more than four medications) or specific xerogenic medications were excluded for saliva collection. Xerogenity of the medications was determined using the medication guides published by Sreebny and Schwartz (1986), Wolff *et al.* (2016) and the Dutch Pharmacotherapeutic Compass [23–25]. The reporting of this study conforms to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement [26].

Study variables

Subjective oral dryness assessment

The Xerostomia Inventory (XI) was used to measure the overall dry-mouth experience. The XI consists of 11 items on a 5-point Likert scale ranging from 1 = "never" to 5 = "very often." The items are about oral dryness and mouth feel. Participants indicate on each item how often they suffer from problems with regard to mouth feel and oral dryness. The scores of the 11 items are summed, resulting in a total XI score that ranges between 11 (no xerostomia) and 55 (extreme xerostomia) [27].

In addition, participants completed the Regional Oral Dryness Inventory (RODI) to measure the intra-oral perceived dryness [8, 9]. This questionnaire contains 9 schematic illustrations of different locations in the oral cavity. Four illustrations represent areas in the upper jaw: the upper lip, anterior part of the palate (including the rugae), inside part of the cheeks and posterior part of the palate (from the rugae up to the end of the soft palate). Four illustrations represent areas in the lower jaw: the lower lip, floor of the mouth, posterior part of the tongue (from vallate papilla up to end of the tongue) and anterior part of the tongue (from the tip of the tongue up to vallate papilla). Finally, one illustration represents the pharynx. At each location, the patient can indicate the severity of the perceived oral dryness using a 5-point Likert scale ranging from 1 = "no dryness" to 5 = "severe dryness" [9, 10].

Sialometry and salivary pH

To limit circadian variations, the saliva measurements were performed between 8:15 and 10:15 A.M in the same room (temperature 20–24 ℃, humidity 50–70%) [28]. The participants were instructed not to eat, drink, chew gum, brush teeth, use mouthwash or smoke at least 1 h before their visit. The unstimulated (UWS) and chew-stimulated salivary flow rates (CH-SWS) were determined as described previously [29]. The pH of saliva was measured immediately after saliva collection using an electronic pH metre (PHM240, pH/ion metre, Meterlab, Copenhagen, Denmark). The samples were kept on ice until analysed.

Determination of the palatal surface area

In order to measure the palatal surface area, an intra-oral scan of the upper jaw including the palate (the whole hard palate and part of the soft palate) was taken using a TRIOS 3 scanner (3Shape, version 21.3.5, Copenhagen, Denmark) using the manufacturer's protocol. Scans were digitally saved as Polygon File Format (PLY) files.

Subsequently, each PLY object was analysed twice in Meshmixer (Autodesk, San Rafael, CA, USA) by one researcher (ZA). This analysis involved the manual separation of the palate by using the vibrating line including visible fovea palatine as a cut-off for the length of the palate. Besides, all palatal mucosa including the gingiva around the upper teeth were included in the palatal surface. After segmentation, the palatal surface areas (in mm^2) were determined.

Measuring the salivary film thickness

Determination of the salivary film was performed as described in previous studies [2, 11–14, 18–21]. At least 15 min after the collection of the whole saliva, the salivary film was collected at different intra-oral locations using Sialopaper filter paper strips (Oraflow, New York, USA).

The filter strips were handled with gloved hands at all times. Five mucosal surfaces were selected based on previous studies [9, 10]: The anterior tongue was sampled in the middle of the tongue approximately 5 mm from the tongue tip, the anterior palate in the middle at the papilla incisive, the posterior palate in the middle at the vibrating line, the inside cheek 1 cm from the right chelion at the occlusal plane and the floor of the mouth at the right sublingual caruncula. The salivary film was collected twice at each location. Participants were instructed to swallow each time before a Sialopaper was applied to the surface for 5 s. The volume of fluid absorbed on the strip was measured electronically using a calibrated micro-moisture metre (Periotron 8000; Oraflow, Hewlett, NY, USA) and stored in Eppendorf tubes (Eppendorf, Cambridge, UK). Participants were instructed to swallow, and a second sample was collected at the same location. Samples were kept on ice until analysed. The salivary film thicknesses were calculated by dividing the collected saliva by the surface area of a Sialostrip (44.15 mm^2).

Measuring MUC5B levels

The MUC5B levels were determined essentially as described before [6, 30–34]. High-binding 96-well polystyrene microplates (Greiner Bio-One) were used for all ELISAs. The unstimulated saliva samples were vortexed for approximately 10 s and centrifuged (10 min, at 10.000 g). The supernatant was transferred to a new vial. Supernatants were diluted 1:200 in coating buffer (0.1 M N aHCO3, pH 9.6), and per sample 100 µL/well was coated in duplicate on the microplates. MUC5B was eluted from the Sialopapers with MilliQ water (210 µL) with an efficiency of 84 ± 15% (data not shown) and then diluted in 210-µL coating buffer. Afterwards, eluted samples (100 µL/well) were coated in duplicate on the 96-well microplates, and all wells were serially diluted in coating buffer. Afterwards, all microplates were subsequently incubated at 37 ℃ for 2 h. Then the wells were rinsed with PBS–0.1% Tween 20 (PBST) for three times. The plates were then blocked for 1 h with 100 µL per well with 1% gelatin in PBST (PBSTG). After removing the blocking solution, 100 µL per well of 1:40 mAb F2, recognising the terminal part of the carbohydrate moiety, sulfoLewis-A SO_3-3Gal_1-3GlcNAc in PBSTG [5, 30, 31, 33, 34]. The microplates were then incubated for 1 h at 37 ℃. After washing, the microplates were incubated for 1 h with rabbit-anti-mouse

IgG-HRP conjugate (Rockland Immunochemicals Inc., Pottstown, PA, USA) 1:2000 in PBSTG. After washing with PBST and distilled water, 100 µL TMB solution (3,3',5,5'-tetramethyl-benzidine; 125 µg/ml in sodium acetate buffer (100 mM, pH 5.0) with 0.05% v/v H_2O_2) was added to each well. After 10 min, the reaction was stopped by adding 50 µL 2 M H_2SO_4 per well. Absorbance was measured at 450 nm with a plate spectrophotometer reader (Multiskan FC, Thermo Scientific, Waltham, MA, USA). Arbitrary units (AU) MUC5B were calculated using a reference sample, as described before [6, 30, 35].

Statistical analysis

The data were processed in an electronic clinical data-management platform (CastorEdc, Castor, Amsterdam, the Netherlands) and then converted into SPSS version 27.0 (IBM Corp SPSS Statistics, Armonk, NY, USA) for the statistical analysis. The Shapiro–Wilk test was used to assess the normality of the data. The data were presented as median and their interquartile range (IQR), as most of the parameters were not normally distributed. The mean and standard deviation were also reported to clarify relatively small differences.

The intraclass correlation coefficient (ICC) was used to determine the degree of agreement between two measurements for the palatal surface area. A two-way mixed, absolute agreement, average-measures ICC was calculated for these measurements [36, 37]. The ICC is indicative of poor (values less than 0.5), moderate (between 0.5 and 0.75), good (between 0.75 and 0.9) and excellent (greater than 0.90) reliability [38].

The mean of the two palatal surface area measurements, the two salivary film measurements and two MUC5B levels at each location were used for further analysis.

Female-male differences for various saliva characteristics, including the salivary flow rate, total XI score and intra-oral RODI scores, were explored with a Mann–Whitney U test.

A Friedman test was conducted for the salivary film thickness and the MUC5B levels at various intra-oral locations, followed by a Wilcoxon signed-rank test as a post-hoc procedure.

Various possible associations were explored in the current study. These relations were analysed with a bootstrapped Pearson correlation test (1000 × bootstrapping). The Pearson correlation coefficient and bias-corrected accelerated (Bca) 95% confidence interval were extracted. The following correlations were investigated: between the salivary film thickness with the MUC5B levels at the five corresponding intraoral locations, between the salivary film thickness of the palate with the palatal surface area and between the

MUC5B levels of the palate with the palatal surface area. Furthermore, the participants were dichotomized based on their sex and the dimensions of the palate. The median of the palatal surface area was used to create two equal groups: 'small' palatal surface area (< 2138.0 mm^2) and 'large' palatal surface area (≥ 2138.0 mm^2). The size of the correlation coefficient was interpreted as poor (r = 0.1–0.2), fair (r = 0.3–0.5), moderate (r = 0.6–0.7) or very strong (r = 0.8–0.9) correlation [39].

Furthermore, a multivariate analysis, multiple linear regression, was performed to investigate the possible association between the salivary film thickness and all independent variables. The salivary film thicknesses of both the anterior and the posterior palate were considered as dependent variables, while the palatal surface area, sex, the UWS and CH-SWS flow rate were considered as independent variables. All these independent variables were chosen because they could affect the thickness of the salivary film. To identify the degree of multicollinearity among the independent variables, the variance inflation factor (VIF) was calculated. The VIF for these variables was < 5, which indicates that there is no multicollinearity present among these variables [40, 41]. Additionally, the R square was reported.

No multiple regression was conducted for the MUC5B levels of the anterior palate as the variance of the residuals was not constant and also multivariate normality was not met (residuals were not normally distributed).
All significance levels (P) were set at 0.05.

RESULTS

Fifty-one volunteers signed up for this study (Fig. 1). The average age of female and male participants did not differ significantly (Mann–Whitney U test p > 0.05). Eleven volunteers had a systemic disease and/or were taking various medications that could initiate dry-mouth symptoms (Fig. 1). After the exclusion of these volunteers, the average age of the remaining 40 volunteers was 40.1 ± 13.4 years. The average age of the female and male participants did not differ significantly (Mann–Whitney U test p > 0.05). Ten of the remaining 18 female volunteers used contraceptive medication.

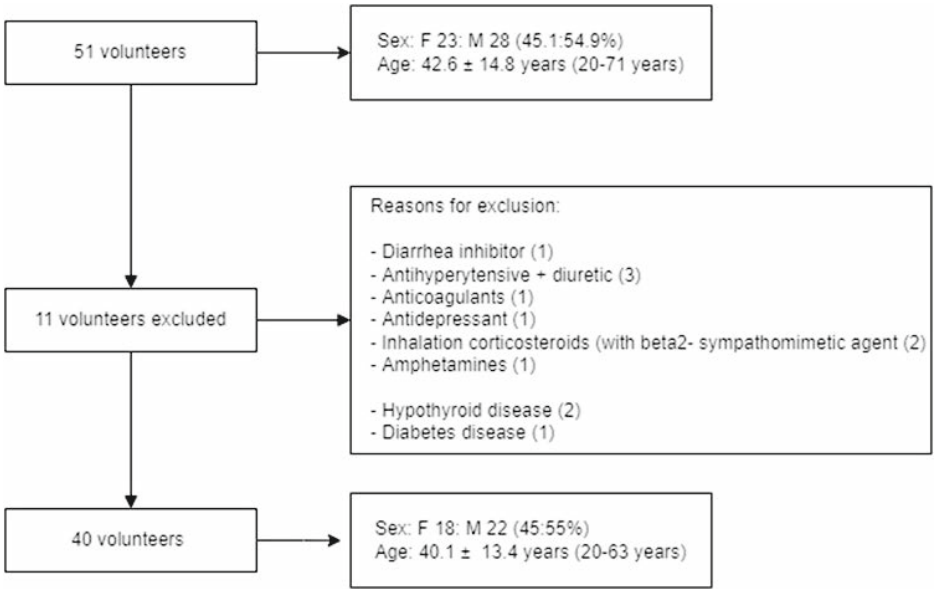


Fig. 1 Flow chart showing the reason for exclusion of some volunteers and the characteristics of the included volunteers

Sialometry, salivary pH, dry-mouth experience and palatal surface area measurement

Table 1 reports the salivary secretion rates and pH, the overall dry-mouth experience as measured with XI and the palatal surface area measurement. The median UWS salivary flow rate for all participants was 0.25 ± 0.16–0.37 mL/min, while the median CH-SWS flow rate was approximately 5 times more than that of the UWS. The median salivary pH for the CH-SWS (pH = 7.14) was higher than the UWS (pH = 6.60). Female and male participants did not show any significant difference with regard to the salivary flow rate and pH of both UWS and CH-SWS (Mann–Whitney U test $p > 0.05$).

The median XI score was 19.5 out of the maximum of 55 (Table 1). The XI-values for female and male participants also did not differ significantly (Mann–Whitney U test $p > 0.05$).

The intra-oral regions with the highest RODI scores were the upper lip (M = 1.68 ± 0.86, Mdn = 1.00 ± 1.00–2.00), the posterior palate (M = 1.63 ± 0.74, Mdn = 1.50 ± 1.00–2.00), the lower lip (M = 1.60 ± 0.78, Mdn = 1.00 ± 1.00–2.00) and the pharynx (M = 1.60 ± 0.67, Mdn = 1.50 ± 1.00–2.00). In contrast, the floor of mouth had the lowest RODI score (M = 1.10 ± 0.30, Mdn = 1.00 ± 1.00–1.00). The RODI scores for all intra-oral locations were < 2, indicating that the volunteers did not experience any intra-oral dryness. Females and male participants did

not differ significantly in RODI scores for each of the intra-oral regions (Mann–Whitney U test $p > 0.05$).

The median palatal surface area was 2138.0 ± 1975.5–2247.6 mm^2. The ICC for the surface area measurements was 0.96, which is in the excellent range. The palatal surface area showed significant differences for both sexes (Mann–Whitney U test $p < 0.05$), whereby male participants had a significantly larger palatal surface area than females (Table 1).

Salivary film thickness and MUC5B levels at various intra-oral locations

The salivary film thickness showed considerable differences by intra-oral location. For example, the salivary film at the anterior tongue was six times thicker than that at the anterior palate (Table 2, Wilcoxon signed-rank tests: $p < 0.05$). Moreover, the salivary film thickness of the floor of the mouth differed significantly from all other intra-oral locations (Wilcoxon signed-rank tests $p < 0.05$). Besides, there was a significant difference in film thickness between the anterior and posterior palate, as the saliva film on the posterior palate was 2.6 times thicker than the film on the anterior palate (Table 2, Wilcoxon signed-rank tests: $p < 0.05$).

The salivary film thickness differed significantly between males and females only for the anterior palate and the inside cheeks, whereby the salivary film in these two regions was thicker for male participants.

Total unstimulated saliva contained the highest levels of MUC5B, *i.e.* 0.345 ± 0.177–0.716 AU/mL. The MUC5B levels in total saliva of female participants (0.369 ± 0.176–0.762 AU/mL, N = 18) did not differ significantly from male participants (0.331 ± 0.171–0.555 AU/ mL, N = 22) (Mann–Whitney U test $p > 0.05$). Significant differences in MUC5B levels between the intra-oral locations were found (Table 3). The MUC5B level at the anterior tongue was 42 times higher than at the anterior palate, where the lowest level was measured. MUC5B level at the anterior palate showed significant sex differences, with female participants having lower MUC5B level than male participants (Mann–Whitney U test $p < 0.05$) (Table 3).

Table 1: Characteristics of the total study population (40 volunteers) and stratified according to sex. The total XI-scores, the unstimulated whole saliva (UWS), chewing-stimulated whole saliva (CH-SWS) flow rate (mL/min), salivary pH, and palatal surface area (mm2) were reported. Data are expressed as medians with the corresponding interquartile ranges (IQR) and as means with standard deviations (SD). The ICC is reported only for the palatal surface area measurement.

Saliva		Total study population Mean ± SD	Total study population Median ± IQR	Females Median ± IQR (N=18)	Males Median ± IQR (N=22)
UWS	Flow rate	0.28 ± 0.15	0.25 ± 0.16-0.37	0.31 ± 0.16-0.43	0.23 ± 0.15-0.30
	pH	6.56 ± 0.29	6.60 ± 6.39-6.77	6.66 ± 6.45-6.82	6.59 ± 6.29-6.71
CH-SWS	Flow rate	1.26 ± 0.67	1.19 ± 0.74-1.58	1.30 ± 0.85-1.58	1.14 ± 0.72-1.61
	pH	7.12 ± 0.31	7.14 ± 6.91-7.33	7.15 ± 6.98-7.34	7.13 ± 6.82-7.32
XI-total		19.9 ± 5.2	19.5 ± 16.3-23.8	19.5 ± 17.8-24.3	19.5 ± 15.8-23.3
Surface area (mm2)					
Palatal	ICC=0.96	2123.8 ± 221.9	2138.0 ± 1975.5-2247.6	2050.6 ± 1868.4-2177.9*	2176.8 ± 2018.9-2260.5

** Female vs. male difference Mann-Whitney U test p-value<0.05*

Table 2: The salivary film thickness at five intra-oral location, stratified according to sex. N indicates the number of participants in each group. Data are presented as median with corresponding interquartile range (IQR).

Intra-oral loacations	Salivary film thickness in µm (N=40) Median ± IQR	Female salivary film thickness in µm (N=18) Median ± IQR	Male salivary film thickness in µm (N=22) Median ± IQR
Anterior part of the tongue	68.9 ± 57.6-77.4	65.2 ± 57.4-73.4	74.0 ± 59.4-79.3
Anterior part of the palate	11.3 ± 5.2-19.1[a]	6.0 ± 4.1-13.8**	15.7 ± 9.2-22.0
Posterior part of the palate	29.7 ± 17.4-44.3[a,b]	28.0 ± 15.7-39.0	31.4 ± 21.1-53.3
Inside cheeks	44.0 ± 34.8-58.5[a,b,c]	40.0 ± 27.1-50.0**	55.4 ± 39.7-68.5
Floor of the mouth	62.5 ± 46.3-78.7[a,b,c,d]	52.5 ± 33.5-78.9	67.4 ± 54.4-78.6

*** Female vs. male difference Mann-Whitney U test p-value <0.01*
a Wilcoxon signed-rank tests: p < 0.05 vs. anterior part of the tongue
b Wilcoxon signed-rank tests: p < 0.05 vs. anterior part of the palate
c Wilcoxon signed-rank tests: p < 0.05 vs. posterior part of the palate
d Wilcoxon signed-rank tests: p < 0.05 vs. inside cheeks

Table 3: The MUC5B levels at five intra-oral locations, stratified according to sex. N indicates the number of participants in each group. Data are presented as median with corresponding interquartile range (IQR).

Intra-oral locations	MUC5B in AU/mL (N=34)# Median ± IQR	Female MUC5B in AU/mL (N=16)# Median ± IQR	Male MUC5B in AU/mL (N=18)# Median ± IQR
Anterior part of the tongue	0.127 ± 0.040-0.353	0.089 ± 0.037-0.173	0.273 ± 0.040-0.638
Anterior part of the palate	0.003 ± 0.000-0.011[a]	0.000 ± 0.000-0.003*	0.006 ± 0.000-0.017
Posterior part of the palate	0.020 ± 0.009-0.121[a,b]	0.018 ± 0.005-0.039	0.027 ± 0.012-0.208
Inside cheeks	0.008 ± 0.000-0.034[a,b]	0.008 ± 0.000-0.032	0.010 ± 0.000-0.075
Floor of the mouth	0.007 ± 0.000-0.029[a,b,c]	0.002 ± 0.000-0.030	0.012 ± 0.000-0.029

** Female vs. male difference Mann-Whitney U test p-value <0.05*
a Wilcoxon signed-rank tests: p < 0.05 vs. anterior part of the tongue
b Wilcoxon signed-rank tests: p < 0.05 vs. anterior part of the palate
c Wilcoxon signed-rank tests: p < 0.05 vs. posterior part of the palate
 # The total number differs as MUC5B samples were not available for all participants.

Association between salivary film thickness and the MUC5B levels

The salivary film thickness of all intra-oral locations showed significant correlations with the MUC5B levels of the associated regions (Table 4). The correlation coefficients varied between 0.48 and 0.66, which can be considered as fair to moderate. A positive correlation indicates that when MUC5B levels are high, the salivary film thickness at the associated region is also high. Only the floor of the mouth did not have any significant correlation between the salivary film thickness and MUC5B levels for the total study sample. However, when this group was stratified on sex, it was found that females had a significant correlation for the floor of the mouth. For all other intra-oral regions, it was found that the correlation coefficient of both sex groups lay in the same range as the total study sample. Only females did not have any significant correlation between the salivary film thickness and the MUC5B levels at the anterior tongue (Table 4).

Table 4: The correlation between the salivary film thickness at five intra-oral regions with the MUC5B level at the associated regions. Data are expressed as the Pearson correlation coefficient and bias-corrected accelerated (Bca) 95% confidence interval.

Correlation between film thickness and MUC5B levels at associated regions	Correlation coefficient (total study population)	Correlation coefficient (females)	Correlation coefficient (males)
Anterior part of the tongue	0.57 (0.42-0.74)**	NS	0.63 (0.34-0.84)**
Anterior part of the palate	0.66 (0.46-0.86)**	0.57 (-0.06-0.89)*	0.63 (0.33-0.87)**
Posterior part of the palate	0.56 (0.33-0.78)**	0.61 (-0.11-0.90)*	0.59 (0.24-0.90)**
Inside cheeks	0.48 (0.21-0.75)**	0.67 (0.08-0.85)**	0.54 (0.14-0.85)*
Floor of the mouth	NS	0.52 (0.18-0.82)*	NS

NS=Not Significant
**Pearson correlation test p -value <0.05*
*** Pearson correlation test p -value <0.01*

Association between the salivary film thickness and the MUC5B levels at the palate with the palatal surface area

The salivary film thickness and MUC5B levels at the anterior and posterior palate did not have any significant correlation with the palatal surface area (Pearson correlation p > 0.05). Because male participants had a significantly larger palatal surface area, this analysis was repeated after stratifying the participants based on their sex. The palatal surface areas of both female and male did not have any significant correlation with the salivary film thickness and/or MUC5B levels of the anterior and posterior palate (Pearson correlation

p > 0.05). Besides, the two palatal dimensions (small vs. large surface area) did not have any significant correlation with the salivary film thickness and/or MUC5B levels of the palate as well (Pearson correlation p > 0.05).

A multivariate regression analysis was performed, taking the palatal surface area, sex, the UWS and CH-SWS flow rate into consideration. For both the anterior and posterior salivary film thickness, no association was found with any of the independent variables (regression p > 0.05). The R squared for the anterior palate was 0.19 and for the posterior palate 0.09. So, the palatal surface area did not affect the salivary film thickness on either the anterior or posterior palate. The same applied to all other independent variables.

DISCUSSION

The results of this study, in which we explored the salivary film thickness and MUC5B levels at various locations in the oral cavity in healthy volunteers, demonstrated that both are unequally distributed over the various intra-oral surfaces. The anterior tongue had the thickest salivary film and contained the highest levels of MUC5B, while the anterior palate had the thinnest salivary film and lowest MUC5B levels. Furthermore, the palatal surface area did not correlate with the palatal salivary film thickness or the palatal MUC5B levels, indicating that in healthy individuals, a larger surface area was not associated with a relatively thinner salivary film and/or lower MUC5B levels. Therefore, our hypothesis should be rejected.

The mean UWS flow rate of the included participants was 0.28 mL/min, which was comparable with the average values of 0.3–0.4 mL/min previously reported [42].

The median XI score was 19.5, indicating that included participants on average did not experience serious dry-mouth complaints. The current XI scores were comparable with the XI scores found in other studies with healthy volunteers (age from 18 to 92 years), ranging between 16.0 and 20.82 [43–49]. Also, the RODI scores for all intra-oral locations were < 2, indicating they did not experience any intra-oral dryness. Dry-mouth patients in previous studies showed RODI scores ≥ 3 for most intra-oral locations [9, 10]. So, although the salivary flow rate seems to deviate slightly from earlier reports, it can be stated the included volunteers had healthy salivary flow rates and experienced no dry-mouth complaints.

The average palatal surface area found was 2123.8 mm^2, which was comparable with other studies, who included adults with an average of 1990–

2010 mm^2 [15, 16, 50]. In these studies, the palatal surface areas were determined using foil impressions taken from stone models, while another study used CBCT imaging and digital analysis [15, 16, 50]. Apparently, all methods used so far reveal comparable and representative results as their surface areas are in the same range. In addition, the technique presented in the current study, using an intra-oral scanner, adds up to this line of methods as it had very good reproducibility, as indicated by the excellent range of the ICC. However, future studies, which investigate and compare the validity and the reliability of various methods including the intra-oral scanner for measuring the intra-oral surface area, seem warranted.

The pattern of salivary film distribution over intra-oral locations found in the current study was comparable with the distribution of the salivary film in healthy volunteers reported previously [2, 11–14, 18–21]. Also, comparable patterns were seen in the current study, as the tongue and/or the floor of the mouth had the thickest salivary film, while the anterior palate had the thinnest salivary film. The reason why the tongue has the highest level of wetness is probably because of its anatomical location near the caruncle of the Wharton's ducts [2, 13, 18]. Here, saliva from the many minor glands in this region and the nasopalatine glands as well as the secretions of the submandibular and sublingual glands is collected [2]. Besides, the von Ebner's glands, with their ducts opening into the sulci of the circumvallate and foliate papillae, produce serous saliva that contributes to the moistening of the tongue [51, 52]. In contrast, several factors make the anterior palate more susceptible to having a thinner salivary film than other intra-oral locations: lack of hard palatal salivary glands, and evaporation, especially during speaking and breathing [18, 53, 54]. Moreover, gravity forces part of the excreted saliva to pool on the floor of the mouth between swallowing episodes. As a consequence, the palate can be moistened less sufficiently [2].

Two previous studies investigated MUC5B levels at various intra-oral locations in healthy controls [11, 14]. However, different techniques were used in these studies compared to our study: Firstly, SDS-PAGE was performed on the eluted Sialopapers with subsequent PAS staining. Then, software analysis was used, scanning lanes of PAS-stained mucin glycoprotein bands, and analysed for colour intensity, gauging the amount of mucin [11, 14]. In contrast, we applied ELISA using an antibody, *i.e.* F2, to specifically measure MUC5B levels. However, it seemed difficult to compare our findings to those of Chaudhury et al. [11] because they expressed the MUC5B levels in MUC5B glycan/protein proportion. In contrast, we calculated arbitrary units/volume of fluid on Sialopaper [11]. In the study by Pramanik *et al.*, contradictory results compared to our study

were found; they found the highest MUC5B levels at the anterior hard palate and the lowest levels at the lower labial mucosa and the anterior tongue [14]. In contrast, in our study, the anterior tongue had the highest levels, and the anterior palate had the lowest levels of MUC5B. This is difficult to explain as to a large extend MUC5B is secreted by the submandibular and sublingual salivary glands with their sublingual caruncle lying on the floor of the mouth [55, 56], in which the tongue is embedded. As mentioned before, the anterior hard palate lacks the presence of salivary glands [22], and MUC5B found on the anterior palate is translocated there mainly by tongue movements.

Surprisingly, the floor of the mouth contained approximately 18 times less MUC5B levels than the anterior tongue, despite the fact that the caruncles of both submandibular and sublingual glands are located on the floor of the mouth. Gravity forces help the floor of the mouth to create a reservoir for all the saliva that does not adhere to the various surfaces. So, the saliva on the floor of the mouth is a mix of various salivary glands. Especially after swallowing episodes, not all the saliva is swallowed; the salivary clearance is approximately 28% [57, 58], indicating that the majority of saliva remains in the mouth. Additionally, the structure of the tongue helps to adhere to all the mucins, as the dorsal (superior) surface has a rough structure of stratified squamous epithelium with numerous circumvallate, filiform and fungiform papillae. Potentially, this rough or plicated surface offers the MUC5B glycoprotein a surface to which it can reside more effectively during oro-facial movements, such as swallowing, than to the smooth structure of the floor of the mouth.

An interesting finding in the current study was the significant correlation between the salivary film thickness and the MUC5B levels. MUC5B forms hydrophilic polymer brushes causing water retention [59]. For this reason, MUC5B is considered as the key lubricant in saliva. So, it could be expected that higher MUC5B levels will influence the increment of the salivary film thickness.

Another interesting finding was the lack of correlation between the palatal surface area and the palatal salivary film thickness and/or the palatal MUC5B levels. We hypothesised that individuals with a larger palatal surface area would have a thinner salivary film at the palate and also less availability of MUC5B glycoproteins. However, we found that all individuals showed comparable salivary film thickness and MUC5B levels. This last result could be explained by the palatal saliva that contained relatively high levels of MUC5B [60]. Palatal saliva is excreted by the orifices of the palatal glands, which are all located near the right and left maxillary second and third molars [22]. The palatal saliva including MUC5B is propelled towards the anterior part

of the palate during swallowing; this can possibly explain why the salivary film thickness and the MUC5B level are not particularly low in individuals with larger palatal dimensions. Additionally, the palatal salivary film is not only formed by the palatal salivary glands, but it is also dependent on the salivary film of the tongue. The tongue also plays an important role in moistening and lubricating the palate. As the salivary film thickness at the tongue is already 2.3–6 times thicker than that of the palate, this will promote the transfer of additional saliva from the tongue to the palate. Finally, the retention of saliva by the anterior palate also plays a possible role. The structural orientation of the anterior palate, especially of the rugae with their irregular, asymmetric ridges [61], causes the retention of mucins and moisture despite the negative effect of gravity.

A possible limitation of the current study is the use of Sialopapers for the collection of MUC5B. Although the elution efficiency of MUC5B from the Sialopapers is good (84 ± 15%), it has to be noted that the absorption of all MUC5B glycoproteins from the mucosal surfaces to the Sialopaper seems virtually impossible. Namely, the oral mucosal surfaces are more or less covered with a double layer: a lower surface-bound layer, which is the mucosal pellicle, and an upper salivary film, loosely attached to the mucosal pellicle [4]. It is plausible to assume that the efficiency of absorption of MUC5B from the loosely attached salivary layer to the Sialopaper is probably more effective than for MUC5B from the mucosal pellicle. In this light, it also has to be noted that oral epithelial cells express membrane-bound mucin (MUC1), which can interact with MUC5B to develop the mucosal pellicle [4]. Consequently, this interaction hinders the adsorption of MUC5B of the mucosal pellicle to the Sialopaper. Transmission Electron Microscopy and immunogold labelling could be applied to study these interactions and shed light on the absorption efficiency [4]. These techniques already have successfully been applied for buccal epithelial cells, but not for other intra-oral surfaces [62].

A recent study revealed that the intra-oral scanner was a suitable instrument to investigate the palatal soft tissue in terms of shape, colour and curvature [63]. In line with our experience, the shape of the palatal surface, especially the palatal rugae, was documented very precisely with the intra-oral scanner. Yet, it has to be noted that the intra-oral scanner lacks the resolution to analyse the full microstructure of the palatal surface, and this could lead to a slight underestimation of the total palatal surface area determined in the current study.

MAIN CONCLUSIONS

The salivary film and MUC5B levels were not equally distributed over the mouth. The anterior tongue had the thickest salivary film and also the highest levels of MUC5B, while the anterior palate had the thinnest salivary film and lowest MUC5B levels. There was no association between the palatal surface area and the salivary film thickness at the palate, also when sex and salivary flow rate were taken into consideration. These findings indicate that a larger surface area is not associated with a relatively thinner salivary film.

REFERENCES

1. Dawes C, Pedersen AM, Villa A, Ekstrom J, Proctor GB, Vissink A, *et al.* The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. Archives of oral biology. 2015;60(6):863-74. doi: 10.1016/j.archoralbio.2015.03.004.
2. Wolff M, Kleinberg I. Oral mucosal wetness in hypo- and normosalivators. Archives of oral biology. 1998;43(6):455-62. doi: 10.1016/s0003-9969(98)00022-3.
3. Kho HS. Understanding of xerostomia and strategies for the development of artificial saliva. The Chinese journal of dental research : the official journal of the Scientific Section of the Chinese Stomatological Association (CSA). 2014;17(2):75-83.
4. Hannig C, Hannig M, Kensche A, Carpenter G. The mucosal pellicle - An underestimated factor in oral physiology. Archives of oral biology. 2017;80:144-52. doi: 10.1016/j.archoralbio.2017.04.001.
5. Tabak LA. In defense of the oral cavity: the protective role of the salivary secretions. Pediatr Dent. 2006;28(2):110-7; discussion 92-8.
6. Vinke J, Oude Elberink M, Stokman MA, Kroese FGM, Nazmi K, Bikker FJ, *et al.* Lubricating properties of chewing stimulated whole saliva from patients suffering from xerostomia. Clin Oral Investig. 2021;25(7):4459-69. doi: 10.1007/s00784-020-03758-8.
7. Saleh J, Figueiredo MA, Cherubini K, Salum FG. Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Archives of oral biology. 2015;60(2):242-55. doi: 10.1016/j.archoralbio.2014.10.004.
8. Niklander S, Veas L, Barrera C, Fuentes F, Chiappini G, Marshall M. Risk factors, hyposalivation and impact of xerostomia on oral health-related quality of life. Brazilian oral research. 2017;31:e14. doi: 10.1590/1807-3107BOR-2017.vol31.0014.
9. Assy Z, Bots CP, Arisoy HZ, Gülveren SS, Bikker FJ, Brand HS. Differences in perceived intra-oral dryness in various dry-mouth patients as determined using the Regional Oral Dryness Inventory. Clin Oral Investig. 2021. doi: 10.1007/s00784-020-03734-2.
10. Assy Z, Jager DHJ, Mashhour E, Bikker FJ, Brand HS. Regional differences in perceived oral dryness as determined with a newly developed questionnaire, the Regional Oral Dryness Inventory. Clin Oral Investig. 2020. doi: 10.1007/s00784-020-03276-7.
11. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH, Proctor GB. Changes in Saliva Rheological Properties and Mucin Glycosylation in Dry Mouth. Journal of dental research. 2015;94(12):1660-7. doi: 10.1177/0022034515609070.
12. Lee SK, Lee SW, Chung SC, Kim YK, Kho HS. Analysis of residual saliva and minor salivary gland secretions in patients with dry mouth. Archives of oral biology. 2002;47(9):637-41. doi: 10.1016/s0003-9969(02)00053-5.
13. Won S, Kho H, Kim Y, Chung S, Lee S. Analysis of residual saliva and minor salivary gland secretions. Archives of oral biology. 2001;46(7):619-24. doi: 10.1016/s0003-9969(01)00018-8.
14. Pramanik R, Osailan SM, Challacombe SJ, Urquhart D, Proctor GB. Protein and mucin retention on oral mucosal surfaces in dry mouth patients. European journal of oral sciences. 2010;118(3):245-53. doi: 10.1111/j.1600-0722.2010.00728.x.
15. Collins LM, Dawes C. The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. Journal of dental research. 1987;66(8):1300-2. doi: 10.1177/00220345870660080201.

16. Kerr WJ, Kelly J, Geddes DA. The areas of various surfaces in the human mouth from nine years to adulthood. Journal of dental research. 1991;70(12):1528-30. doi:10.1177/00220345910700121001.
17. Watanabe S, Dawes C. Salivary flow rates and salivary film thickness in five-year-old children. Journal of dental research. 1990;69(5):1150-3. doi: 10.1177/00220345900690050601.
18. DiSabato-Mordarski T, Kleinberg I. Measurement and comparison of the residual saliva on various oral mucosal and dentition surfaces in humans. Archives of oral biology. 1996;41(7):655-65. doi: 10.1016/s0003-9969(96)00055-6.
19. Osailan S, Pramanik R, Shirodaria S, Challacombe SJ, Proctor GB. Investigating the relationship between hyposalivation and mucosal wetness. Oral diseases. 2011;17(1):109-14. doi: 10.1111/j.1601-0825.2010.01715.x.
20. Chaudhury NM, Proctor GB, Karlsson NG, Carpenter GH, Flowers SA. Reduced Mucin-7 (Muc7) Sialylation and Altered Saliva Rheology in Sjögren's Syndrome Associated Oral Dryness. Mol Cell Proteomics. 2016;15(3):1048-59. doi: 10.1074/mcp.M115.052993.
21. Osailan SM, Pramanik R, Shirlaw P, Proctor GB, Challacombe SJ. Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. Oral surgery, oral medicine, oral pathology and oral radiology. 2012;114(5):597-603. doi: 10.1016/j.oooo.2012.05.009.
22. Hamada T, Kawazoe Y, Sekino K, Nagasawa T, Tsuru H. Palatal gland distribution. Journal of dental research. 1974;53(4):944. doi: 10.1177/00220345740530043701.
23. Sreebny LM, Schwartz SS. A reference guide to drugs and dry mouth. Gerodontology. 1986;5(2):75-99. doi: 10.1111/j.1741-2358.1986.tb00055.x.
24. Wolff A, Joshi RK, Ekström J, Aframian D, Pedersen AM, Proctor G, *et al.* A Guide to Medications Inducing Salivary Gland Dysfunction, Xerostomia, and Subjective Sialorrhea: A Systematic Review Sponsored by the World Workshop on Oral Medicine VI. Drugs R D. 2017;17(1):1-28. doi: 10.1007/s40268-016-0153-9.
25. Zorginstituut Nederland: Geneesmiddelen https://www.farmacotherapeutischkompas.nl/bladeren/preparaatteksten/groep (2022). Accessed.
26. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. International journal of surgery (London, England). 2014;12(12):1495-9. doi: 10.1016/j.ijsu.2014.07.013.
27. Thomson WM, Chalmers JM, Spencer AJ, Williams SM. The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community dental health. 1999;16(1):12-7.
28. Dawes C. Circadian rhythms in human salivary flow rate and composition. J Physiol. 1972;220(3):529-45. doi: 10.1113/jphysiol.1972.sp009721.
29. Navazesh M, Kumar SK. Measuring salivary flow: challenges and opportunities. Journal of the American Dental Association (1939). 2008;139 Suppl:35s-40s. doi: 10.14219/jada.archive.2008.0353.
30. Morquecho-Campos P, Bikker FJ, Nazmi K, de Graaf K, Laine ML, Boesveldt S. Impact of food odors signaling specific taste qualities and macronutrient content on saliva secretion and composition. Appetite. 2019;143:104399. doi: 10.1016/j.appet.2019.104399.

31. Dijkema T, Terhaard CH, Roesink JM, Raaijmakers CP, van den Keijbus PA, Brand HS, *et al.* MUC5B levels in submandibular gland saliva of patients treated with radiotherapy for head-and-neck cancer: a pilot study. Radiat Oncol. 2012;7:91. doi: 10.1186/1748-717x-7-91.
32. Sonesson M, Wickström C, Kinnby B, Ericson D, Matsson L. Mucins MUC5B and MUC7 in minor salivary gland secretion of children and adults. Archives of oral biology. 2008;53(6):523-7. doi: 10.1016/j.archoralbio.2008.01.002.
33. Silva DG, Stevens RH, Macedo JM, Hirata R, Pinto AC, Alves LM, *et al.* Higher levels of salivary MUC5B and MUC7 in individuals with gastric diseases who harbor Helicobacter pylori. Archives of oral biology. 2009;54(1):86-90. doi: 10.1016/j.archoralbio.2008.08.003.
34. Veerman EC, Bolscher JG, Appelmelk BJ, Bloemena E, van den Berg TK, Nieuw Amerongen AV. A monoclonal antibody directed against high M(r) salivary mucins recognizes the SO3-3Gal beta 1-3GlcNAc moiety of sulfo-Lewis(a): a histochemical survey of human and rat tissue. Glycobiology. 1997;7(1):37-43. doi: 10.1093/glycob/7.1.37.
35. Ligtenberg AJM, Meuffels M, Veerman ECI. Effects of environmental temperature on saliva flow rate and secretion of protein, amylase and mucin 5B. Arch. Oral Biol. 2020;109:104593. doi: 10.1016/j.archoralbio.2019.104593.
36. Hallgren KA. Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. Tutorials in quantitative methods for psychology. 2012;8(1):23-34.
37. McGraw K, Wong SP. Forming Inferences About Some Intraclass Correlation Coefficients. Psychological Methods. 1996;1:30-46. doi: 10.1037/1082-989X.1.1.30.
38. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. Journal of chiropractic medicine. 2016;15(2):155-63. doi: 10.1016/j.jcm.2016.02.012.
39. Akoglu H. User's guide to correlation coefficients. Turkish journal of emergency medicine. 2018;18(3):91-3. doi: 10.1016/j.tjem.2018.08.001.
40. Marcoulides KM, Raykov T. Evaluation of Variance Inflation Factors in Regression Models Using Latent Variable Modeling Methods. Educ Psychol Meas. 2019;79(5):874-82. doi: 10.1177/0013164418817803.
41. Kim JH. Multicollinearity and misleading statistical results. Korean J Anesthesiol. 2019;72(6):558-69. doi: 10.4097/kja.19087.
42. Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. Journal of the American Dental Association (1939). 2008;139 Suppl:18s-24s. doi: 10.14219/jada.archive.2008.0351.
43. Assy Z, Brand HS, Ligtenberg AJM. [The relationship between xerostomia and saliva secretion in young adults]. Nederlands tijdschrift voor tandheelkunde. 2020;127(10):573-80. doi: 10.5177/ntvt.2020.10.19121.
44. Thomson WM. Measuring change in dry-mouth symptoms over time using the Xerostomia Inventory. Gerodontology. 2007;24(1):30-5. doi: 10.1111/j.1741-2358.2007.00137.x.
45. Thomson WM, Chalmers JM, Spencer AJ, Slade GD. Medication and dry mouth: findings from a cohort study of older people. Journal of public health dentistry. 2000;60(1):12-20. doi: 10.1111/j.1752-7325.2000.tb03286.x.

46. Thomson WM, Williams SM. Further testing of the xerostomia inventory. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2000;89(1):46-50. doi: 10.1016/s1079-2104(00)80013-x.

47. Wiener RC, Wu B, Crout R, Wiener M, Plassman B, Kao E, *et al.* Hyposalivation and xerostomia in dentate older adults. Journal of the American Dental Association (1939). 2010;141(3):279-84. doi: 10.14219/jada.archive.2010.0161.

48. Enoki K, Matsuda KI, Ikebe K, Murai S, Yoshida M, Maeda Y, *et al.* Influence of xerostomia on oral health-related quality of life in the elderly: a 5-year longitudinal study. Oral surgery, oral medicine, oral pathology and oral radiology. 2014;117(6):716-21. doi: 10.1016/j.oooo.2014.03.001.

49. Ramsay SE, Whincup PH, Watt RG, Tsakos G, Papacosta AO, Lennon LT, *et al.* Burden of poor oral health in older age: findings from a population-based study of older British men. BMJ Open. 2015;5(12):e009476. doi: 10.1136/bmjopen-2015-009476.

50. Assy Z, Klop C, Brand HS, Hoogeveen RC, Koolstra JH, Bikker FJ. Determination of intra-oral surface areas by cone-beam computed tomography analysis and their relation with anthrometric measurements of the head. Surg Radiol Anat. 2020;42(9):1063-71. doi: 10.1007/s00276-020-025307.

51. Carpenter GH. The secretion, components, and properties of saliva. Annu Rev Food Sci Technol. 2013;4:267-76. doi: 10.1146/annurev-food-030212-182700.

52. Piludu M, Lantini MS, Cossu M, Piras M, Oppenheim FG, Helmerhorst EJ, *et al.* Salivary histatins in human deep posterior lingual glands (of von Ebner). Archives of oral biology. 2006;51(11):967-73. doi: 10.1016/j.archoralbio.2006.05.011.

53. Holmberg KV, Hoffman MP. Anatomy, biogenesis and regeneration of salivary glands. Monographs in oral science. 2014;24:1-13. doi: 10.1159/000358776.

54. Kessler AT, Bhatt AA. Review of the Major and Minor Salivary Glands, Part 1: Anatomy, Infectious, and Inflammatory Processes. Journal of clinical imaging science. 2018;8:47. doi: 10.4103/jcis.JCIS_45_18.

55. Piludu M, Rayment SA, Liu B, Offner GD, Oppenheim FG, Troxler RF, *et al.* Electron microscopic immunogold localization of salivary mucins MG1 and MG2 in human submandibular and sublingual glands. J Histochem Cytochem. 2003;51(1):69-79. doi: 10.1177/002215540305100109.

56. Rayment SA, Liu B, Offner GD, Oppenheim FG, Troxler RF. Immunoquantification of human salivary mucins MG1 and MG2 in stimulated whole saliva: factors influencing mucin levels. Journal of dental research. 2000;79(10):1765-72. doi: 10.1177/00220345000790100601.

57. Lagerlöf F, Dawes C. The volume of saliva in the mouth before and after swallowing. Journal of dental research. 1984;63(5):618-21. doi: 10.1177/00220345840630050201.

58. Dawes C. A mathematical model of salivary clearance of sugar from the oral cavity. Caries research. 1983;17(4):321-34. doi: 10.1159/000260684.

59. Boyd H, Gonzalez-Martinez JF, Welbourn RJL, Gutfreund P, Klechikov A, Robertsson C, *et al.* A comparison between the structures of reconstituted salivary pellicles and oral mucin (MUC5B) films. J Colloid Interface Sci. 2021;584:660-8. doi: 10.1016/j.jcis.2020.10.124.

60. Eliasson L, Carlén A. An update on minor salivary gland secretions. European journal of oral sciences. 2010;118(5):435-42. doi: 10.1111/j.1600-0722.2010.00766.x.

61. Chong JA, Mohamed A, Pau A. Morphological patterns of the palatal rugae: A review. J Oral Biosci. 2020;62(3):249-59. doi: 10.1016/j.job.2020.06.003.

62. Morzel M, Siying T, Brignot H, Lherminier J. Immunocytological detection of salivary mucins (MUC5B) on the mucosal pellicle lining human epithelial buccal cells. Microsc Res Tech. 2014;77(6):453-7. doi: 10.1002/jemt.22366.

63. Deferm JT, Schreurs R, Baan F, Bruggink R, Merkx MAW, Xi T, *et al.* Validation of 3D documentation of palatal soft tissue shape, color, and irregularity with intraoral scanning. Clin Oral Investig. 2018;22(3):1303-9. doi: 10.1007/s00784-017-2198-8.

INTERVENTIONS TO RELIEVE ORAL DRYNESS

7

The relationship between the severity of oral dryness and the use of dry-mouth interventions by various subgroups of dry-mouth patients

Z. Assy
H. S. Brand
C. P. Bots
F. J. Bikker

Clinical Oral Investigations, 2022 Mar; 26(3):3097–3108.

ABSTRACT

Objective

Dry-mouth patients use different interventions to relieve their oral dryness. As recent studies showed that various subgroups of dry-mouth patients perceived different intra-oral regions as most dry, the present study investigated whether the use of dry-mouth interventions by various subgroups of dry-mouth patients was related to the perceived oral dryness as well as salivary flow rate.

Materials and methods

Xerostomia Inventory (XI) scores, Regional Oral Dryness Score (RODI) scores and dry-mouth interventions used were extracted from the medical records of 528 patients visiting a saliva clinic. Based on their medical history, they were allocated into 6 subgroups.

Results

The subgroups of dry-mouth patients used a wide range of interventions to relieve their oral dryness. Sjögren's syndrome patients used most interventions more frequently than patients with oral dryness due to use of a limited number of medications and controls. Patients using medications showed associations between the total XI score and dry-mouth interventions aimed at the entire mouth. In medication using patients and controls, the locally applied intervention "using mouth gel" was associated with RODI scores of the anterior tongue.

Conclusion

The use of dry-mouth interventions was associated with dry-mouth feelings. Use of interventions aimed to relieve dryness of the entire mouth was significantly associated with total XI score, while locally applied interventions were significantly associated with the severity of dryness at specific intra-oral regions, the anterior tongue in particular.

Clinical relevance

The results will help clinicians to advice dry-mouth patients about the most suitable interventions for relief of oral dryness complaints.

Keywords

Sjögren's syndrome, Dry-mouth, Xerostomia, Xerostomia Inventory, Dry-mouth interventions, Salivary flow rate

INTRODUCTION

Dry-mouth symptoms are usually the result of (poly)pharmacy, but radiotherapy of the head and neck region, systemic diseases such as Sjögren's syndrome, and chronic stress are also associated with dry-mouth complaints [1–4]. Dry-mouth symptoms may also be induced by a combination of these factors [2]. For example, polypharmacy is common in patients with Sjögren's syndrome.

To relieve the dry-mouth complaints, patients apply a broad spectrum of dry-mouth interventions. When residual salivary function is present, secretion can be stimulated by the use of lozenges and chewing gums or prescription of systemic pharmacotherapies, such as pilocarpine or cevimeline [5–7]. Alternatively, electrostimulation of the salivary glands has been reported to increase saliva production [5, 6]. However, when the salivary gland function is irreversibly impaired, only palliative treatment with, *e.g.* saliva substitutes, gels and oral rinses remains for the relief of dry-mouth symptoms.

The Xerostomia Inventory (XI) is a questionnaire to quantify the overall feeling of oral dryness [8]. In contrast, the recently developed Regional Oral Dryness Inventory (RODI) can be applied to determine dry-mouth perception at specific intra-oral locations [9]. Using the RODI, it was found that Sjögren's syndrome patients, patients on polypharmacy and patients treated with radiotherapy differed in their perceived intra-oral regional dryness [10]. In Sjögren's syndrome patients, the posterior palate was the area, which was experienced as most dry, while in polypharmacy patients, the anterior tongue was the most dry area [10]. In those patients treated with radiotherapy, all intra-oral regions were experienced as dry.

We hypothesized that the choice for specific dry-mouth interventions by various subgroups of dry-mouth patients might be related to intra-oral regional differences in dry mouth perception. Therefore, we investigated the use of dry mouth interventions by subgroups of patients with different causes of oral dryness and explored the possible relation of the applied interventions with intra-oral dryness and salivary flow rate.

MATERIALS AND METHODS

This study was approved by the Ethics Review Committee at the Academic Centre for Dentistry Amsterdam (ACTA, protocol number 201951). Data for this retrospective case series study were collected from patients who visited the

saliva clinic of the Dutch Institute for Salivary Research in Bunschoten, the Netherlands, from October 2012 to April 2019. The following clinical data were retrieved: age, gender, health status, number of medications used, XI scores, RODI scores, unstimulated whole salivary flow rate (UWS), chewing-stimulated whole saliva flow rate (CH-SWS) and citric acid-stimulated whole saliva flow rate (A-SWS). The methods for collection of saliva, the salivary secretion rates and the scores on the questionnaires of the study sample have been described in detail previously [10]. In short, the patients were instructed not to eat, drink, chew gum, brush teeth, use mouthwash or smoke at least 1 h before their visit to the saliva clinic. The UWS was collected by the draining method in a pre-weighed plastic container [11]. Patients were asked to collect unstimulated saliva immediately after an initial swallow, by expectorating into the container as soon as they had collected the saliva in their mouth. During saliva collection, patients were not allowed to swallow. To collect CH-SWS, they were asked to chew a 5 × 5-cm sheet of parafilm (Parafilm M, Pechiney, Chicago, IL, USA) and to expectorate into a pre-weighed plastic container every 30 s. To stimulate A-SWS secretion, a citric acid solution (2% w/v) was applied with cotton buds to the lateral borders of the tongue at 30 s intervals [12]. When the collection period had finished, the plastic containers were reweighted, and the collected volume was determined by subtracting the weight of the container before collection. Salivary flow was calculated by dividing the volume collected (assuming 1 g of saliva equals 1 mL) by the collection time (min). Salivary flow rates were expressed in mL/min [11]. To limit circadian variations, saliva collection took place in all patients between 8:00 and 12:00 A.M. [13].

On the basis of their health status, determined with The European Medical Risk-Related History [14, 15], patients were allocated to subgroups, including patients using limited medications (< 4 different prescription medications; Low Med patients), patients using multiple medications (≥ 4 different prescription medications; High Med patients), patients treated with radiotherapy of head and neck area (RTX patients), patients treated with radiotherapy of head and neck area using multiple medications (RTX + High Med patients), Sjögren's syndrome patients (SS patients), Sjögren's syndrome patients using multiple medications (SS + High Med patients), and controls (none of the conditions listed above; no use of prescription medication). For the current study, the data from the subgroup RTX + High Med patients were not included due to the small number of patients (N = 6).

An additional questionnaire collected data on interventions applied to relieve the feeling of oral dryness [16, 17]. These interventions are presented in Tables 4 and 5. The reported interventions were based on information provided

by the patients upon arrival at the saliva clinic, before examination had taken place. The participants could indicate with yes/no which options they apply for the relief of their dry mouth. With the option "using other interventions", they could report additional interventions applied not included in the questionnaire. Based on a previous study, the dry-mouth interventions were divided into two categories: frequently (> 20%) and less frequently (< 20%) applied interventions [17].

The data were statistically analysed with SPSS, version 26.0 (IBM Corp SPSS statistics, Armonk, NY, USA). The use of dry-mouth interventions by the various patient subgroups has been expressed as percentages. A chi-square test was used to investigate whether there is an overall significant difference in the use of specific dry-mouth interventions between the various patient subgroups, followed by a 2 × 2 chi-square test as post hoc analysis when appropriate. To compensate for multiple testing, Bonferroni corrected p-values have been used during further analysis.

To investigate the association between use of dry-mouth interventions and the total XI, the RODI, the flow rates UWS, CH-SWS and A-SWS, a binary logistic regression analysis was performed for each patient subgroup separately. Only the interventions which showed a significant difference during the chi-square tests were further explored with the binary logistic regression. During the binary logistic regression, the dry-mouth interventions were considered a dependent variable and the total XI score, the RODI scores of each the eight different intra-oral regions and the UWS, CH-SWS and A-SWS flow rates were considered as independent variables.

To identify the degree of multicollinearity among the independent variables, the variance inflation factor (VIF) was calculated. The VIF for these variables was < 5, which indicates that there was no multicollinearity present among them [18, 19].

The backward conditional method was used to analyse these independent variables. If there was a significant association between a dry-mouth intervention and one or more independent variables, then the odds ratio (OR) and the 95% confidence interval (95% CI) were reported. Furthermore, the last step of the Omnibus test chi-square including their degree of freedom (df) and their p-values was reported. Also, the Cox & Snell R square and the Nagelkerke R square were mentioned, if the association was significant.
All significance levels (α) were set at 0.05.

RESULTS

A total 528 health records were included. The mean age of participants was 59.6 ± 16.0 years (N = 522), and the majority of the patients were women (68.4%) (N = 525). The European Medical Risk-Related History questionnaire, used to distinguish the different patient subgroups, was completed by 517 patients.

The saliva secretion rates and scores on the inventories have previously been reported in detail [10] (Tables 1, 2 and 3). In summary, controls had significantly lower total XI scores than all other groups (Table 1), indicating that the overall dry-mouth feeling they experienced was the lowest. On the other hand, SS and SS + High Med patients had the highest XI scores, indicating that their overall dry mouth feeling was significantly more severe than the controls, Low Med patients and High Med patients (Table 1). With regard to the salivary flow rates, there was a trend whereby controls and Low Med patients had the highest salivary flow rates for UWS, CH-SWS and A-SWS, while SS and SS + High Med patients had the lowest (Table 1). The RODI scores also differed among the patient subgroups (Tables 2 and 3). In controls and SS patients, the posterior palate was the driest area, while in Low Med and High Med patients, it was the anterior tongue. The region that was experienced as least dry also differed among the patient subgroups. In Low Med, High Med and SS patients, it was the inside cheeks; in controls, it was the floor of the mouth (Tables 2 and 3). In RTX and SS + High Med patients, there were no significant differences among the intra-oral regions. Besides, the RODI scores for all intra-oral regions were investigated among the various patient subgroups; SS and SS + High Med patients had the highest RODI scores for all regions, while controls and Low Med patients had the lowest (Tables 2 and 3). This difference was significant for all eight intra-oral regions. High Med and SS patients differed only significantly with regard to the RODI scores of the posterior palate, with SS patients experiencing more severe dryness of the posterior palate than High Med patients (Tables 2 and 3).

Table 1: Patients were divided into six different patient groups based on their health status. Several patient characteristics for the six groups are shown; age, distribution of women and men in each group, the total number of medications used, the total XI-scores, salivary flow rates (in mL/min), and the salivary pH of UWS, CH-SWS, and A-SWS. The age is presented as mean with standard deviation (SD.) For the number of medications used, the total XI-scores, salivary flow rates, and salivary pH the median with corresponding interquartile range (IQR) is shown. The distribution of women and men is given in percentages. N indicates the total subjects for each patient group.

Patient groups (N=517)	N	Age: mean±SD.*	% of woman: men*	Numberof medication: median±IQR *	Total XI-scores: median±IQR*	UWS: median±IQR*	pH UWS: median±IQR	CH-SWS: median±IQR*	pH CH-SWS: median±IQR	A-SWS: median±IQR*	pH A-SWS: median±IQR*
Controls#	136	50.6±17.7	64.2: 35.8	-	27.0±19.0-34.0	0.22±0.07 -0.36	6.50±6.10-7.00	0.76±0.44 -1.33	7.00±6.70-7.00	1.62±1.01 -2.20	4.70±4.40-5.80
Low Med patients	157	*60.7±14.8[a]*	68.2: 31.8	*2±1-2[a]*	*30.0±22.0 -37.0[a]*	0.17±0.07-0.32	6.50±6.10-7.00	0.72±0.39-1.18	7.00±6.50-7.00	*1.17±0.71-1.80[a]*	4.70±4.00 -6.10
High Med patients	140	*65.9±13.1[a,b]*	65.7: 34.3	*6±4-9[a,b]*	*33.0±24.0-40.0[a,b]*	*0.11±0.04-0.30[a,b]*	6.5±5.80-6.90	*0.57±0.27-1.08[a]*	7.00-6.10-7.00	*0.95±0.44-1.50[a,b]*	*4.40±4.00-5.00[a,b]*
RTX patients	10	58.7±17.9	40.0: 60.0	*1±0-1[a,b,c]*	*37.5±31.0-43.8[a,b]*	0.13±0.05-0.23	6.10±5.65-6.90	0.45±0.29-0.76	7.00±6.40-7.00	*0.52±0.24-0.95[a,b]*	4.55±4.15 -5.08
SS patients	46	*61.7±14.0[a]*	*84.8: 15.2[a,b,c,d]*	*2±1-2[a,b,c,d]*	*44.0±37.0-49.8[a,b,c]*	*0.08±0.03-0.16[a,b]*	6.50±6.00-6.80	*0.30±0.05-0.61[a,b,c]*	6.90±6.10-7.00	*0.50±0.23-1.15[a,b,c]*	*4.40±4.00 -5.00[a,b]*
SS + High Med patients	22	*62.1±9.1[a]*	*95.5: 4.5[a,b,c,d]*	*7±5-9[a,b,d,e]*	*46.0±37.5-49.5[a,b,c,d]*	*0.06±0.03-0.16[a,b]*	6.50±5.50-7.00	*0.28±0.14-0.56[a,b,c]*	7.00±6.50-7.00	*0.48±0.23-1.35[a,b]*	*4.40±4.00-4.70[a,b]*

 # controls: subjects visiting a saliva clinic who did not use prescription medication, were not treated with radiotherapy in head and/or neck region and were not diagnosed with Sjögren's syndrome.

** Indicates significant differences between the six patient groups, Kruskal Wallis test p<0.01.*

Mann-Whitney U test: [a] p<0.05 vs. controls, [b] p<0.05 vs. Low Med patients, [c] p<0.05 vs. High Med patients, [d] p<0.05 vs. RTX patients, and [e] p<0.05 vs. SS patients.

Reprinted with permission from Clinical *Oral Investigations 2021;25:4031–4043*

Table 2: Perceived oral dryness in four different intra-oral regions of the upper jaw as determined with the Regional Oral Dryness Inventory (RODI) in six different patient groups. Data are presented as median with corresponding interquartile range (IQR) and as a mean with standard deviation (SD.). N indicates the total number of subjects per intra-oral region.

Patient groups	Upper lip: mean±SD. (N)*	Upper lip: median±IQR	Anterior palate: mean±SD. (N)*	Anterior palate: median±IQR	Posterior palate: mean±SD. (N)*	Posterior palate: median±IQR	Inside cheeks: mean±SD. (N)*	Inside cheeks: median±IQR
Controls# (N=136)	2.40±1.31 (N=113)	2.00±1.00-3.00	2.34±1.25 (N=114)	2.00±1.00-3.00	2.64±1.23 (N=115)	3.00±1.00-4.00	2.05±1.25 (N=113)	1.00±1.00-3.00
Low Med patients (N=157)	2.68±1.21 (N=134)	3.00±2.00-4.00	*2.74±1.26 (N=131)[a]*	3.00±2.00-4.00	2.81±1.32 (N=135)	3.00±1.00-4.00	2.28±1.30 (N=130)	2.00±1.00-3.00
High Med patients (N=140)	*3.08±1.28 (N=121)[a,b]*	3.00±2.00-4.00	*3.23±1.35 (N=119)[a,b]*	4.00±2.00-4.00	3.23±1.32 *(N=122)[a,b]*	4.00±2.00-4.00	*2.78±1.34 (N=121)[a,b]*	3.00±2.00-4.00
RTX patients (N=10)	3.00±1.05 (N=10)	3.00±2.00-4.00	3.00±1.00 (N=9)	3.00±2.50-4.00	3.30±1.42 (N=10)	4.00±1.75-4.00	*3.30±0.82 (N=10)[a,b]*	3.50±2.75-4.00
SS patients (N=46)	*3.40±0.98 (N=40)[a,b]*	3.00±3.00-4.00	*3.33±1.14 (N=40)[a,b]*	4.00±3.00-4.00	*3.79±0.90 (N=42)[a,b,c]*	4.00±3.00-4.00	*2.98±1.07 (N=40)[a,b]*	3.00±2.00-4.00
SS + High Med patients (N=22)	*3.50±1.04 (N=18)[a,b]*	4.00±2.75-4.00	*3.72±0.75 (N=18)[a,b]*	4.00±3.00-4.00	*3.84±0.50 (N=19)[a,b]*	4.00±4.00-4.00	*3.42±1.07 (N=19)[a,b,c]*	4.00±3.00-4.00

 # controls: subjects visiting a saliva clinic who did not use prescription medication, were not treated with radiotherapy in head and/or neck region and were not diagnosed with Sjögren's syndrome.

** Indicates significant differences between the six patient groups; Kruskal Wallis test p<0.01.*

Mann-Whitney U test: [a] p<0.05 vs. controls, [b] p<0.05 vs. Low Med patients, [c] p<0.05 vs. High Med patients, [d] p<0.05 vs. RTX patients, and [e] p<0.05 vs. SS patients.

Reprinted with permission from Clinical Oral Investigations 2021;25:4031–4043

Table 3: Perceived oral dryness in four different intra-oral regions of the lower jaw as determined with the Regional Oral Dryness Inventory (RODI) in six different patient groups. Data are presented as median with corresponding interquartile range (IQR) and as a mean with standard deviation (SD.). N indicates the total number of subjects per intra-oral region.

Patient groups	**Lower lip: mean±SD. (N)***	**Lower lip: median±IQR**	**Anterior tongue: mean±SD. (N)***	**Anterior tongue: median±IQR**	**Posterior tongue: mean±SD. (N)***	**Posterior tongue: median±IQR**	**Floor mouth: mean±SD. (N)***	**Floor mouth: median±IQR**
Controls# (N=136)	2.38±1.27 (N=111)	2.00±1.00-3.00	2.48±1.33 (N=112)	2.50±1.00-4.00	2.57±1.34 (N=113)	3.00±1.00-4.00	2.01±1.20 (N=112)	2.00±1.00-3.00
Low Med patients (N=157)	*2.70±1.28 (N=135)[a]*	3.00-1.00-4.00	*2.83±1.29 (N=132)[a]*	3.00±2.00-4.00	2.79±1.34 (N=134)	3.00±1.00-4.00	*2.42±1.31 (N=132)[a]*	2.00±1.00-4.00
High Med patients (N=140)	*3.05±1.27 (N=120)[a,b]*	3.00±2.00-4.00	*3.25±1.29 (N=118)[a,b]*	3.00±2.75-4.00	*3.24±1.30 (N=120)[a,b]*	3.00±2.00-4.00	*2.91±1.35 (N=118)[a,b]*	3.00±2.00-4.00
RTX patients (N=10)	3.10±1.10 (N=10)	3.50±2.00-4.00	3.00±1.41 (N=10)	4.00±1.00-4.00	3.10±1.37 (N=10)	3.50±1.75-4.00	2.70±1.16 (N=10)	3.00±1.75-4.00
SS patients (N=46)	*3.40±1.03 (N=40)[a,b]*	3.00±3.00-4.00	*3.41±1.14 (N=41)[a,b]*	4.00±3.00-4.00	*3.43±1.21 (N=42)[a,b]*	4.00±3.00-4.00	*3.07±1.27 (N=41)[a,b]*	3.00±2.00-4.00
SS + High Med patients (N=22)	*3.53±1.12 (N=19)[a,b]*	4.00±3.00-4.00	*3.72±1.02 (N=18)[a,b]*	4.00±3.00-4.00	*4.00±0.67 (N=19)[a,b,c]*	4.00±4.00-4.00	*3.68±0.95 (N=19)[a,b,c,d]*	4.00±3.00-4.00

 # controls: subjects visiting a saliva clinic who did not use prescription medication, were not treated with radiotherapy in head and/or neck region and were not diagnosed with Sjögren's syndrome.

** Indicates significant differences between the six patient groups, Kruskal Wallis test p<0.01.*

Mann-Whitney U test: [a] p<0.05 vs. controls, [b] p<0.05 vs. Low Med patients, [c] p<0.05 vs. High Med patients, [d] p<0.05 vs. RTX patients, and [e] p<0.05 vs. SS patients.

Reprinted with permission from Clinical Oral Investigations 2021;25:4031–4043

Dry-mouth intervention strategies in the total samle

Tables 4 and 5 show the dry-mouth interventions that were applied by the total study sample. Most of the patients (87.9%) reported the use of multiple interventions (≥ 2) to relieve their dry-mouth complaints. Only 3.2% reported use of a single intervention and 8.9% of the patients did not use any intervention. Obviously, "drinking water" was the most frequently used intervention by all patients, followed by "moistening the lips" and "rinsing the mouth". Less frequently used interventions were "using pilocarpine" and "using acupuncture". The spontaneously reported "other interventions" included "oil pulling", "using fluoride in a fluoride tray", "using specialized toothpaste or mouth washes", "using milk", "using a nasal spray", "changing diet" and "doing yoga, cycling or walking".

Dry-mouth interventions in the various patient subgroups

Table 4 displays the dry-mouth interventions frequently used for each of the patient subgroups. The interventions "drinking tea", "sucking candies", "chewing gum" and "focusing on other activities" did not differ significantly among the various patients. On the other hand, SS and SS + High Med patients used all other interventions significantly more often than the controls or Low Med patients. High Med patients used interventions, such as "rinsing the mouth", "eating fruit", "drinking small volume" and "drinking coffee", significantly more than controls.

No significant differences were observed between Low and High Med patients, except for "drinking small volumes" to relieve oral dryness. Also, SS, SS + High Med and High Med patients did not show any significant difference in the percentage of used dry-mouth interventions, except for "moistening the lip", which was more often used by SS and SS + High Med patients. The use of interventions by RTX patients did not differ significantly from all other patient subgroups.

For the less frequently used interventions (Table 5), the patient subgroups only differed in "no intervention"; however, for "no intervention", there were no significant differences among the subgroups after Bonferroni correction.

Table 4: Frequently (>20%) used interventions to relieve dry-mouth symptoms, stratified for the six patient subgroups. Data are expressed as percentages. N indicates the total number of respondents in each patient subgroup.

Patients	Drinking water* (%)	Moistening the lips** (%)	Rinsing of the mouth** (%)	Drinking tea (%)	Sucking candies (%)	Chewing gum (%)	Eating fruit* (%)	Drinking small volumes** (%)	Drinking coffee** (%)	Using mouth gel** (%)	Focusing on other activities (%)
Controls[#] (N=136)	79.2	45.4	43.1	42.3	33.8	36.9	24.6	20.8	19.2	18.5	22.3
Low Med patients (N=157)	78.8	55.0	50.3	47.7	47.0	43.0	31.8	25.2	35.1[a]	15.9	19.9
High Med patients (N=140)	84.8	62.1	66.7[a]	52.3	47.7	38.6	42.4[a]	41.7[a,b]	40.2[a]	29.5	25.0
RTX patients (N=10)	90.0	60.0	60.0	60.0	40.0	50.0	30.0	60.0	20.0	30.0	0.0
SS patients (N=46)	100.0[a,b]	81.8[a,b]	63.6	56.8	43.2	47.7	31.8	56.8[a,b]	38.6	45.5[a,b]	22.7
SS + High Med patients (N=22)	86.4	90.9[a,b,c]	63.6	68.2	40.9	36.4	54.5	59.1[a,b]	27.3	27.3	27.3
Total study population (N=517)	83.2	58.1	54.7	49.4	42.9	40.7	33.4	33.2	32.2	24.3	22.1

 # controls: subjects visiting a saliva clinic who did not use prescription medication, were not treated with radiotherapy in head and/or neck region and were not diagnosed with Sjögren's syndrome.
*Overall Chi-square test among the six patient subgroups: * p<0.05*
*Overall Chi-square test among the six patient subgroups: ** p<0.01*
Post-hoc 2X2 Chi-square test: a Bonferroni-corrected p< 0.05 vs controls, b Bonferroni-corrected p< 0.05 vs Low Med patients, c Bonferroni-corrected p< 0.05 vs High Med patients, d Bonferroni-corrected p< 0.05 vs RTX patients, e Bonferroni-corrected p< 0.05 vs SS patients.

Table 5: Less frequently (<20%) used interventions to relieve dry-mouth symptoms, stratified for the six patient subgroups. Data are expressed as percentages. N indicates the total number of respondents in each patient subgroup.

Patients	Using mouth spray (%)	Using other interventions (%)	Drinking soft drinks (%)	Avoiding talking (%)	Drinking lemonade (%)	No intervention* (%)	Drinking beer (%)	Sucking ice cubes (%)	Using acupuncture (%)	Using pilocarpine (%)
Controls# (N=136)	15.4	10.8	11.5	12.3	10.0	31.8	4.6	3.8	4.6	0.8
Low Med patients (N=157)	16.6	17.9	11.9	12.6	9.9	14.6	5.3	4.6	6.0	1.3
High Med patients (N=140)	22.7	20.5	21.2	12.1	12.9	5.3	4.5	3.8	1.5	2.3
RTX patients (N=10)	20.0	20.0	10.0	10.0	20.0	0.0	10.0	0.0	0.0	0.0
SS patients (N=46)	25.0	27.3	9.1	15.9	9.1	2.3	4.5	2.3	2.3	6.8
SS + High Med patients (N=22)	4.5	13.6	4.5	18.2	22.7	0.0	0.0	4.5	0.0	9.1
Total study population (N=517)	18.4	17.8	13.6	13.0	11.5	8.9	4.9	4.0	3.6	2.4

 # controls: subjects visiting a saliva clinic who did not use prescription medication, were not treated with radiotherapy in head and/or neck region and were not diagnosed with Sjögren's syndrome.
*Overall Chi-square test among the six patient subgroups: * $p<0.05$*

The association between the use of dry-mouth interventions, subjective oral dryness and salivary flow rates

In Table 6, the significant associations between the dry-mouth interventions and the independent variables were reported for four patient subgroups. Due to the small numbers, it was not possible to perform a binary logistic regression for the RTX, SS + High Med and RTX + High Med patient subgroups. For SS patients, none of the independent variables was significantly associated with any intervention.

The UWS flow rate of all patient subgroups did not have an significant association with any intervention, while the CH-SWS or A-SWS flow rates had significant associations with "drinking water", "rinsing of the mouth" or "drinking coffee". For Low Med patients, the CH-SWS or A-SWS flow rate was significant associated with more than one intervention, while for controls and High Med patients, the SWS flow rate was associated with only one intervention: "drinking coffeetab" and "drinking water", respectively. All the associations between salivary flow rates and these interventions had an odds ratio of < 1, except for the controls, indicating that patients with a low CH-SWS or a low A-SWS flow rate are more likely to use these interventions. As controls had an odds ratio > 1 (OR = 5.16) for "drinking coffee" and the salivary flow rate of CH-SWS, this indicates that controls with a low CH-SWS flow rate are less likely to drink coffee.

For the total XI scores, Low Med and High Med patients showed an significant association between XI and most of the interventions, except for "drinking coffee", "drinking small volumes" (Low Med) and "using mouth gel" (High Med). The odds ratio between XI and most of the interventions was > 1, indicating that patients with more severe xerostomia (higher XI scores) are more likely to use these interventions. On the other hand, controls had significant associations between the total XI scores and the interventions "moistening the lips", "drinking small volumes" and "drinking coffee" only. The odds ratios for these associations were also > 1.

As for the RODI score, all eight intra-oral regions had a significant association with 1 or more dry-mouth interventions. An odds ratio of > 1 indicates that patients with a drier intra-oral region (higher RODI score) are more likely to use a specific intervention, while an odds ratio of < 1 indicates patients with a less dry intra-oral region (lower RODI scores) are more likely to use a specific intervention. The anterior tongue and the upper lip had the most significant associations with several interventions. For controls, Low Med and High Med patients, the RODI scores of the anterior tongue were significantly associated with "using a mouth gel". RODI scores of the upper lip in the three patient subgroups were significantly associated with "drinking water", "moistening

Table 6: The odds ratios of the significant independent variables (RODI-scores, total XI-scores, flow rates of UWS, CH-SWS or A-SWS) of the frequently used interventions with significant difference among the patient subgroups. The odds ratio including the 95% CI is reported. For the significant associations also the last step of the Omnibus test Chi-square including the df and p-values were reported. Furthermore, the Coxx & Snell and the Nagelkerke R square were mentioned.

Patient subgroups	Drinking water	Moistening the lips	Rinsing of the mouth	Eating fruit	Drinking small volumes	Drinking coffee	Using mouth gel
Controls#	PP*: 2.62 (1.24-5.55)[a] AP*: 3.37 (1.17-9.71)[a] FoM*: 0.41 (0.17-0.95)[a]	Total XI**: 1.13 (1.05-1.22)[d]	IC*: 1.74 (1.07-2.82)[g] FoM*: 1.87 (1.09-3.18)[g]	AT*: 1.59 (1.10-2.32)[j]	Total XI**: 1.12 (1.04-1.21)[m] UL*: 3.57 (1.19-10.74)[m] LL*: 0.21 (0.06-0.70)[m]	Total XI**: 1.12 (1.03-1.22)[o] AP*: 0.50 (0.26-0.97)[o] CH-SWS*: 5.16 (1.38-19.33)[o]	AT*: 1.84 (1.15-2.92)[r]
Low Med patients	Total XI*: 1.09 (1.01-1.19)[b] PT*: 2.12 (1.12-4.01)[b] CH-SWS*: 0.19 (0.05-0.82)[b]	Total XI*: 1.07 (1.01-1.13)[e] UL**: 1.97 (1.30-2.98)[e]	Total XI*: 1.05 (1.00-1.10)[h] A-SWS*: 0.56 (0.34-0.91)[h]	Total XI*: 1.09 (1.02-1.17)[k] LL*: 0.37 (0.16-0.84)[k] AT**: 2.17 (1.24-3.81)[k]	NS	PT*: 1.66 (1.10-2.50)[p] FoM*: 1.92 (1.14-3.25)[p] A-SWS*: 0.58 (0.34-0.98)[p]	Total XI**: 1.13 (1.04-1.23)[s] AT*: 2.18 (1.15-4.11)[s]
High Med patients	Total XI**: 1.21 (1.06-1.39)[c] UL*: 0.44 (0.20-0.99)[c] A-SWS**: 0.10 (0.02-0.46)[c]	Total XI**: 1.14 (1.05-1.23)[f] UL*: 1.96 (1.13-3.39)[f] IC*: 0.42 (0.20-0.92)[f]	Total XI**: 1.08 (1.02-1.13)[i]	Total XI*: 1.06 (1.01-1.11)[l]	Total XI*: 1.06 (1.01-1.12)[n]	AP*: 1.49 (1.02-2.16)[q]	UL**: 2.54 (1.29-4.99)[t] AP*: 0.39 (0.18-0.84)[t] AT**: 4.24 (1.89-9.52)[t]
SS patients	NS	NS	NS	NS	NS	NS	NS

 # controls: subjects visiting a saliva clinic who did not use prescription medication, were not treated with radiotherapy in head and/or neck region and were not diagnosed with Sjögren's syndrome.

NS: none of the independent variables was significant, PP: posterior palate of the RODI, AP: anterior palate of the RODI, FoM: floor of mouth of the RODI, IC: inside cheeks of the RODI, PT: posterior tongue of the RODI, AT: anterior tongue of the RODI, UL: upper lip of the RODI, LL: lower lip of the RODI.

*Binary logistic regression: * p<0.05*
*Binary logistic regression: ** p<0.01*
[a] *Omnibus test χ^2 =29.6, df=5, p<0.01; Cox & Snell R2=0.29; Nagelkerke R2=0.44*
[b] *Omnibus test χ^2 =37.4, df=4, p<0.01; Cox & Snell R2=0.29; Nagelkerke R2=0.47*
[c] *Omnibus test χ^2 =20.8, df=4, p<0.01; Cox & Snell R2=0.22; Nagelkerke R2=0.44*
[d] *Omnibus test χ^2 =24.4, df=3, p<0.01; Cox & Snell R2=0.25; Nagelkerke R2=0.33*
[e] *Omnibus test χ^2 =28.1, df=3, p<0.01; Cox & Snell R2=0.23; Nagelkerke R2=0.30*
[f] *Omnibus test χ^2 =31.9, df=4, p<0.01; Cox & Snell R^2=0.32; Nagelkerke R^2=0.43*
[g] *Omnibus test χ^2 =24.4, df=2, p<0.01; Cox & Snell R^2=0.24; Nagelkerke R^2=0.33*
[h] *Omnibus test χ^2 =20.4, df=3, p<0.01; Cox & Snell R^2=0.17; Nagelkerke R^2=0.23*
[i] *Omnibus test χ^2 =9.4, df=1, p<0.01; Cox & Snell R^2=0.11; Nagelkerke R^2=0.15*
[j] *Omnibus test χ^2 =6.3, df=1, p<0.05; Cox & Snell R^2=0.07; Nagelkerke R^2=0.10*
[k] *Omnibus test χ^2 =26.2, df=6, p<0.01; Cox & Snell R^2=0.21; Nagelkerke R^2=0.29*
[l] *Omnibus test χ^2 =6.0, df=1, p<0.05; Cox & Snell R^2=0.07; Nagelkerke R^2=0.09*
[m] *Omnibus test χ^2 =20.5, df=3, p<0.01; Cox & Snell R^2=0.21; Nagelkerke R^2=0.34*
[n] *Omnibus test χ^2 =16.1, df=2, p<0.01; Cox & Snell R^2=0.17; Nagelkerke R^2=0.23*
[o] *Omnibus test χ^2 =14.0, df=4, p<0.01; Cox & Snell R^2=0.15; Nagelkerke R^2=0.25*
[p] *Omnibus test χ^2 =26.0, df=4, p<0.01; Cox & Snell R^2=0.21; Nagelkerke R^2=0.29*
[q] *Omnibus test χ^2 =8.0, df=2, p<0.05; Cox & Snell R^2=0.09; Nagelkerke R^2=0.12*
[r] *Omnibus test χ^2 =7.3, df=1, p<0.01; Cox & Snell R^2=0.08; Nagelkerke R^2=0.14*
[s] *Omnibus test χ^2 =21.1, df=3, p<0.01; Cox & Snell R^2=0.17; Nagelkerke R^2=0.30*
[t] *Omnibus test χ^2 =35.3, df=4, p<0.01; Cox & Snell R^2=0.34; Nagelkerke R^2=0.48*

For controls and Low Med patients, the RODI score of the anterior tongue was significantly associated with "eating fruit", while the the lips", "drinking small volumes" and "using a mouth gel". Also, the RODI score of the anterior palate had several significant associations for various interventions. These associations were found in controls and High Med patients for the interventions "drinking water", "drinking coffee" and "using a mouth gel". The RODI scores of the floor of mouth were significantly associated with "drinking water" and "rinsing of the mouth" in controls. The RODI scores of the inside cheeks had a significant association with "moistening the lips" in High Med patients and "rinsing of the mouth" in controls. Finally, the lower lip was significantly associated with "eating fruit" in Low Med patients and "drinking small volumes" in controls.

DISCUSSION

Despite the wide variety of dry-mouth interventions, efficient and satisfying treatment of oral dryness still seems to be lacking [20]. Therefore, in our view, understanding the choice for dry-mouth interventions might contribute to a more tailored advice contributing to improved dry-mouth treatments. The use of dry-mouth interventions has been investigated in different groups of dry-mouth patients; some studies included various dry-mouth patients without differentiating between patients, while others included only a specific group of dry-mouth patients, such as Sjögren's syndrome [5–7, 21, 22]. To the best of our knowledge, no studies have compared the use of interventions by various dry-mouth patient subgroups differentiated on the aetiology of oral dryness.

The present study explored the use of dry-mouth interventions in various subgroups of dry-mouth patients and the potential associations with dry-mouth feelings and salivary flow rates. The dry-mouth patients used a wide variety of interventions to relieve their oral dryness. The interventions "drinking water", "moistening the lips" and "rinsing of the mouth" were the most frequently used. There was a significant difference between the subgroups of patients in the chosen interventions to relieve their dry mouth. In general, SS (+ High Med) patients used more interventions than Low Med patients and controls. Moreover, some patient subgroups showed associations between the XI score and/or the RODI score and the use of specific dry-mouth interventions.

Some interventions were frequently used, which might be related to the fact that they are easily accessible and devoid of negative side-effects. For example, drinking water, tea or coffee and sugar-free chewing gum are easy and safe to use. In turn, it can be anticipated that the interventions "drinking

soft drinks or beer", "sucking ice cubes" and "acupuncture" could be used less frequently due to the fact that they were less convenient or less easy to use. The cost of interventions could also influence the choice. For example, the costs of acupuncture are around $50–70 per session [23], while the effectiveness is questionable [5, 24, 25]. Side-effects, such as nausea, sweating or headache, have frequently been reported for individuals taking pilocarpine, which will negatively affect the number of patients choosing this intervention [5]. Application of saliva substitutes, such as mouth sprays and gels, provides a moisture-retaining coating over the mouth. However, these products need to be applied frequently during the day and also the flavour is often experienced as unpleasant [7, 20]. Also, reimbursement of specific interventions for a particular patient group may also have affected their use.

"Drinking water", "moistening the lips" and "rinsing of the mouth" were the most frequently used interventions by all patient subgroups. Apparently, these interventions seem more popular than using chewing gum or using salivary substitutes. Obviously, drinking water can only temporarily relieve the sensation of a dry mouth [26, 27], because the viscosity of water does not change with increasing shear like saliva [28]. Besides, the moistening and lubrication effect of water is limited, due to limited surface retention and evaporation by the absence of salivary MUC5B [29].

Since irradiation in the head and neck region can damage the salivary glands [1, 2], it was expected that RTX would use chewing gum to relieve dry-mouth complaints less frequently than patients with oral dryness due to medication, where mechanical stimulation of the saliva secretion is possible. Surprisingly, there was no significant difference between these patient subgroups for "chewing gum". The SS subgroup where progressive immune-mediated self-destruction of the salivary glands occurs also did not differ in the use of chewing gum than High and Low Med subgroups (Table 4). This might be related to the severe overall dry-mouth experience in RTX patients and SS patients, as indicated by their extreme high XI scores and RODI scores [10]. However, in the early stages of Sjögren's syndrome, some improvements can be obtained in salivary secretion by gustatory or pharmacological stimulation [30].

Several studies have reported that the effectiveness of mouth gel for the relief of dry-mouth seems to be limited as their use did not result in a satisfactory alleviation of dry-mouth complaints [6, 7, 22]. However, in the current study, a substantial number of patients used this product (ranging between 27.3 and 45.5%). In particular, SS, RTX and High Med patients had the highest percentages for the use of mouth gels. It is possible that these patient

subgroups use mouth gels to improve other oral problems such as a burning mouth, and difficulty with mastication and swallowing [31].

The use of dry-mouth interventions was significantly associated with the total XI score and the flow rate of CHSWS and A-SWS. Besides, the use of one or more dry-mouth interventions was significantly associated with all eight intra-oral regions of the RODI questionnaire. UWS was not associated with any dry-mouth intervention. In several groups of patients, it has been shown that UWS flow rate does not have a strong correlation with the severity of their xerostomia [32–34]. It was also found that the RODI scores and salivary flow rates were not correlated [9]. This suggests that advice to a patient for use of dry-mouth interventions should be based on the severity of their oral dryness complaints experienced, not on the UWS salivary flow rate. Nevertheless, health care professionals should still consider the UWS flow rate important, as a severely low secretion rate is a major risk factor for the development of oral health problems like caries, oral discomfort, taste alterations and candidiasis [1, 2, 29].

In almost all patients, the total XI score was strongly associated with the use of almost all dry-mouth interventions. As discussed earlier, the overall dry-mouth experience is an important determining factor for the use of interventions. As almost all these interventions are aimed to relieve dryness of the entire mouth, like the interventions "drinking water", "rinsing of the mouth", "eating fruit" and "drinks small volumes", they have significant associations with the total XI score which represents the overall dry-mouth feeling.

On the other hand, specific intra-oral regions of the RODI had an significant association with locally applied interventions. Surprisingly, "using mouth gel" was significantly associated with the RODI scores of the anterior tongue for all patient subgroups. It seems that, the tongue plays an important role in dry-mouth perception [17], and mouth gels can be easily applied to this intra-oral region. "Moistening the lips" was significant associated with the RODI scores of the upper lip. In this anatomical area, an intervention can easily be applied as well.

In summary, the use of dry-mouth interventions was significantly associated with the dry-mouth experience of patients, especially the overall (total XI score) and intra-oral (RODI scores) dry-mouth feeling. Interventions aimed to relieve dryness of the entire mouth, such as "drinking water" and "rinsing of the mouth", were significantly associated with total XI score, while locally applied interventions (for example "using mouth gel") were significant associated with dryness of anterior tongue. This finding will help clinicians in advising dry-mouth patients depending on dryness of intra-oral region(s) in

combination with the severity of their overall mouth dryness. Dryness of the mouth is associated with dryness of other regions of the body, such as the nose or eyes [35]. Therefore, it seems interesting to investigate which therapies people apply for dryness of these body parts, and to explore their relationship with the interventions against oral dryness.

Surprisingly, no significant associations were found for SS in relation to any intervention. This outcome is contrary to a previous study which revealed in SS patients that dryness of the inside cheeks was significantly associated with the use of mouth gels, while dryness of posterior palate, anterior and posterior tongue was associated with drinking water, rinsing the mouth or drinking small volumes [17]. This discrepancy is probably attributed to the difference in included subjects in both studies. In the previous study, 87 SS patients were included, while in the present study, a much smaller number of SS patients was included, negatively affecting the statistical power of the analysis.

A possible limitation of the present study is that the control subjects who visited the saliva clinic were a rather heterogeneous group. The reasons why they were referred to the clinic were diverse, varying from an inexplicably high caries incidence, having a metal taste to dry-mouth complaints which were not due to Sjögren's syndrome, radiotherapy or medication. It is possible that their dry-mouth symptoms might be due to other reasons like (chronic) stress and depression, which can significantly reduce the salivary flow rate and cause xerostomia [36]. However, the median flow rate of the control subjects for unstimulated saliva was 0.22 mL/min, which is in the normal range [37]. Their mean dry-mouth experience (XI score) was 27.0, which was the lowest than other patient subgroups. This suggests that the possible contribution of dry-mouth patients to the control groups might be limited.

Another potential limitation of the present study is that no data were available about the frequency of use, which may differ between different dry-mouth interventions. To exemplify, it is possible that Sjögren's syndrome patients drank water multiple times a day, while they moistened their lips only once or twice a day. Knowledge of the frequency of use can contribute to tailored advice for dry-mouth patients in even more detail. Also, no data on the perceived effectiveness of the dry-mouth interventions were available. Therefore, future studies should include these parameters, for example by asking the patients to register the use of interventions in a diary for some time and to rate the effectiveness on their oral dryness using a Likert scale. In future research, it is important to investigate the frequency and efficacy of dry-mouth interventions in detail.

Unfortunately, the duration of the dry-mouth complaints was not available. In this light, it cannot be excluded that, for example patients who suffered for a relative long period from dry mouth, used different types of interventions than patients with more recent complaints. Besides, the included patients were allocated to the various dry-mouth patient groups based on their self-reported answers to the European Medical Risk-Related History questionnaire [14, 14]. Despite the high validity (sensitivity of 88–92% and specificity of 98–99%) of the European Medical Risk-Related History questionnaire [38, 39], this questionnaire does not provide information about the criteria that have been used for the diagnosis Sjögren's syndrome nor information about the cumulative dose of radiotherapy received. Obtaining this information from the attending physician in future studies might improve the allocation of patients to different subgroups and thereby improve the validity of the data for each group of patients.

MAIN CONCLUSIONS

Various dry-mouth patients used a wide range of interventions to relieve their oral dryness. The use of these dry-mouth interventions was significantly associated with the overall (total XI score) and intra-oral (RODI scores) dry-mouth feeling. The use of interventions aimed to relieve dryness of the entire mouth, such as "drinking water" and "rinsing of the mouth", was significantly associated with total XI score, while locally applied interventions, for example "using mouth gel", were significantly associated with dryness of anterior tongue in particular.

These findings might help clinicians to give more specific and patient-tailored advice about interventions for the relief of oral dryness complaints.

REFERENCES

1. Saleh J, Figueiredo MA, Cherubini K, Salum FG (2015) Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol 60(2):242–255. https://doi.org/10.1016/j.archoralbio.2014.10.004
2. Ying Joanna ND, Thomson WM (2015) Dry mouth - an overview. Singapore Dent J 36:12–17. https://doi.org/10.1016/j.sdj.2014.12.001
3. Porter SR, Scully C, Hegarty AM (2004) An update of the etiology and management of xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 97(1):28–46. https://doi.org/10.1016/j.tripleo.2003.07.010
4. Tanasiewicz M, Hildebrandt T, Obersztyn I (2016) Xerostomia of various etiologies: a review of the literature. Adv Clin Exp Med 25(1):199–206. https://doi.org/10.17219/acem/29375
5. Al Hamad A, Lodi G, Porter S, Fedele S, Mercadante V (2019) Interventions for dry mouth and hyposalivation in Sjögren's syndrome: a systematic review and meta-analysis. Oral Dis 25(4):1027–1047. https://doi.org/10.1111/odi.12952
6. Furness S, Bryan G, McMillan R, Worthington HV (2013) Interventions for the management of dry mouth: non-pharmacological interventions. Cochrane Database Syst Rev (8):Cd009603. https://doi.org/10.1002/14651858.CD009603.pub2
7. Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R (2011) Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev (12):Cd008934. https://doi.org/10.1002/14651858.CD008934.pub2
8. Thomson WM, Chalmers JM, Spencer AJ, Williams SM (1999) The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health 16(1):12–17
9. Assy Z, Jager DHJ, Mashhour E, Bikker FJ, Brand HS (2020) Regional differences in perceived oral dryness as determined with a newly developed questionnaire, the Regional Oral Dryness Inventory. Clin Oral Investig. https://doi.org/10.1007/s00784-020-03276-7
10. Assy Z, Bots CP, Arisoy HZ, Gülveren SS, Bikker FJ, Brand HS (2021) Differences in perceived intra-oral dryness in various dry mouth patients as determined using the Regional Oral Dryness Inventory. Clin Oral Investig 25(6):4031–4043. https://doi.org/10.1007/s00784-020-03734-2
11. Navazesh M, Kumar SK (2008) Measuring salivary flow: challenges and opportunities. J Am Dent Assoc 139 Suppl:35s-40s. https://doi.org/10.14219/jada.archive.2008.0353
12. Kalk WW, Vissink A, Spijkervet FK, Bootsma H, Kallenberg CG, Nieuw Amerongen AV (2001) Sialometry and sialochemistry: diagnostic tools for Sjogren's syndrome. Ann Rheum Dis 60(12):1110–1116. https://doi.org/10.1136/ard.60.12.1110
13. Dawes C (1972) Circadian rhythms in human salivary flow rate and composition. J Physiol 220(3):529–545. https://doi.org/10.1113/jphysiol.1972.sp009721
14 Smeets EC, de Jong KJ, Abraham-Inpijn L (1998) Detecting the medically compromised patient in dentistry by means of the medical risk-related history. A survey of 29,424 dental patients in The Netherlands. Prev Med 27(4):530–535. https://doi.org/10.1006/pmed.1998.0285

15. Abraham-Inpijn L, Russell G, Abraham DA, Backman N, Baum E, Bullon-Fernandez P, Declerck D, Fricain JC, Georgelin M, Karlsson KO, Lamey PJ, Link-Tsatsouli I, Rigo O (2008) A patient-administered Medical Risk Related History questionnaire (EMRRH) for use in 10 European countries (multicenter trial). Oral Surg Oral Med Oral Pathol Oral Radiol Endod 105(5):597–605. https://doi.org/10.1016/j.tripleo.2007.09.032
16. Brand HS, Bots CP, Veerman ECI (2011) Therapies for xerostomia in Sjögren's disease are age- and gender-dependent. J Dent Res 90 (Special Issue A):1347
17. Assy Z, Bikker FJ, Picauly O, Brand HS (2021) The association between oral dryness and use of dry-mouth interventions in Sjögren's syndrome patients. Clin Oral Investig. https://doi.org/10.1007/s00784-021-04120-2
18. Marcoulides KM, Raykov T (2019) Evaluation of variance inflation factors in regression models using latent variable modeling methods. Educ Psychol Meas 79(5):874–882. https://doi.org/10.1177/0013164418817803
19. Kim JH (2019) Multicollinearity and misleading statistical results. Korean J Anesthesiol 72(6):558–569. https://doi.org/10.4097/kja.19087
20. Brand HS, Ouzzine R, Bots CP (2013) Sticky saliva products. Br Dent J 214(3):95. https://doi.org/10.1038/sj.bdj.2013.118
21. Gil-Montoya JA, Silvestre FJ, Barrios R, Silvestre-Rangil J (2016) Treatment of xerostomia and hyposalivation in the elderly: a systematic review. Med Oral Patol Oral Cir Bucal 21(3):e355-366. https://doi.org/10.4317/medoral.20969
22. Brito-Zerón P, Retamozo S, Kostov B, Baldini C, Bootsma H, De Vita S, Dörner T, Gottenberg JE, Kruize AA, Mandl T, Ng WF, Seror R, Tzioufas AG, Vitali C, Bowman S, Mariette X, Ramos-Casals M (2019) Efficacy and safety of topical and systemic medications: a systematic literature review informing the EULAR recommendations for the management of Sjögren's syndrome. RMD Open 5(2):e001064. https://doi.org/10.1136/rmdopen-2019-001064
23. Sasportas LS, Hosford DN, Sodini MA, Waters DJ, Zambricki EA, Barral JK, Graves EE, Brinton TJ, Yock PG, Le QT, Sirjani D (2013) Cost-effectiveness landscape analysis of treatments addressing xerostomia in patients receiving head and neck radiation therapy. Oral Surg Oral Med Oral Pathol Oral Radiol 116(1):e37-51. https://doi.org/10.1016/j.oooo.2013.02.017
24. Assy Z, Brand HS (2018) A systematic review of the effects of acupuncture on xerostomia and hyposalivation. BMC Complement Altern Med 18(1):57. https://doi.org/10.1186/s12906-018-2124-x
25. Ni X, Tian T, Chen D, Liu L, Li X, Li F, Liang F, Zhao L (2020) Acupuncture for radiation-induced xerostomia in cancer patients: a systematic review and meta-analysis. Integr Cancer Ther 19:1534735420980825. https://doi.org/10.1177/1534735420980825
26. Jose A, Singh ML, Magnuson B, Farag A, Varghese R, Papas A (2018) A randomized controlled study to evaluate an experimental moisturizing mouthwash formulation in participants experiencing dry mouth symptoms. Oral Surg Oral Med Oral Pathol Oral Radiol 126(3):231-239.e235. https://doi.org/10.1016/j.oooo.2018.05.007
27. Purdie J, Carpenter MD, Noll JJ, Stephens C, Taylor YJ, Napenas JJ, Brennan MT (2021) Xerostomia symptoms and treatment strategies associated with salivary flows. Oral Surg Oral Med Oral Pathol Oral Radiol 131(4):e116. https://doi.org/10.1016/j.oooo.2020.10.047
28. Carpenter GH (2013) The secretion, components, and properties of saliva. Annu Rev Food Sci Technol 4:267–276. https://doi.org/10.1146/annurev-food-030212-182700

29. Roblegg E, Coughran A, Sirjani D (2019) Saliva: an all-rounder of our body. Eur J Pharm Biopharm 142:133–141. https://doi.org/10.1016/j.ejpb.2019.06.016
30. Gravenmade EJ, Vissink A (1992) Management of the oral features of Sjögren's syndrome. Neth J Med 40(3–4):117–124
31. Alves MB, Motta AC, Messina WC, Migliari DA (2004) Saliva substitute in xerostomic patients with primary Sjögren's syndrome: a single-blind trial. Quintessence Int 35(5):392–396
32. Kho HS (2014) Understanding of xerostomia and strategies for the development of artificial saliva. Chin J Dent Res 17(2):75–83
33. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH, Proctor GB (2015) Changes in saliva rheological properties and mucin glycosylation in dry mouth. J Dent Res 94(12):1660–1667. https://doi.org/10.1177/0022034515609070
34. Fox PC, Busch KA, Baum BJ (1987) Subjective reports of xerostomia and objective measures of salivary gland performance. J Am Dent Assoc 115(4):581–584. https://doi.org/10.1016/s0002-8177(87)54012-0
35. Ito K, Takamatsu K, Nohno K, Sugano A, Funayama S, Katsura K, Kaneko N, Ogawa M, Meurman JH, Inoue M (2017) Factors associated with mucosal dryness in multiple regions and skin: a web-based study in women. J Obstet Gynaecol Res 43(5):880–886. https://doi.org/10.1111/jog.13290
36. Gholami N, Hosseini Sabzvari B, Razzaghi A, Salah S (2017) Effect of stress, anxiety and depression on unstimulated salivary flow rate and xerostomia. Journal of dental research, dental clinics, dental prospects 11(4):247–252. https://doi.org/10.15171/joddd.2017.043
37. Humphrey SP, Williamson RT (2001) A review of saliva: normal composition, flow, and function. J Prosthet Dent 85(2):162–169.https://doi.org/10.1067/mpr.2001.113778
38. de Jong KJ, Abraham-Inpijn L, Vinckier F, Declerck D (1997) The validity of a medical risk-related history for dental patients in Belgium. Int Dent J 47(1):16–20. https://doi.org/10.1111/j.1875-595x.1997.tb00672.x
39. de Jong KJ, Borgmeijer-Hoelen A, Abraham-Inpijn L (1991) Validity of a risk-related patient-administered medical questionnaire for dental patients. Oral Surg Oral Med Oral Pathol 72(5):527–533. https://doi.org/10.1016/0030-4220(91)90488-x

8

The association between oral dryness and use of dry-mouth interventions in Sjögren's syndrome patients

Z. Assy
F. J. Bikker
O. Picauly
H. S. Brand

Clinical Oral Investigations, 2022 Feb;26(2):1465-1475.

ABSTRACT

Objective

Sjögren's syndrome patients use different dry-mouth interventions for the relieve of their oral dryness. Recently, it was shown that patients with dry-mouth complaints have regional differences in perceived intra-oral dryness. Therefore, the aim of the present study was to investigate whether the use of dry-mouth interventions is related to the perceived regional oral dryness.

Materials and methods

A cross-sectional study was performed with Sjögren's patients. Volunteers could anonymously administer various questionnaires, including the Regional Oral Dryness Inventory (RODI), Xerostomia Inventory (XI), Bother Index (BI) and a list of dry-mouth interventions.

Results

Sjögren's syndrome patients use a wide variety for the relief of oral dryness. "Drinking water" and "moistening the lips" were used most frequently. Dry-mouth interventions, "drinking water", "rinsing of the mouth", and "drinking small volumes" were associated with the RODI scores of the posterior palate, and anterior and posterior tongue, respectively. On the other hand, "using mouth gel" had an significant association with the RODI scores of the inside cheeks.

Conclusion

Sjögren's syndrome patients are more likely to use mouth gels when their inside cheeks were experienced as most dry, while they drank water, rinsed their mouth or drank small volumes if the posterior palate, and anterior and posterior tongue were considered as dry. It can be concluded that intra-oral dryness affects dry-mouth perception and thereby also the use of the various dry-mouth interventions.

Clinical relevance

The therapeutic choice of dry-mouth interventions by Sjögren's syndrome patients seems to some extent to be related to dryness at specific intra-oral regions.

Keywords:

Sjögren's syndrome, Dry mouth, Xerostomia, Xerostomia Inventory, Bother Index, Dry-mouth interventions

INTRODUCTION

Sjögren's syndrome is an autoimmune disease that affects the exocrine lacrimal and salivary glands [1, 2]. As a result of progressive immune-mediated damage to the salivary glands, Sjögren's syndrome is associated with hyposalivation and xerostomia [1]. Both hyposalivation and xerostomia may induce comorbidities such as difficulty with swallowing, speaking and sleeping. Loss of the protective and antimicrobial properties of saliva may also increase the risk of oral diseases such as dental caries and oral candidiasis [1, 3]. This negatively affects the oral health and the quality of life [1, 4]. In order to relieve their dry mouth complaints, Sjögren's syndrome patients seek for effective care and treatment.

In early stages of Sjögren's syndrome, when residual salivary function is still present, salivary flow can be stimulated, *e.g.* by the use of lozenges and chewing gums. Upon prescription, systemic pharmacotherapies, such as pilocarpine or cevimeline, might be used [4–6]. Alternatively, electrostimulation of the salivary glands and acupuncture have been reported to increase saliva production [4, 5]. However, when the salivary function is irreversibly impaired, only the use of saliva substitutes remains for the relief of oral problems. For this purpose, a wide range of salivary substitutes such as mouth sprays, gels and mouthwashes is available.

Despite the fact that several dry-mouth interventions are available, their effectiveness seems to be limited. Although the use of pilocarpine is associated with a reduction in dry mouth symptoms, the effect size, clinical significance and duration of the effect remain unclear [4]. Furthermore, for cevimeline and electrostimulation, there is limited evidence with respect to increasing the salivary flow in Sjögren's syndrome patients [4]. Besides, adverse events such as nausea, sweating or headache are commonly reported for individuals taking pilocarpine and cevimeline [4]. Additionally, these pharmacotherapies may be contraindicated in patients with comorbidity like chronic respiratory, cardiovascular or renal disease [6]. Taken together, there is no robust evidence that any of the treatments known is fully effective or leads to a widely supported satisfaction to relieve dry mouth complaints [5–7]. As a consequence, therapeutic advice of healthcare professionals to patients with Sjögren's syndrome is difficult and generally based on a combination of dentist's opinion, scientific literature, patients' personal experience and availability of products [4]. The advice is usually related to the overall oral dryness severity. However, we have recently shown that there are important regional differences in perceived intra-oral dryness [8, 9]. Dry-mouth patients experienced the oral

dryness of the posterior palate as most severe, while the floor of the mouth and the inside cheeks were experienced as less dry. Accordingly, the aim of the present study was to investigate possible associations between the use of dry-mouth interventions and the perceived oral dryness, both overall and regional, of Sjögren's syndrome patients. We anticipate that this information will contribute in developing more tailored advice about dry-mouth intervention(s) for Sjögren's syndrome patients.

MATERIALS AND METHOD

Study design

A cross-sectional study was performed among Sjögren's syndrome patients who visited the annual meeting of the Dutch Sjögren Patients Federation on October 5th, 2019 (Dutch: Nederlandse Vereniging van Sjögren Patiënten). Volunteers could anonymously fill in the questionnaire described below and return it in a designated mailbox during the meeting or return the questionnaire by mail using an enclosed prepaid envelope.
The local Ethics Review Committee of the Academic Centre for Dentistry Amsterdam (ACTA) confirmed that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study (protocol number 201930). The reporting of this study conforms to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement [10].

Study variables

The questionnaire, developed for this study, consisted of five parts. First, some general questions with regard to age, sex and year in which Sjögren's syndrome had been diagnosed by a physician.

Second, the Regional Oral Dryness Inventory (RODI) questionnaire was used to determine differences in dry-mouth perception at different intra-oral locations. The RODI questionnaire contains nine schematic illustrations of different locations in the oral cavity [8, 9]. Four illustrations represent areas in the upper jaw: the upper lip, the posterior part of the palate (from the rugae up to the end of the soft palate), the anterior part of the palate (including the rugae) and the inside part of the cheeks. Four other illustrations represent areas in the lower jaw: the lower lip, the anterior part of the tongue (from the tip of the tongue up to the vallate papilla), the posterior part of the tongue (from the vallate papilla up to end of the tongue) and the floor of the mouth. Finally, one illustration represents the pharynx. At each location, the patient can indicate

the severity of the intra-oral dryness on a 5-point Likert scale ranging from 1—"No dryness" to 5—"Severe dryness" [8, 9].

The third part was the Xerostomia Inventory (XI), consisting of 11 items on a 5-point Likert scale ranging from 1—"Never" to 5—"Very often". The items concern patients' oral dryness and mouth feel. Per item, patients indicate how often they experience problems regarding mouth feel and oral dryness. The scores of the 11 items are summed to produce a total XI score that ranges between 11 (no xerostomia) and 55 (extreme xerostomia) [11].

The fourth part consisted of the Bother Index (BI). In the BI, the patient is asked to rate the severity of dry mouth on a scale from 0 to 10 [12–16].

Finally, the questionnaire included a list of potential interventions to relieve the feeling of a dry mouth [17]. These interventions are summed up in Table 2 and divided into two categories: the frequently (> 20%) and less frequently used (< 20%) interventions. The participants could indicate with yes/no which options they apply for the relief of their dry mouth. With the option "using other interventions'', they could report additional interventions applied not listed in the questionnaire. Because some respondents did not answer all items of the questionnaires, the total number for items may differ.

Data analysis

The data were statistically analyzed with SPSS, version 26.0 (IBM Corp SPSS statistics, Armonk, NY, USA). The Shapiro–Wilk test was used to assess the normality of the data. As not all variables were normally distributed, the data are presented as medians and their interquartile range (IQR). To clarify relatively small differences, the mean and standard deviation (SD) are also reported.

A Friedman test was conducted for the RODI scores of the total study sample, followed by a Wilcoxon signed rank test as a post hoc procedure.

The possible relationships between frequently used dry-mouth interventions and the perceived oral dryness (RODI and XI) and patients' discomfort (BI) were investigated initially by using a univariate analysis, using Mann–Whitney U tests. Only the significant interventions found in the univariate analysis were further explored in the multivariate analysis, the binary logistic regression. The dry-mouth interventions were considered as dependent variable and the total XI score, BI-score and RODI scores of the nine intra-oral regions were considered as independent variables. To identify the degree of multicollinearity among the independent variables, the variance inflation factor (VIF) was calculated. The VIF for these variables was < 5, which indicates that there is no multicollinearity present among these variables [18, 19], so they do not influence each other.

The backward conditional method was used to analyze these independent variables. If there was a significant association between a dry-mouth intervention and one or more independent variables, then the odds ratio and the 95% confidence interval (95% CI) were reported. Furthermore, the last step of the Omnibus test chi-square, and the Hosmer and Lemeshow (H–L) test chi-square including their degree of freedom (df) and their p-values were reported. Also, the Cox & Snell R square and the Nagelkerke R square were reported, if the association was significant.

All significance levels (α) were set at 0.05.

RESULTS

At the yearly meeting of the patient federation, 176 questionnaires were distributed. In total, 91 questionnaires were returned, a response rate of 51.7%. Most of the respondents were female (N = 81, 89.0%), while 6 were male and 4 did not indicate their gender. The mean age of the respondents was 64 ± 10 years, with age ranging from 35 to 84 years. Almost all patients (N = 87, 95.6%) reported that they had been diagnosed with Sjögren's syndrome by a physician, while 4 respondents did not answer this question. After excluding these four respondents, the final study sample consisted of 78 females, 6 males and 3 without any indication of their gender.

Removal of these respondents did not affect the mean age.

Perceived oral dryness and patients' oral discomfort

The perceived oral dryness at various intra-oral locations, as determined with the RODI questionnaire, is presented in Table 1. Perceived oral dryness in the total study sample differed significantly among the nine intra-oral regions (Friedman test $p < 0.05$, followed by Wilcoxon signed-rank test). The perceived intra-oral dryness was most severe for the posterior palate and the pharynx. In contrast, the floor of the mouth and the inside of the cheeks were experienced as least dry.

The mean overall XI score was 42.8 ± 8.7, with a median score of 45.0 and IQR of 38.0–48.5 (N = 85). The mean BI score was 7.1 ± 2.4, with a median score of 8.0 and IQR of 6.0–9.0 (N = 87).

Table 1: Perceived oral dryness in nine intra-oral regions as determined with the Regional Oral Dryness Inventory (RODI) in the study population. Data are presented as median with corresponding interquartile range (IQR) and as a mean with standard deviation (SD). N indicates the total number of respondents for each intra-oral region.

Intra-oral regions	Mean	SD	Median	IQR	N
Upper lip	3.25	1.00	3.0	3.0-4.0	85
Inside cheeks	3.13	1.04	3.0	2.0-4.0	86
Anterior palate[b]	3.40	1.04	3.0	3.0-4.0	86
Posterior palate[a,b,c]	3.67	0.99	4.0	3.0-4.0	86
Lower lip[c,d]	3.19	0.98	3.0	3.0-4.0	83
Floor of the mouth[a,c,d]	3.02	1.09	3.0	2.0-4.0	83
Anterior tongue[a,e,f]	3.46	1.10	4.0	3.0-4.0	83
Posterior tongue[a,b,e,f]	3.54	1.00	4.0	3.0-4.0	84
Pharynx[a,b,e,f]	3.61	1.03	4.0	3.0-4.0	83

[a] *Wilcoxon signed-rank tests: $p < 0.05$ vs. upper lip*
[b] *Wilcoxon signed-rank tests: $p < 0.05$ vs. inside cheeks*
[c] *Wilcoxon signed-rank tests: $p < 0.05$ vs. anterior palate*
[d] *Wilcoxon signed-rank tests: $p < 0.05$ vs. posterior palate*
[e] *Wilcoxon signed-rank tests: $p < 0.05$ vs. lower lip*
[f] *Wilcoxon signed-rank tests: $p < 0.05$ vs. floor of the mouth*
[g] *Wilcoxon signed-rank tests: $p < 0.05$ vs. anterior part of the tongue*
[h] *Wilcoxon signed-rank tests: $p < 0.05$ vs. posterior part of the tongue*

Dry-mouth interventions strategies

Most respondents use one or more interventions for the relief of their dry mouth complaints (Table 2). The most frequently used interventions (> 20%) to relieve dry mouth complaints were "drinking water'' and "moistening the lips''. Less frequently used interventions (< 20%) by Sjögren's syndrome patients were "keeping lemon slices in the mouth'' and "putting olive oil in the mouth''. Most reported "using other medications'' that Sjögren's syndrome patients used included the use of Xylimelts®, oral adhering discs that release xylitol and cellulose gum upon use. The spontaneously reported "using other interventions'' included "drinking chocolate milk", "using mouth wash", "using specialized toothpaste" and "using different kind of candies".

Table 2: Frequently (>20%) and less frequently used (<20%) interventions by Sjögren's syndrome patients to relieve dry-mouth symptoms. Data are expressed as percentages.

Frequently used intervention for dry mouth	**%**	**Less frequently used intervention for dry mouth**	**%**
Drinking water	90.5	Focusing on other activities	13.1
Moistening the lips	72.6	Using other interventions	12.2
Drinking tea	60.7	Using other medications	11.9
Rinsing of the mouth	50.0	Using pilocarpine	8.3
Chewing gum	48.8	Drinking lemonade	4.8
Drinking small volumes	48.8	Drinking soft drinks	3.6
Using mouth gel	42.9	Using acupuncture	3.6
Eating fruit	40.5	Drinking beer	3.6
Using mouth spray	27.4	No intervention	3.6
Drinking coffee	25.0	Sucking ice cubes	2.4
Sucking sour candies	23.8	Putting olive oil in the mouth	1.2
		Keeping lemon slices in the mouth	1.2

The association of oral dryness and patients' discomfort with dry-mouth intervention strategies

The association between the perceived oral dryness of Sjögren's syndrome patients and the frequently used intervention strategies (used by more than 20% of the sample) to relieve dry mouth was further explored. In Tables 3 and 4, the associations are presented between the use of these interventions and the perceived dryness at different intra-oral locations (RODI scores). Respondents who rinsed their mouth and those who refrained from rinsing their mouth showed significant differences in RODI scores for all intra-oral regions except for the anterior palate. The RODI scores of patients who use water were higher for all intra-oral regions except for the anterior palate, lower lip and the anterior tongue than the RODI scores of patients who did not drink water. For other dry-mouth interventions, only a few regions showed significant differences between patients who applied an intervention and patients who did not apply that intervention. Interestingly, for "using mouth gel'', significant differences were only observed for the inside cheeks and the anterior palate. Intra-oral dryness was not related to the use of the following dry-mouth interventions: "sucking sour candies'', "chewing gum'', "eating fruit'', "moistening the lips'' and "using mouth spray''.

Table 5 shows the association between the total XI scores and frequently used dry-mouth intervention strategies. Interventions that are associated with

significant higher total XI scores are “rinsing of the mouth”, “drinking water”, “eating fruit” and “using mouth gel”, indicating that the patients who use these interventions suffer from more severe overall dry mouth than patients who refrain from them.

All other interventions did not show any relation with the XI scores.

Table 6 presents the BI-scores of patients who apply frequently used dry-mouth interventions versus patients who do not use these interventions. Only Sjögren’s patients who rinsed their mouth and/or who drank water had significantly higher BI-scores and thereby more dry-mouth discomfort than patients who refrained from these interventions. For all other interventions, there were no significant differences between patients who use a specific intervention or those who refrain from that intervention.

Table 3: The RODI-scores of the upper jaw for Sjögren's syndrome patients who report the use of a specific intervention for the relieve of dry mouth complaints versus patients who do not use that intervention. Data are expressed as mean scores with standard deviation (SD).

Intervention	Use	Upper lip	Inside cheek	Anterior palate	Posterior palate
Drinking water	Yes	3.4 ± 0.9*	3.2 ± 1.0*	3.5 ± 1.0	3.8 ± 0.9**
	No	2.3 ± 1.1	2.1 ± 1.1	2.4 ± 1.5	2.4 ± 1.4
Moistening the lips	Yes	3.4 ± 1.0	3.2 ± 1.0	3.4 ± 1.1	3.7 ± 0.9
	No	3.0 ± 1.1	3.0 ± 1.1	3.4 ± 1.1	3.6 ± 1.1
Drinking tea	Yes	3.2 ± 0.9	3.0 ± 1.0	3.3 ± 1.0*	3.6 ± 0.9
	No	3.3 ± 1.1	3.3 ± 1.1	3.6 ± 1.1	3.7 ± 1.1
Rinsing of the mouth	Yes	3.6 ± 0.9**	3.5 ± 1.0**	3.6 ± 1.0	3.9 ± 0.9**
	No	3.0 ± 1.0	2.8 ± 1.0	3.2 ±1.1	3.4 ± 1.0
Chewing gum	Yes	3.4 ± 1.0	3.2 ± 1.1	3.6 ± 1.0	3.6 ± 0.9
	No	3.2 ± 1.0	3.1 ± 1.0	3.2 ± 1.1	3.7 ± 1.0
Drinking small volumes	Yes	3.4 ± 0.9	3.3 ± 1.0	3.5 ± 0.9	3.9 ± 0.8
	No	3.2 ± 1.1	2.9 ± 1.1	3.3 ± 1.28	3.4 ± 1.1
Using mouth gel	Yes	3.5 ± 0.8	3.5 ± 0.8**	3.7 ± 0.9*	3.8 ± 1.0
	No	3.1 ± 1.1	2.9 ± 1.1	3.2 ± 1.1	3.5 ± 1.0
Eating fruit	Yes	3.4 ± 0.9	3.2 ± 1.0	3.7 ± 1.0	3.9 ± 0.8
	No	3.2 ± 1.1	3.1 ± 1.1	3.2 ± 1.1	3.5 ± 1.1
Using mouth spray	Yes	3.3 ± 0.9	3.3 ± 1.1	3.5 ± 1.0	3.7 ± 1.0
	No	3.3 ± 1.0	3.1 ± 1.1	3.4 ± 1.1	3.7 ± 1.0
Drinking coffee	Yes	3.4 ± 0.9	3.1 ± 0.8	3.6 ± 0.8	3.9 ± 0.8
	No	3.2 ± 1.0	3.1 ± 1.1	3.3 ± 1.1	3.6 ± 1.0
Sucking sour candies	Yes	3.2 ± 0.9	3.1 ± 0.8	3.6 ± 1.1	3.7 ± 0.9
	No	3.3 ± 1.0	3.1 ± 1.1	3.3 ± 1.0	3.6 ± 1.0

** p <0.05 compared to the RODI-score of patients who do not use the intervention, Mann-Whitney U test*
*** p <0.01 compared to the RODI-score of patients who do not use the intervention, Mann-Whitney U test*

Table 4: The RODI-scores of the lower jaw and throat for Sjögren's syndrome patients who report the use of a specific intervention for the relieve of dry-mouth complaints versus patients who do not use that intervention. Data are expressed as mean scores with standard deviation (SD).

Intervention	Use	Lower lip	Floor of the mouth	Anterior tongue	Posterior tongue	Pharynx
Drinking water	Yes	3.3 ± 0.9	3.1 ± 1.1	3.5 ± 1.1	3.6 ± 0.9**	3.7 ± 0.9*
	No	2.6 ± 1.3	2.0 ± 1.2*	2.6 ± 1.3	2.4 ± 1.1	2.4 ± 1.5
Moistening the lips	Yes	3.3 ± 0.9	3.1 ± 1.1	3.5 ± 1.1	3.5 ± 1.0	3.7 ± 1.0
	No	2.9 ± 1.1	2.8 ± 1.2	3.3 ± 1.2	3.6 ± 1.1	3.4 ± 1.1
Drinking tea	Yes	3.2 ± 1.0	3.0 ± 1.1	3.4 ± 1.1	3.6 ± 0.9	3.7 ± 1.0
	No	3.2 ± 1.0	3.1 ± 1.2	3.5 ± 1.2	3.4 ± 1.1	3.6 ± 1.1
Rinsing of the mouth	Yes	3.5 ± 0.9*	3.4 ± 0.9**	3.9 ± 0.8**	3.8 ± 0.9*	3.9 ± 1.0*
	No	3.0 ± 1.1	2.7 ± 1.2	3.0 ± 1.2	3.3 ± 1.1	3.4 ± 1.1
Chewing gum	Yes	3.3 ± 1.1	3.2 ± 1.2	3.6 ± 1.1	3.6 ± 1.0	3.5 ± 1.0
	No	3.1 ± 0.9	2.9 ± 1.0	3.3 ± 1.1	3.5 ± 1.0	3.8 ± 1.1
Drinking small volumes	Yes	3.3 ± 1.0	3.2 ± 1.0	3.7 ± 1.0	3.8 ± 0.9**	3.9 ± 0.8*
	No	3.1 ± 1.0	2.8 ± 1.2	3.2 ± 1.2	3.2 ± 1.0	3.3 ± 1.2
Using mouth gel	Yes	3.5 ± 0.9	3.3 ± 1.0	3.8 ± 1.0	3.8 ± 1.1	3.7 ± 1.1
	No	3.0 ± 1.0	2.8 ± 1.2	3.2 ± 1.2	3.4 ± 0.9	3.5 ±1.0
Eating fruit	Yes	3.3 ± 0.9	3.2 ± 1.0	3.8 ± 0.8	3.7 ± 0.9	3.8 ± 1.0
	No	3.2 ± 1.0	2.9 ± 1.2	3.2 ± 1.3	3.4 ± 1.1	3.5 ± 1.1
Using mouth spray	Yes	3.5 ± 0.8	3.2 ± 1.1	3.7 ± 0.9	3.7 ± 1.0	3.9 ± 1.1
	No	3.1 ± 1.0	2.9 ± 1.1	3.4 ± 1.2	3.5 ± 1.0	3.5 ± 1.0
Drinking coffee	Yes	3.5 ± 0.9	3.5 ± 0.7*	3.6 ± 1.0	3.9 ± 0.8	3.9 ± 1.0
	No	3.1 ± 1.0	2.9 ± 1.2	3.4 ± 1.1	3.4 ± 1.0	3.5 ± 1.1
Sucking sour candies	Yes	3.2 ± 0.8	3.1 ± 0.7	3.5 ± 0.8	3.5 ± 0.8	3.8 ± 0.6
	No	3.2 ± 1.0	3.0 ± 1.2	3.4 ± 1.2	3.5 ± 1.1	3.6 ± 1.1

** p <0.05 compared to the RODI-score of patients who do not use the intervention, Mann-Whitney U test*
*** p <0.01 compared to the RODI-score of patients who do not use the intervention, Mann-Whitney U test*

Table 5: The total XI-scores of Sjögren's syndrome patients who report the use of a specific intervention for the relieve of dry mouth complaints versus patients who do not use that intervention. Data are expressed as mean scores with standard deviation (SD).

Intervention	XI-total of patients who use intervention (mean ± SD)	XI-total of patients who do not use intervention (mean ± SD)
Drinking water	43.7 ± 7.5*	32.9 ± 13.4
Moistening the lips	43.5 ± 8.2	40.5 ± 10.1
Drinking tea	42.4 ± 7.7	43.1 ± 10.3
Rinsing of the mouth	45.9 ± 6.6**	39.5 ± 9.6
Chewing gum	43.7 ± 7.6	41.7 ± 9.7
Drinking small volumes	44.4 ± 6.2	40.9 ± 10.6
Using mouth gel	45.4 ± 6.1*	40.5 ± 9.9
Eating fruit	45.8 ± 6.7**	40.6 ± 9.4
Using mouth spray	44.6 ± 7.3	41.9 ± 9.2
Drinking coffee	44.8 ± 7.3	42.0 ± 9.2
Sucking sour candies	45.7 ± 4.8	41.8 ± 9.5

** p <0.05 compared to XI-total of patients who do not use the intervention, Mann-Whitney U test*
*** p <0.01 compared to XI-total of patients who do not use the intervention, Mann-Whitney U test*

Table 6: The BI-scores of Sjögren's syndrome patients who report the use of a specific intervention for the relieve of dry mouth complaints versus patients who do not use that intervention. Data are expressed as mean scores with standard deviation (SD).

Intervention	BI-score of patients who use intervention (mean ± SD)	BI-score of patients who do not use intervention (mean ± SD)
Drinking water	7.4 ± 2.1**	3.9 ± 3.5
Moistening the lips	7.1 ± 2.5	7.0 ± 2.4
Drinking tea	6.8 ± 2.3	7.5 ± 2.7
Rinsing of the mouth	8.0 ± 1.8**	6.1 ± 2.7
Chewing gum	7.1 ± 2.5	7.0 ± 2.5
Drinking small volumes	7.7 ± 1.8	6.4 ± 2.8
Using mouth gel	7.6 ± 2.1	6.6 ± 2.6
Eating fruit	7.6 ± 1.9	6.7 ± 2.7
Using mouth spray	7.5 ± 1.5	6.9 ± 2.7
Drinking coffee	7.2 ± 1.8	7.0 ± 2.7
Sucking sour candies	7.7 ± 2.3	6.9 ± 2.5

** p <0.05 compared to BI-score of patient who do not use the intervention, Mann-Whitney U test*
*** p <0.01 compared to BI-score of patients who do not use the intervention, Mann-Whitney U test*

Multivariate analysis of the association of oral dryness and patients' discomfort with dry-mouth interventions strategies

In Table 7, the odds ratios for the dry-mouth interventions are reported. Interestingly, general interventions such as "drinking water'', "rinsing the mouth'' and "drinking small volumes'' had significant odds ratios for respectively the RODI scores of the posterior palate, anterior and posterior tongue areas. This result indicates that patients having more severe dryness at these intra-oral regions would more likely use these general dry-mouth interventions.

For "using a mouth gel'', there was only a significant association with the RODI scores of the inside cheeks. As for "eating fruit'', there was an association with the total XI score, indicating that overall oral dryness could influence the use of the dry-mouth intervention "eating fruit''. Only "drinking coffee'' had significant associations with two intra-oral regions, the inside cheeks and the floor of the mouth. However, the RODI score of the inside cheeks was below 1 (0.25), while the RODI score of the floor of the mouth was larger than 1 (2.82). This indicates that higher RODI scores for the floor of the mouth and lower scores of the inside cheeks will probably affect drinking coffee by

Sjögren's patients. The dry-mouth intervention "drinking tea'' did not have any significant association with any of the included independent variables. Also, the independent variable, BI-score, did not have any significant association with any dry-mouth intervention.

Table 7: The odds ratio of several independent variables (RODI-scores, total XI-scores, BI-score) for the significant interventions after univariate analysis. The odds ratio including the 95% CI is reported. For the significant associations also the last step of the Omnibus and H-L test Chi-square including their df and p-values were reported. Furthermore, the Coxx & Snell and the Nagelkerke R square were mentioned.

	Drinking water	Drinking tea	Rinsing of the mouth	Drinking small volumes	Using mouth gel	Eating fruit	Drinking coffee
RODI-score of upper lip	NS	NS	NS		NS	NS	NS
RODI-score of inside cheeks	NS	NS	NS	NS	1.97 (1.19-3.27)**,d	NS	0.25 (0.09-0.70)**,f
RODI-score of anterior palate	NS	NS	NS	NS	NS	NS	NS
RODI-score of posterior palate	4.90 (1.70-14.08)**,a	NS	NS	NS	NS	NS	NS
RODI-score of lower lip	NS	NS	NS	NS	NS	NS	NS
RODI-score of floor of the mouth	NS	NS	NS	NS	NS	NS	2.82 (1.10-7.19)*,f
RODI-score of anterior tongue	NS	NS	1.90 (1.09-3.30)*,b	NS	NS	NS	NS
RODI-score of posterior tongue	NS	NS	NS	2.00 (1.21-3.31)**,c	NS	NS	NS
RODI-score of pharynx	NS	NS	NS	NS	NS	NS	NS
Total XI-score	NS	NS	NS	NS	NS	1.09 (1.02-1.17)**,e	NS
BI-score	NS	NS	NS	NS	NS	NS	NS

NS=none of the independent variables was significant
*Binary Logistic regression: * p <0.05*
*Binary Logistic regression: ** p <0.01*
[a] *H-L test χ^2 =12.4, df=2, p<0.01; Omnibus test χ^2 =12.5, df=1, p<0.01; Cox & Snell R2=0.15; Nagelkerke R2=0.33*
[b] *H-L test χ^2 =11.6, df=6, p>0.05; Omnibus test χ^2 =16.3, df=2, p<0.01; Cox & Snell R2=0.19; Nagelkerke R2=0.26*
[c] *H-L test χ^2 =2.5, df=2, p>0.05; Omnibus test χ^2 =8.3, df=1, p<0.01; Cox & Snell R2=0.10; Nagelkerke R2=0.14*
[d] *H-L test χ^2 =3.7, df=3, p>0.05; Omnibus test χ^2 =7.9, df=1, p<0.01; Cox & Snell R2=0.10; Nagelkerke R2=0.13*
[e] *H-L test χ^2 =8.2, df=7, p>0.05; Omnibus test χ^2 =8.6, df=1, p<0.01; Cox & Snell R2=0.11; Nagelkerke R2=0.14*
[f] *H-L test χ^2 =4.2, df=8, p>0.05; Omnibus test χ^2 =14.3, df=3, p<0.01; Cox & Snell R2=0.17; Nagelkerke R2=0.25*

DISCUSSION

The present study was designed to explore the possible associations between the perceived (regional) oral dryness of Sjögren's syndrome patients, and patients' use of dry-mouth interventions. Sjögren's syndrome patients use various interventions to relieve their oral dryness. Of those interventions, "drinking water" and "moistening the lips'' were the most frequently used. Besides, there were some clear associations between perceived oral dryness and some interventions applied, illustrated by the significant odds ratios between general dry-mouth interventions, "drinking water'', "rinsing of the mouth", and "drinking small volumes" and the RODI scores of the posterior palate, anterior and posterior tongue, respectively. On the other hand, "using mouth gel'' was significantly associated with the RODI scores of the inside cheeks. This observation could indicate that the use of these dry-mouth interventions is affected by the intra-oral dryness, measured by the RODI questionnaire.

The Sjögren's syndrome patients in the current study experienced the posterior palate and the pharynx as most dry. This observation could be explained by the fact that several factors make the hard palate more susceptible to oral dryness than other intra-oral locations. These factors include paucity of palatal glands, gravity, and evaporation during open-mouth breathing [20–22]. Besides, it is envisaged that saliva-related changes also contribute to the dry mouth feeling of Sjögren's syndrome patients: an altered sialochemical composition, such as higher concentrations of sodium, chloride and phosphate [23]; a higher protein concentration on the palate [24]; a significantly reduced saliva film on the hard palate; a reduced spinnbarkheit of unstimulated whole saliva; and an altered glycosylation of salivary mucins [16]. All these factors seem to negatively influence the wetting of the posterior palate and the pharynx.

In contrast, the Sjögren's syndrome patients experienced the floor of the mouth and inside cheeks as least dry. These regions include the orifices of the major salivary gland [20]. Because of their proximity to the orifices of the salivary glands, the saliva film in these regions is probably more moisturizing than the saliva film on the palate [21, 25–27].

The current study findings are consistent with our previous study which reported the perceived intraoral dryness for various dry-mouth patients [8], including Sjögren's patients as well as patients with polypharmacy and patients treated with radiotherapy. In that previous study, it was also found that the posterior palate was also the most dry in Sjögren's syndrome patients, while

the floor of the mouth and the inside cheeks were experienced as least dry [8]. This supports the suggestion that use of the RODI might add in screening or diagnosis of Sjögren syndrome.

The current study found that the use of dry-mouth interventions is influenced by intra-oral dryness (RODI questionnaire) in Sjögren's patients. For almost all dry-mouth interventions, there was a significant association with the RODI scores except for "eating fruit'' (Table 7). Only "eating fruit'' was significantly associated with the overall mouth dryness (total XI score); however, the odds ratio was only slightly above 1 (1.09). While for all other associations between dry-mouth interventions and RODI scores, the odds ratios were around 2 or above (Table 7). On the other hand, patients' discomfort was not significantly associated with any dry-mouth interventions. These results show that the intra-oral dryness, measured by the RODI questionnaire, can be a helpful tool in advising dry-mouth interventions for Sjögren's syndrome patients.

An interesting significant association could be seen for the dry-mouth interventions "drinking water'', "rinsing of the mouth", and "drinking small volumes'' with some intra-oral regions. However, it is expected that these generic dry interventions would be significantly associated with the overall mouth dryness (XI score) and not with the intra-oral dryness. In a previous study, it was found that the XI scores of Sjögren's patients had the highest correlations with the RODI scores of the posterior palate, anterior and posterior tongue, and floor of the mouth [8]. When looking to the other dry-mouth patients, it was found that the RODI scores of the anterior and posterior tongue and the floor of the mouth had the highest correlations with total XI scores [8]. This finding indicates that the tongue and possibly also the posterior palate play an important role in dry-mouth perception. A different study that used the Clinical Oral Dryness Score (CODS), a clinical tool to semi-quantitatively assess oral dryness, found that the items "fissured or depapillated tongue'' and "lack of saliva pooling in the floor of the mouth'' are signs of hyposalivation [28]. Other clinical features of their study, such as a mirror sticking to the tongue, a lack of saliva pooling in the floor of the mouth and a tongue showing loss of papillae, can be associated with a moderate but significant reduction in mucosal wetness [28]. Taken together, this suggests that the tongue might play an important role in dry-mouth perception. This may explain why Sjögren's patients have a significant association between "rinsing of the mouth", "drinking small volumes" and the RODI scores of the anterior and posterior tongue, respectively. The significant association between "drinking water'' and the RODI scores of the posterior palate is explained by the high RODI scores of this region. Of all intra-oral regions, the posterior palate was considered the

most dry than all other intra-oral regions except the anterior and posterior tongue and the pharynx (Table 1). This result shows that dryness of the posterior palate in combination with dryness of the anterior and posterior tongue seems to play a major role in choosing a dry-mouth intervention, much more than the total XI score.

Other interesting findings were the significant associations between "using mouth gel'' and the RODI score of the inside cheeks (Table 7). As seen in Table 1, the inside cheeks were considered as least dry region. However, when this region becomes more dry (Table 3, RODI score ≥ 3.5), patients tend to use a mouth gel that can be applied to this region to relieve its dryness.

The frequently used dry-mouth interventions by Sjögren's syndrome patients were "drinking water'' and "moistening the lips''. Drinking water was the most used (90.5%) intervention compared to all other dry-mouth interventions. As mentioned earlier by several systematic reviews, dry mouth products are not effective to relieve dry mouth [4, 6, 7]. Especially salivary substitutes, such as mouth gels and sprays, are not effective in reducing dry mouth symptoms or increasing the salivary flow [4, 6, 7]. This is in line with previous research that interviewed Sjögren's syndrome patients in the Netherlands about their saliva substitutes usage [29]. These patients reported that they discontinued use of saliva substitutes after a short period of time due to lack of effectiveness [29]. Possibly for this reason, Sjögren's syndrome patients prefer to drink water instead of using other dry-mouth interventions. Water is widely accessible at low costs. Drinking water can temporarily relieve the subjective sensation of dry mouth [30, 31]. However, the effectiveness and longevity of this strategy are limited [27], because the viscosity of water does not change with increasing shear [32]. In contrast, the viscosity of saliva decreases with increasing shear. In practice, this allows saliva to be easily spread on the oral surfaces as well as to be retained and not easily washed off oral surfaces [32]. For the reason, saliva has important lubricating properties in contrast to water. As a consequence, the effectivity of drinking water as a dry-mouth intervention is limited compared to saliva.

Although the RODI scores for the upper and lower lip were lower than other regions such as the posterior palate, anterior and posterior tongue and the pharynx, patients frequently moisten their lips (Table 1). Maintaining moist lips appears to be important for patients and can be helped by the administration of simple water-based gels and ensuring humidification [33]. Another study concluded that scheduled use of ice water oral swabs and lip moisturizer with menthol may lessen thirst intensity and dry mouth [34].

Sjögren's syndrome is an autoimmune disease that predominantly affects women. The female to male ratio of Sjögren's syndrome is 10:1 [35]. This means that vast majority of female respondents in the present study (89%) is a good representation of the gender distribution of Sjögren's syndrome in the Dutch population.

A possible limitation of the present study could be that the recruitment of the participants may have introduced a certain bias into the study. It can be assumed that Sjögren's syndrome patients who visited the annual meeting of the patient federation suffer significantly from their disease and want their stories and problems to be heard. The response rate of these participants was 52%, whereas a response rate of 70–80% is envisaged to be ideal to eliminate a potential nonresponse bias [36], though the current response rate is comparable with the response rates of a previous study using a questionnaire (56%) which investigated health problems, health information sought and attendance of general practice in elderly patients with approximately the same age as our study sample(70 years vs 64 ± 10 years in the current study) [37]. If a reminder was sent to the participants, then it could positively have affected the response rate. Several studies have shown that sending a reminder increased the response rate [38, 39]. However, sending reminders was not possible in the current study due to the General Data Protection Regulation (GDPA) restrictions with regard to collect personal data such as name and address. Therefore, it is possible that the study sample in the present study is not representative for the total Sjögren's population, as part of the opinion of the silent part of the population may not be present.

Additionally, patients attending the annual meeting may be more interested in their oral health than other Sjögren's syndrome patients. This may have introduced an additional bias in the questionnaire responses that may have led to an overestimation of their perceived oral dryness.

A limitation of the current study could be that some specific interventions were not included in the questionnaire. For example, the low number of patients that reported the use of Xylimelts could be due to the fact that this intervention was not included. Also, the frequency and efficiency of the dry-mouth interventions were not included in the questionnaire. *E.g.*, it is possible that Sjögren's syndrome patients drank water many times a day, while they moistened their lips only one or twice a day. The perceived effectiveness of the dry-mouth interventions should also be evaluated, for example by asking the patients to rate this on a Likert scale. As the effectiveness of dry-mouth interventions might be related to the degree to which the salivary glands are still sensitive to stimulation [31], it is important that prospective studies

also assess the relation between salivary flow rates and use of dry-mouth interventions.

MAIN CONCLUSIONS

The present study shows that Sjögren's syndrome patients used a wide range of interventions to relieve their oral dryness, especially "drinking water'' was a frequently used intervention care. As for the association between dry-mouth interventions with oral dryness and patients' discomfort, only intra-oral dryness was significantly associated with the use of dry-mouth interventions. "Drinking water'', "rinsing of the mouth", and "drinking small volumes" had significant associations with the RODI scores of the posterior palate, and anterior and posterior tongue, respectively, while the "use of a mouth gel'' had a significant association with the RODI scores of the inside cheeks. These results indicate that dryness of the posterior palate and the anterior and posterior tongue will influence Sjögren's syndrome patients to use general dry-mouth interventions, such as "drinking water'', "rinsing of the mouth" and "drinking small volumes". On the other hand, dryness of the inside cheeks will cause patients to use a mouth gel. It can be concluded that Sjögren's syndrome patients are more likely to use mouth gels when their inside cheeks were experienced as most dry, while they drank water, rinsed their mouth or drank small volumes if the posterior palate, anterior and posterior tongue were considered as dry. This finding has provided a deeper insight into the association between the use of dry-mouth interventions and mouth dryness, as intra-oral dryness affects dry-mouth perception and thereby also the use of the various dry-mouth interventions. Altogether, the therapeutic choice of a dry-mouth intervention by Sjögren's syndrome patients seems to some extent to be related to dryness at specific oral regions.

REFERENCES

1. Roblegg E, Coughran A, Sirjani D (2019) Saliva: an all-rounder of our body. Eur J Pharm Biopharm 142:133–141. https://doi.org/10.1016/j.ejpb.2019.06.016
2. Saleh J, Figueiredo MA, Cherubini K, Salum FG (2015) Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol 60(2):242–255. https://doi.org/10.1016/j.archoralbio.2014.10.004
3. Tincani A, Andreoli L, Cavazzana I, Doria A, Favero M, Fenini MG, Franceschini F, Lojacono A, Nascimbeni G, Santoro A, Semeraro F, Toniati P, Shoenfeld Y (2013) Novel aspects of Sjögren's syndrome in 2012. BMC Med 11:93. https://doi.org/10.1186/1741-7015-11-93
4. Al Hamad A, Lodi G, Porter S, Fedele S, Mercadante V (2019) Interventions for dry mouth and hyposalivation in Sjögren's syndrome: a systematic review and meta-analysis. Oral Dis 25(4):1027–1047. https://doi.org/10.1111/odi.12952
5. Furness S, Bryan G, McMillan R, Worthington HV (2013) Interventions for the management of dry mouth: non-pharmacological interventions. Cochrane Database Syst Rev (8):Cd009603. https://doi.org/10.1002/14651858.CD009603.pub2
6. Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R (2011) Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev (12):Cd008934. https://doi.org/10.1002/14651858.CD008934.pub2
7. Brito-Zerón P, Retamozo S, Kostov B, Baldini C, Bootsma H, De Vita S, Dörner T, Gottenberg JE, Kruize AA, Mandl T, Ng WF, Seror R, Tzioufas AG, Vitali C, Bowman S, Mariette X, Ramos-Casals M (2019) Efficacy and safety of topical and systemic medications: a systematic literature review informing the EULAR recommendations for the management of Sjögren's syndrome. RMD Open 5(2):e001064. https://doi.org/10.1136/rmdopen-2019-001064
8. Assy Z, Bots CP, Arisoy HZ, Gülveren SS, Bikker FJ, Brand HS (2021) Differences in perceived intra-oral dryness in various dry mouth patients as determined using the Regional Oral Dryness Inventory. Clin Oral Investig 25(6):4031–4043. https://doi.org/10.1007/s00784-020-03734-2
9. Assy Z, Jager DHJ, Mashhour E, Bikker FJ, Brand HS (2020) Regional differences in perceived oral dryness as determined with a newly developed questionnaire, the Regional Oral Dryness Inventory. Clin Oral Investig. https://doi.org/10.1007/s00784-020-03276-7
10. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Int J Surg 12(12):1495–1499. https://doi.org/10.1016/j.ijsu.2014.07.013
11. Thomson WM, Chalmers JM, Spencer AJ, Williams SM (1999) The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health 16(1):12–17
12. Bulthuis MS, Jan Jager DH, Brand HS (2018) Relationship among perceived stress, xerostomia, and salivary flow rate in patients visiting a saliva clinic. Clin Oral Investig 22(9):3121–3127. https://doi.org/10.1007/s00784-018-2393-2

13. Jager DHJ, Bots CP, Forouzanfar T, Brand HS (2018) Clinical oral dryness score: evaluation of a new screening method for oral dryness. Odontology 106(4):439–444. https://doi.org/10.1007/s10266-018-0339-4
14. Challacombe S, Bds P, Bsc P (2015) Clinical Scoring Scales for Assessment of Dry Mouth. In. pp 119–132. https://doi.org/10.1007/978-3-642-55154-3_8
15. Chaudhury NM, Proctor GB, Karlsson NG, Carpenter GH, Flowers SA (2016) Reduced Mucin-7 (Muc7) sialylation and altered saliva rheology in Sjögren's syndrome associated oral dryness. Mol Cell Proteomics 15(3):1048–1059. https://doi.org/10.1074/mcp.M115.052993
16. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH, Proctor GB (2015) Changes in saliva rheological properties and mucin glycosylation in dry mouth. J Dent Res 94(12):1660–1667. https://doi.org/10.1177/0022034515609070
17. Brand HS, Bots CP, Veerman ECI (2011) Therapies for xerostomia in Sjögren's disease are age- and gender-dependent. J Dent Res 90(Special Issue A):1347
18. Marcoulides KM, Raykov T (2019) Evaluation of variance inflation factors in regression models using latent variable modelling methods. Educ Psychol Meas 79(5):874–882. https://doi.org/10.1177/0013164418817803
19. Kim JH (2019) Multicollinearity and misleading statistical results. Korean J Anesthesiol 72(6):558–569. https://doi.org/10.4097/kja.19087
20. Holmberg KV, Hofman MP (2014) Anatomy, biogenesis and regeneration of salivary glands. Monogr Oral Sci 24:1–13. https://doi.org/10.1159/000358776
21. DiSabato-Mordarski T, Kleinberg I (1996) Measurement and comparison of the residual saliva on various oral mucosal and dentition surfaces in humans. Arch Oral Biol 41(7):655–665. https://doi.org/10.1016/s0003-9969(96)00055-6
22. Kessler AT, Bhatt AA (2018) Review of the major and minor salivary glands, part 1: anatomy, Infectious, and inflammatory processes. J Clin Imaging Sci 8:47. https://doi.org/10.4103/jcis.JCIS_45_18
23. Kalk WW, Vissink A, Spijkervet FK, Bootsma H, Kallenberg CG, Nieuw Amerongen AV (2001) Sialometry and sialochemistry: diagnostic tools for Sjogren's syndrome. Ann Rheum Dis 60(12):1110–1116. https://doi.org/10.1136/ard.60.12.1110
24. Pramanik R, Osailan SM, Challacombe SJ, Urquhart D, Proctor GB (2010) Protein and mucin retention on oral mucosal surfaces in dry mouth patients. Eur J Oral Sci 118(3):245–253. https://doi.org/10.1111/j.1600-0722.2010.00728.x
25. Won S, Kho H, Kim Y, Chung S, Lee S (2001) Analysis of residual saliva and minor salivary gland secretions. Arch Oral Biol 46(7):619–624. https://doi.org/10.1016/s0003-9969(01)00018-8
26. Wolf M, Kleinberg I (1998) Oral mucosal wetness in hypo- and normosalivators. Arch Oral Biol 43(6):455–462. https://doi.org/10.1016/s0003-9969(98)00022-3
27. Lee SK, Lee SW, Chung SC, Kim YK, Kho HS (2002) Analysis of residual saliva and minor salivary gland secretions in patients with dry mouth. Arch Oral Biol 47(9):637–641. https://doi.org/10.1016/s0003-9969(02)00053-5
28. Osailan SM, Pramanik R, Shirlaw P, Proctor GB, Challacombe SJ (2012) Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. Oral Surg Oral Med Oral Pathol Oral Radiol 114(5):597–603. https://doi.org/10.1016/j.oooo.2012.05.009

29. Brand HS, Ouzzine R, Bots CP (2013) Speelselsubstituten: voor verbetering vatbaar!. Ned Tijdschr Tandheelkd 120(1):4

30. Jose A, Singh ML, Magnuson B, Farag A, Varghese R, Papas A (2018) A randomized controlled study to evaluate an experimental moisturizing mouthwash formulation in participants experiencing dry mouth symptoms. Oral Surg Oral Med Oral Pathol Oral Radiol 126(3):231-239.e235. https://doi.org/10.1016/j.oooo.2018.05.007

31. Purdie J, Carpenter MD, Noll JJ, Stephens C, Taylor YJ, Napenas JJ, Brennan MT (2021) Xerostomia symptoms and treatment strategies associated with salivary flows. Oral Surg Oral Med Oral Pathol Oral Radiol 131(4):e116. https://doi.org/10.1016/j.oooo.2020.10.047

32. Carpenter GH (2013) The secretion, components, and properties of saliva. Annu Rev Food Sci Technol 4:267–276. https://doi.org/10.1146/annurev-food-030212-182700

33. Fleming M, Craigs CL, Bennett MI (2020) Palliative care assessment of dry mouth: what matters most to patients with advanced disease? Support Care Cancer 28(3):1121–1129. https://doi.org/10.1007/s00520-019-04908-9

34. VonStein M, Buchko BL, Millen C, Lampo D, Bell T, Woods AB (2019) Effect of a scheduled nurse intervention on thirst and dry mouth in intensive care patients. Am J Crit Care 28(1):41–46. https://doi.org/10.4037/ajcc2019400

35. Baer AN, Walitt B (2018) Update on Sjögren syndrome and other causes of sicca in older adults. Rheum Dis Clin North Am 44(3):419–436. https://doi.org/10.1016/j.rdc.2018.03.002

36. Parashos P, Morgan MV, Messer HH (2005) Response rate and nonresponse bias in a questionnaire survey of dentists. Community Dent Oral Epidemiol 33(1):9–16. https://doi.org/10.1111/j.1600-0528.2004.00181.x

37. Wensing M, Schattenberg G (2005) Initial nonresponders had an increased response rate after repeated questionnaire mailings. J Clin Epidemiol 58(9):959–961. https://doi.org/10.1016/j.jclinepi.2005.03.002

38. Sahlqvist S, Song Y, Bull F, Adams E, Preston J, Ogilvie D (2011) Effect of questionnaire length, personalisation and reminder type on response rate to a complex postal survey: randomised controlled trial. BMC Med Res Methodol 11:62. https://doi.org/10.1186/1471-2288-11-62

39. Koitsalu M, Eklund M, Adolfsson J, Grönberg H, Brandberg Y (2018) Effects of pre-notification, invitation length, questionnaire length and reminder on participation rate: a quasi-randomised controlled trial. BMC Med Res Methodol 18(1):3. https://doi.org/10.1186/s12874-017-0467-5

9

Preferences of Sjögren's syndrome patients regarding potential new saliva substitutes

Z. Assy
F. J. Bikker
E. Mashhour
M. Asadi
H. S. Brand

Clinical Oral Investigations, 2022 Oct;26(10):6245-6252.

ABSTRACT

Objective

Sjögren's syndrome (SS) patients should be involved in the development of new saliva substitutes at an early stage. The purpose of the current study was to explore the preferences of these patients regarding various product characteristics of potential new saliva substitutes.

Materials and methods

A questionnaire was distributed among SS patients. They could anonymously indicate their preferences for saliva substitute characteristics using 5-point Likert scales.

Results

Fifty-nine SS patients filled in the questionnaire. According to their opinion, the most ideal saliva substitute has a thin-watery consistency with a neutral flavour that should be applied as a spray. Patients demand a prolonged alleviation of dry mouth complaints and neutralization of harmful bacteria. The patients mainly object against the presence of artificial sweeteners and alcohol in saliva substitutes, but have limited objections against the presence of vegetable-based ingredients and natural enzymes. Major objections were against the potential side-effects "bitter taste" and "discoloration of teeth". Age and severity of xerostomia affected preferences flavour. Younger patients preferred menthol flavour, while respondents with severe xerostomia preferred the use of "neutral flavours" significantly more.

Conclusion

The most ideal saliva substitute has thin-watery consistency in spray form with a neutral flavour and provides long alleviation of dry mouth complaints. It should not contain artificial sweeteners or alcohol, and should not have a bitter taste or cause discoloration of the teeth.

Clinical relevance

Investigating the opinion of SS patients provides tailored insights into their preference, which may contribute to the development of more effective saliva substitutes.

Keywords

Sjögren's syndrome, Dry mouth, Xerostomia, Saliva substitutes, Patient preferences, Ingredients.

INTRODUCTION

Sjögren's syndrome is an autoimmune disease that causes progressive damage to the exocrine glands including the salivary glands. As a consequence, Sjögren's syndrome leads to hyposalivation and/or xerostomia [1, 2]. The resulting dry mouth may induce comorbidities such as difficulties with mastication, swallowing, speaking, and sleeping. In addition, the reduction of the protective properties of saliva may also increase the risk of developing dental caries and oral candidiasis [1, 3].

At early stages of Sjögren's syndrome, when residual salivary function is still present, salivary flow can be stimulated, *e.g.* by the use of lozenges and chewing gums, systemic pharmacotherapy, or electrostimulation of the salivary glands [4–6]. However, in case of an advanced disease process, when the salivary function is irreversibly impaired, saliva substitutes such as mouth sprays, gels, and mouthwashes can be applied for the relief of oral complaints [6, 7]. A substantial number of Sjögren's syndrome patients are using or have used a saliva substitute in the past. In a recent study, this percentage ranged between 42.9 and 45.5% for the use of a mouth gel, while for the use of a mouth spray it ranged between 25.0 and 27.4% [7, 8].

The currently available saliva substitutes contain animal- and vegetable-based lubricants and thickeners like porcine gastric mucins, hydroxyethyl cellulose, or aloe vera [9]. However, these ingredients have limited ability to retain water and require specific environmental conditions to be effective. For example, porcine gastric mucins are effective only at an acidic pH and in a low ionic strength environment [9]. Besides, some compounds are easily removed from the oral cavity by swallowing or drinking, leading to limited duration of moistening and lubrication. Additionally, a number of substitutes have flavours such as "apple", "lemon", and "strawberry". A reason for manufacturers using these flavours is that they can stimulate salivary secretion due to their gustatory effect [10]. However, more than the half of Sjögren's syndrome patients reported that they discontinued the use of saliva substitutes after a short period of time. An unpleasant taste and sticky consistency were main reasons for their discontinuation [11, 12]. The sticky consistency may compromise masticatory function [13]. Also, the presence of animal-based products in salivary substitutes could induce objections in people from certain religious, cultural, and social backgrounds because these products may be against their beliefs [14].

However, to the best of our knowledge, no studies have investigated patients' preference for characteristics of saliva substitutes, such as taste,

consistency, and objections for specific ingredients. Investigating the opinion of the users at an early stage of the development of new saliva substitutes might provide tailored insights into preference criteria which may contribute to the development of more effective saliva substitutes. For this reason, the purpose of the current study was to explore the preferences of Sjögren's syndrome patients for various product characteristics of potential new saliva substitutes, especially the important functions of possible substitutes, objections against certain ingredients, desired flavours for the substitutes, objections against potential side-effects of saliva substitutes, and the preferred method of administration. The unpleasant taste of saliva substitutes is a major reason for discontinuation of use of these products [11, 12]. Therefore, we explored the preferences for a wide range of possible flavours. As the amount of saliva present in the oral cavity may affect taste, we hypothesized that Sjögren's syndrome patients with less severe dry-mouth experience will prefer different flavours than patients with more severe dryness. In addition, we hypothesized that Sjögren's patients will have more objections against the presence of specific animal-based ingredients than for non-animal–based products.

MATERIALS AND METHOD

A cross-sectional study was performed among Sjögren's syndrome patients who visited the website of the Dutch Sjögren Patients Federation (Dutch: Nederlandse Vereniging van Sjögren Patiënten). Volunteers could anonymously fill in the questionnaire described below during a period of 7 weeks. Only volunteers with diagnosed Sjogren's syndrome were eligible to fill in the questionnaire.

The local Ethics Review Committee of the Academic Centre for Dentistry Amsterdam (ACTA) confirmed that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study (protocol number 2017001). The reporting of this study conforms to the Strengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement [15].

A priori sample size calculation was performed using G*Power software, version 3.1.9.4 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany); with a medium effect size (0.5) and a power of 80%, 148 participants were needed.

Study variables

The questionnaire, developed for this study, consisted of eight parts. First, several general questions with regard to age, sex, and year when Sjögren's syndrome had been diagnosed by a physician.

The second part was the internationally accepted and validated Xerostomia Inventory (XI), consisting of 11 items on a 5-point Likert scale ranging from 1 = "Never" to 5 = "Very often". The items concern patients' oral dryness and mouth feel. Per item, patients indicate how often they experience problems regarding mouth feel and oral dryness. The scores of the 11 items are summed to produce a total XI score that ranges between 11 (no xerostomia) and 55 (extreme xerostomia) [16]. XI had showed adequate content and concurrent validity [16].

The remaining parts of the questionnaire contained questions regarding various product characteristics of hypothetical new saliva substitutes.

The third part explored the importance of different functions of saliva substitutes. The patients could indicate the importance of each function by using a 5-point Likert scale, ranging from 1 = "Unimportant" to 5 = "Very important". All investigated possible functions of salivary substitutes are presented in Table 1.

The fourth part consisted of a question about the preferred consistency of saliva substitutes; thin-watery or thick-liquid-like or gel-like.

The fifth part explored how much the patients object the presence of certain ingredients in saliva substitutes using 5-point Likert scales, ranging from 1 = "No objection" to 5 = "Insurmountable objections". Table 2 presents all potential ingredients investigated.

The sixth part consisted of an item on the desired flavour of saliva substitutes. A 5-point Likert scale was used to indicate the importance of the availability of each flavour, ranging from 1 = "Unimportant" to 5 = "Very important". The desired flavours investigated are presented in Table 3.

The seventh part of questionnaire was about potential side-effects of saliva substitutes. For each side-effect, the patient could indicate if they would experience it as unpleasant by using a 5-point Likert scale ranging from 1 = "Not unpleasant" to 5 = "Very unpleasant". Table 4 presents the investigated potential side-effects of salivary substitutes.

Finally, a question was included about the preferred method of administration of the saliva substitutes, whereby patient could choose a mouth gel, a mouth spray, an oral rinse, or a tablet.

Data analysis

The data were statistically analysed with SPSS, version 28.0 (IBM Corp SPSS statistics, Armonk, NY, USA). The Shapiro–Wilk test was used to assess the normality of the data. As not all variables were normally distributed, the data are presented as medians and their interquartile range (IQR). To clarify relatively small differences, the mean and standard deviation (SD) are also reported.

The respondents were dichotomized based on their age and the severity of their xerostomia. The two xerostomia groups were used to test the hypothesis whether Sjögren's syndrome patients with less severe dry-mouth experience prefer other flavours than patients with more severe dryness. The median of these two parameters was used to divide them into two groups: birth year ≤ 1958 versus birth year ≥ 1959 and mouth dryness with a XI score ≤ 46 versus mouth dryness with a XI score ≥ 47. A Mann–Whitney U test was used to explore whether the subgroups of respondents varied based on their respective answers.

All significance levels (α) were set at 0.05.

RESULTS

At the time the questionnaire was distributed to the patients, the patients' association had 2115 members. In the period when the questionnaire was available online, the association's website was visited by 1485 people. During this period, 59 Sjögren's syndrome patients completed the questionnaire. Almost all respondents were women (N = 58, 98%). The mean age of the respondents was 55.7 ± 12.0 years, ranging from 25 to 79 years. The respondents reported that the Sjögren's syndrome had been diagnosed between 1 and 36 years ago. The total XI score of all patients had a median of 47.0 with IQR of 43.0–51.0.

Table 1 describes the opinion of Sjögren's syndrome patients regarding the importance of different functions of saliva substitutes. Most of the possible functions were considered important (score ≥ 4), while functions such as "provides fast alleviation of dry mouth", "gives prolonged alleviation of dry mouth", "protects the mucosa", "facilitates speaking", "neutralizes harmful bacteria", and "optimizes the mouth flora" were considered very important (score ≥ 4.5). On the other hand, functions such as "available in different flavours" and "can be used unnoticed" were considered relatively less important. The two age groups did not show any significant differences regarding possible functions of saliva substitutes (Mann–Whitney U test $p > 0.05$). Respondents with a more severe xerostomia, indicated by a higher XI score, considered the function "gives a prolonged alleviation of dry mouth" more important than the lower XI-group (mean = 4.73 ± 0.83, median = 5.0 ± 5.0–5.0, N = 30, versus mean = 4.55 ± 0.63, median = 5.0 ± 4.0–5.0, N = 29, Mann–Whitney U test $p < 0.05$). All other functions did not show any significant difference for the two XI-groups.

Table 1: Sjögren's syndrome patients opinon regarding the importance of the potential functions of saliva substitutes, using a Likertscale (from 1= "Unimportant'' to 5= "Very important''). Data are expressed as as mean score with standard deviation (SD.) and median scores with the corresponding interquartile range (IQR). N indicates the number of participants in each group.

Possible functions	**Total mean ± SD. (N)**	**Total median ± IQR**
Helps to prevent tooth decay	4.03 ± 1.36 (N=59)	5.0 ± 3.0-5.0
Helps painful swallowing	4.15 ± 1.20 (N=59)	5.0 ± 5.0-4.0
Provides fast alleviation of dry mouth	4.49 ± 1.01 (N=59)	5.0 ± 5.0-4.0
Gives prolonged alleviation of dry mouth	4.64 ± 0.74 (N=59)	5.0 ± 5.0-4.0
Protects the mucosa	4.49 ± 0.88 (N=59)	5.0 ± 5.0-4.0
Treats bleeding gingiva	3.54 ± 1.29 (N=59)	4.0 ± 5.0-3.0
Facilitates speaking	4.48 ± 1.02 (N=59)	5.0 ± 5.0-4.0
Improves the taste	3.95 ± 1.15 (N=58)	4.0 ± 5.0-3.0
Available in different flavours	3.09 ± 1.38 (N=59)	3.0 ± 4.0-2.0
Stimulates saliva secretion	4.41 ± 0.97 (N=59)	5.0 ± 5.0-4.0
Neutralizes harmful bacteria	4.63 ± 0.79 (N=59)	5.0 ± 5.0-4.0
Optimizes the mouth flora	4.60 ± 0.77 (N=58)	5.0 ± 5.0-4.0
Contains natural saliva enzymes	4.17 ± 1.09 (N=58)	5.0 ± 3.8-5.0
Gives a balanced pH	4.29 ± 0.86 (N=58)	4.5 ± 5.0-4.0
Is practical and handy in use	4.46 ± 0.95 (N=57)	5.0 ± 5.0-4.0
Can be used with little effort	4.40 ± 0.90 (N=58)	5.0 ± 5.0-4.0
Can be used unnoticed	2.98 ± 1.54 (N=59)	3.0 ± 4.0-2.0

As for the consistency of salivary substitutes, most of the respondents preferred thin-watery consistency (52.5%) followed by gel-like consistency (33.9%). Only 8.5% of respondents preferred a thick-liquid consistency. Age or XI-groups did not influence the preference of the consistency.

In Table 2, the objections of the respondents against the presence of certain ingredients are reported. The respondents mainly objected against the presence of "artificial sweeteners", "alcohol", "foaming agents", and "preservatives". They objected less against the presence of "vegetable-based ingredients", "natural enzymes", and "fluoride". The two XI-groups only showed a significant difference with regard to the objections against vegetable-based ingredients (Mann–Whitney U test $p < 0.05$). Respondents with relatively low xerostomia (mean = 1.31 ± 0.89, median = 1.0 ± 1.0–1.0, N = 29) had less objections against the presence of vegetable-based ingredients than the respondent with more severe xerostomia (mean = 1.86 ± 1.27, median = 1.0 ± 1.0–3.0, N = 28).

Table 2: Sjögren's syndrome patients objections against certain ingredients in saliva substitutes, using a 5-point Likert scale (from 1 = "No objection'' to 5 = "Insurmountable objections''). Data are expressed as mean score with standard deviation (SD.) and as median scores with the corresponding interquartile range (IQR). N indicates the number of participants in each group.

Potential ingredients	Total mean ± SD. (N)	Total median ± IQR
Alcohol	3.39 ± 1.46 (N=57)	3.0 ± 5.0-2.0
Preservatives	3.14 ± 1.38 (N=57)	3.0 ± 5.4-2.0
Fluoride	3.03 ± 1.47 (N=57)	1.0 ± 3.0-1.0
Urea	2.82 ± 1.17 (N=57)	3.0 ± 3.0-2.0
Foaming agents	3.30 ± 1.35 (N=57)	3.0 ± 2.5-4.0
Artificial sweeteners	3.40 ± 1.52 (N=57)	4.0 ± 5.0-2.0
Gluten	2.67 ± 1.57 (N=57)	3.0 ± 4.0-1.0
Natural enzymes	1.72 ± 1.22 (N=57)	1.0 ± 5.2-1.0
Vegetable-based ingredients	1.58 ± 1.12 (N=57)	1.0 ± 2.0-1.0
Ingredients from chicken eggs	2.32 ± 1.38 (N=57)	2.0 ± 3.0-1.0
Ingredients from cattle	2.58 ± 1.40 (N=57)	3.0 ± 5.3-1.0
Ingredients from pigs	3.04 ± 1.49 (N=57)	3.0 ± 1.5-4.5
Ingredients from fish	2.82 ± 1.42 (N=56)	3.0 ± 4.0-1.0

Table 3: Sjögren's syndrome patients' opinion regarding the desired flavours of saliva substitutes, using a Likertscale (from 1= "Unimportant" to 5= "Very important"). Patients were stratified in subgroups based on their age and severity of xerostomia. For the total study population, the data are expressed as mean score with standard deviation (SD.) and as median scores with the corresponding interquartile range (IQR). For both ages groups and XI-groups the mean score with SD. were reported. N indicates the number of participants in each group.

Possible flavours	Total mean ± SD. (N)	Total median ± IQR	Birthyear ≤1958 (N)	Birthyear ≥1959 (N)	p-value*	XI-score ≤46 (N)	XI-score ≥47 (N)	p-value*
Strawberry	1.81 ± 1.27 (N=57)	1.0 ± 1.0-3.0	1.77 ± 1.24 (N=26)	1.84 ± 1.32 (N=31)	0.55	1.79 ± 1.18 (N=29)	1.82 ± 1.39 (N=28)	0.54
Apple	2.03 ± 1.36 (N=58)	1.0 ± 1.0-3.0	1.89 ± 1.31 (N=27)	2.16 ± 1.42 (N=31)	0.29	2.17 ± 1.47 (N=29)	1.90 ± 1.26 (N=29)	0.40
Banana	1.82 ± 1.31 (N=57)	1.0 ± 1.0-3.0	1.77 ± 1.24 (N=26)	1.87 ± 1.38 (N=31)	0.49	1.86 ± 1.27 (N=29)	1.79 ± 1.37 (N=28)	0.38
Blueberry	2.09 ± 1.43 (N=56)	1.0 ± 1.0-3.0	2.08 ± 1.50 (N=26)	2.10 ± 1.40 (N=30)	0.64	2.41 ± 1.57 (N=29)	1.74 ± 1.20 (N=27)	0.03
Lemon	2.31 ± 1.50 (N=58)	1.5 ± 1.0-4.0	2.33 ± 1.59 (N=27)	2.29 ± 1.44 (N=31)	0.83	2.69 ± 1.54 (N=29)	1.93 ± 1.39 (N=29)	0.05
Cola	1.49 ± 1.12 (N=57)	1.0 ± 1.0-1.0	1.54 ± 1.21 (N=26)	1.45 ± 1.06 (N=31)	0.56	1.59 ± 1.24 (N=29)	1.39 ± 0.99 (N=28)	0.34
Liquorice	1.81 ± 1.37 (N=57)	1.0 ± 1.0-2.5	1.81 ± 1.36 (N=26)	1.81 ± 1.40 (N=31)	0.75	1.79 ± 1.37 (N=29)	1.82 ± 1.39 (N=28)	0.66
Menthol/ spearmint	3.57 ± 1.55 (N=58)	4.0 ± 3.0-5.0	2.96 ± 1.53 (N=27)	4.10 ± 1.38 (N=31)	0.003	3.66 ± 1.50 (N=29)	3.48 ± 1.62 (N=29)	0.58
No flavour	3.91 ± 1.58 (N=57)	5.0 ± 3.0-5.0	3.88 ± 1.68 (N=26)	3.94 ± 1.53 (N=31)	0.65	3.79 ± 1.66 (N=29)	4.04 ± 1.53 (N=28)	0.89
Neutral flavour	3.98 ± 1.47 (N=58)	5.0 ± 3.0-5.0	3.93 ± 1.57 (N=27)	4.03 ± 1.40 (N=31)	0.74	3.52 ± 1.64 (N=29)	4.45 ± 1.12 (N=29)	0.02

**: p-value of the Mann-Whitney U test*

The opinion of the respondents regarding the desired flavours of salivary substitutes is reported in Table 3. Highly preferred flavours were a "neutral flavour", "no flavour", and "menthol/spearmint flavour", whereas the flavours "cola", "liquorice", and "strawberry" were the least popular. There was a significant difference between the two age groups with regard to preferences of flavours (Mann–Whitney U test $p < 0.05$); the younger respondents preferred "menthol/ spearmint" flavour more than the older age group. The two groups with different levels of xerostomia also showed significant differences (Mann–Whitney U test $p < 0.05$). The respondents with relatively low xerostomia (XI score ≤ 46) preferred the use of flavour "blueberry" more than the respondents with more severe xerostomia (XI score ≥ 47). On the other hand, respondents in the XI ≥ 47 group preferred the use of "neutral flavours" in salivary substitutes significantly more than respondents with a relatively low xerostomia.

Table 4 depicts the opinion of respondents regarding potential side-effects of the use of saliva substitutes. Major objections were against saliva substitutes "causing discoloration of the teeth" and ones "having a bitter taste". The least objections were about using saliva substitutes multiple times a day. The two age groups only differed significantly with regard to "causing discoloration of the teeth" (Mann–Whitney U test $p < 0.05$), whereby the younger age group (mean = 4.74 ± 0.89, median = 5.0 ± 5.0–5.0, N = 31) had more objections than the older age group (mean = 4.25 ± 1.40, median = 5.0 ± 4.0–5.0, N = 28).
Finally, Table 5 presents the preferred method of administration. The respondents preferred a mouth spray followed by a mouth gel or an oral rinse. A minority of the respondents preferred a tablet. These preferences did not differ significantly for the two age groups and the two XI-groups (Mann–Whitney U test $p > 0.05$).

Table 4: Sjögren's syndrome patients' opinion regarding potential side effects of saliva substitutes, using a 5-point Likert scale (1 = "Not unpleasant'' to 5 = "Very unpleasant''). Data are expressed as as mean score with standard deviation (SD.) and as median scores with the corresponding interquartile range (IQR). N indicates the number of participants in each group.

Potential negative effects side effects	Total mean ± SD. (N)	Total median ± IQR
Causing discoloration of the teeth	4.59 ± 1.03 (N=58)	5.0 ± 5.0-5.0
Causing discoloration of the oral mucosa	4.14 ± 1.12 (N=58)	5.0 ± 3.0-5.0
Having a bitter taste	4.47 ± 0.90 (N=58)	5.0 ± 4.0-5.0
Having an aftertaste	4.22 ± 0.94 (N=58)	4.5 ± 4.0-5.0
Using the product multiple times a day	2.45 ± 1.33 (N=58)	3.0 ± 1.0-3.0

Table 5: Sjögren's syndrome patients' opinion regarding the preferred method of administration. Data are presented as percentages.

Method of administration	Percentage
Mouth spray	45.5
Mouth gel	23.6
Oral rinse	23.6
Tablet	7.3

DISCUSSION

The present study was designed to explore criteria for new saliva substitutes according to the preferences of Sjögren's syndrome patients. The most ideal saliva substitute has thin-watery consistency in spray form, with a neutral flavour and providing a prolonged alleviation of dry mouth. Besides, it preferably should not contain artificial sweeteners or alcohol, and should not have a bitter taste and not cause discoloration of the teeth.

Most of the respondents of the present study were female (98%) with average age of 55.7 ± 12.0 years and with severe dry-mouth complaints, as indicated by the high average XI score (47.0 ± 43.0–51.0). This overrepresentation is in line with the female to male ratio of Sjögren's syndrome, which ranged between 20:1 and 9:1 [17]. The average age and the severity of oral dryness in the current study are also comparable with other studies that included Sjögren's syndrome patients with dry-mouth complaints [7, 18]. The average age in these previous studies varied between 61.7 ± 14.0 and 64 ± 10 years. As for the severity of xerostomia, the mean XI scores in these previous studies were between 44.0 ± 37.0–49.8 and 45.0 ± 38.0–48.5 [7, 18]. In summary, this suggests that the respondents in the current study form a good representation of Sjögren's syndrome patients in the Dutch population.

Several systematic reviews have reported that the effectiveness of currently available saliva substitutes for the relief of dry mouth seems to be limited [4, 6, 19]. For this reason, in the present study the Sjögren's syndrome patients indicated that prolonged alleviation of dry mouth is the most essential function of saliva substitutes. Unfortunately, most of the available saliva substitutes now provide only a temporary relief [9]; as the lubrication time of a typical saliva substitute, such as Dentaid Xeros, is around 0.5 min [9]. However, recently a promising new supercharged polypeptide-based salivary lubrication enhancer has been reported which could prolong the lubrication time up to 21 ± 7.3 min [9].

When developing a new saliva substitute, it is also important to try to mimic the complex biological properties of natural saliva, including neutralizing harmful bacteria and optimizing the mouth flora. Many Sjögren's syndrome patients with a reduced salivary flow have alterations in the composition of the oral bacterial plaque despite good oral hygiene measures [20, 21], which causes an increased risk of caries and candidiasis [20, 21]. Moreover, the salivary pH, bicarbonate concentration, and buffer capacity were significantly lower in the Sjögren's syndrome patients than healthy controls [21]. Besides an increased caries risk, these patients have also a higher risk of tooth erosion, as they experience a greater decline in salivary pH after exposure to acidic challenges. These factors might explain the urge of Sjögren's syndrome patients for a salivary substitute that "neutralizes harmful bacteria" and "optimizes the mouth flora".

Saliva plays a major role in taste perception, as the hypotonicity of unstimulated saliva allows the taste buds to perceive different tastes without being masked by normal plasma sodium levels [22]. Moreover, saliva is very important for the solubilization of flavours in saliva, for the chemical interaction between flavours and salivary ingredients, and for the dilution and/or the diffusion of flavours in saliva [23]. Based on these factors, it is conceivable that taste sensitivity is easily affected by changes in saliva [23], especially in Sjögren's syndrome patients with a low unstimulated salivary flow rate [24–30] and altered rheological properties of saliva [31]. This altered taste sensitivity may explain why these patients had objections against the presence of "artificial sweeteners" and "alcohol" and why they preferred a "neutral flavour" or "no flavour" at all. Previous studies showed that an unpleasant taste is a major reason for Sjögren's syndrome patients to discontinue the use of saliva substitutes [12]. Sjögren's syndrome patients having sicca syndrome are recommended to avoid alcohol [32], which may explain why patients prefer saliva substitutes without alcohol. Given these reasons, it is important to develop new saliva substitutes with a "neutral flavour" without "artificial sweeteners" nor "alcohol". In contrast to our expectation described in the "Introduction", the presence of specific animal-based ingredients seems of very limited importance, compared to other ingredients such as "artificial sweeteners" or "alcohol".

Sjögren's syndrome patients reported major objections against discoloration of the teeth or the oral mucosa as potential side-effects of the use of saliva substitutes. Discoloration was not mentioned in a study reporting side-effects of some saliva substitutes [33]. Possibly, these objections against

discoloration might be related that white teeth are important for people in general, as demonstrated by others [34].

In the current study, the flavours of the available saliva substitutes such as "apple", "lemon", and "strawberry" were the least preferred, although a previous study has showed that a malic acid ("apple acid") containing spray significantly stimulated salivary flow rate in patients using antihypertensive medication and improved their xerostomia [35]. However, this positive effect on oral dryness will be less or completely absent in Sjögren's syndrome patients with an advanced disease process.

As mentioned in the Introduction, the severity of the dry-mouth feeling, as measured with XI, may influence the preference of desired flavours. Patients with low xerostomia preferred the use of the flavour "blueberry" more than the respondents with more severe xerostomia. On the other hand, respondents' severe xerostomia preferred the use of "neutral flavours" in salivary substitutes more. This confirms the hypothesis that severity of oral dryness may play a major role in the preferred saliva substitute flavours.

A possible limitation of the current study is that the reported preferences for saliva substitutes are only based on the opinion of Sjögren's syndrome patients. However, saliva substitutes are also used by patients suffering from oral dryness due to other conditions, including patients using xerogenic medications or polypharmacy, and patients irradiated in the head and neck region [2, 36–38]. Further research should investigate whether the preferences of these other dry-mouth patients are similar to those of Sjögren's syndrome patients.

Another possible limitation of the current study is that the Sjögren's syndrome patients, who filled in this questionnaire, may be more interested in oral health than other Sjögren's syndrome patients, or suffer from more severe xerostomia. This may have resulted in an above-average oral dryness which may have affected the preferences of new saliva substitutes. Besides, it is unknown which diagnosis criteria have been used by the patients' physician to establish the diagnosis of Sjögren's syndrome, and whether they suffered from primary or secondary Sjögren's disease.

Finally, another limitation is that the actual number of participants in the current study is lower than the number calculated a priori. This indicates that the power of the current study is relatively low, and so all results in which no significant differences were found between the two age or XI-groups should be interpreted with caution.

MAIN CONCLUSION

The current study has identified preferences criteria of Sjögren's syndrome patients for various product characteristics for new saliva substitutes. The most ideal saliva, according to Sjögren's syndrome patients, has thin-watery consistency in spray form with a neutral flavour and providing long alleviation of dry mouth complaints. Besides, it should not contain artificial sweeteners or alcohol, and should not have a bitter taste or cause discoloration of the teeth.

REFERENCES

1. Roblegg E, Coughran A, Sirjani D (2019) Saliva: an all-rounder of our body. Eur J Pharm Biopharm 142:133–141. https://doi.org/ 10.1016/j. ejpb.2019.06.016
2. Saleh J, Figueiredo MA, Cherubini K, Salum FG (2015) Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol 60(2):242–255. https://doi.org/10.1016 /j.archoral https://doi.org/10.1016/j.archoralbio.2014.10.004bio. 2014. 10. 004
3. Tincani A, Andreoli L, Cavazzana I, Doria A, Favero M, Fenini MG et al (2013) Novel aspects of Sjögren's syndrome in 2012. BMC Med 11:93. https:// doi. org/ 10. 1186/ 1741- 7015- 11- 93
4. Al Hamad A, Lodi G, Porter S, Fedele S, Mercadante V (2019) Interventions for dry mouth and hyposalivation in Sjögren's syndrome: a systematic review and meta-analysis. Oral Dis 25(4):1027–1047. https:// doi. org/ 10. 1111/ odi. 12952
5. Furness S, Bryan G, McMillan R, Worthington HV (2013) Interventions for the management of dry mouth: non-pharmacological interventions. Cochrane Database Syst Rev 8:Cd009603. https://doi. org/ 10. 1002/14651 858.CD009 603. pub2
6. Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R (2011) Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev 12:Cd008934. https://doi.org/ 10. 1002/ 14651 858. CD008 934. pub2
7. Assy Z, Bikker FJ, Picauly O, Brand HS (2021) The association between oral dryness and use of dry-mouth interventions in Sjögren's syndrome patients. Clin Oral Investig. https://doi.org/10.1007/s00784- 021- 04120-2
8. Assy Z, Bots CP, Arisoy HZ, Gülveren SS, Bikker FJ, Brand HS (2021) Differences in perceived intra-oral dryness in various dry-mouth patients as determined using the Regional Oral Dryness Inventory. Clin Oral Invest. https:// doi.org/ 10. 1007/ s00784- 020- 03734-2
9. Wan H, Ma C, Vinke J, Vissink A, Herrmann A, Sharma PK (2020) Next generation salivary lubrication enhancer derived from recombinant supercharged polypeptides for xerostomia. ACS Appl Mater Interfaces 12(31):34524–34535. https:// doi. org/ 10. 1021/ acsami. 0c061 59
10. Pedersen AM, Bardow A, Jensen SB, Nauntofte B (2002) Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral Dis 8(3):117–129. https://doi.org/10.1034/j.1601-0825. 2002. 02851.x
11. Brand HS, Ouzzine R, Bots CP (2013) Speelselsubstituten: voor verbetering vatbaar!. Ned Tijdschr Tandheelkd 120(1):4
12. Brand HS, Ouzzine R, Bots CP (2013) Sticky saliva products. Br Dent J 214(3):95. https:// doi.org/ 10. 1038/ sj. bdj. 2013. 118
13. Kho HS (2014) Understanding of xerostomia and strategies for the development of artificial saliva. Chin J Dent Res: Off J Sci Sect Chin Stomatol Assoc (CSA) 17(2):75–83
14. Ali K, Gupta P, Turay E, Burns L, Brookes Z, Raja M (2022) Dentistry in a multicultural society: the impact of animal-based products on person-centred care. Br Dent J 232(4):269–272. https://doi.org/ 10. 1038/ s41415- 022- 3982-7

15. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Int J Surg 12(12):1495–1499. https://doi.org/10.1016/j. ijsu. 2014. 07. 013

16. Thomson WM, Chalmers JM, Spencer AJ, Williams SM (1999) The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health 16(1):12–17

17. Harris VM, Sharma R, Cavett J, Kurien BT, Liu K, Koelsch KA et al (2016) Klinefelter's syndrome (47, XXY) is in excess among men with Sjögren's syndrome. Clin Immunol 168:25–29. https:// doi. org/ 10. 1016/j. clim. 2016. 04. 002

18. Assy Z, Bots CP, Arisoy HZ, Gülveren SS, Bikker FJ, Brand HS (2021) Differences in perceived intra-oral dryness in various dry mouth patients as determined using the Regional Oral Dryness Inventory. Clin Oral Investig 25(6):4031–4043. https:// doi. org/ 10. 1007/ s00784- 020- 03734-2

19. Brito-Zerón P, Retamozo S, Kostov B, Baldini C, Bootsma H, De Vita S et al (2019) Efficacy and safety of topical and systemic medications: a systematic literature review informing the EULAR recommendations for the management of Sjögren's syndrome. RMD Open 5(2):e001064. https://doi.org/1 0 .1136 /r mdop en- 2019- 001064

20. López-Pintor RM, Fernández Castro M, Hernández G (2015) Oral involvement in patients with primary Sjögren's syndrome. Multidisciplinary care by dentists and rheumatologists. Reumatol Clin 11(6):387–94. https://doi.org/10. 1016/j. reuma. 2015. 03. 010

21. Pedersen AM, Bardow A, Nauntofte B (2005) Salivary changes and dental caries as potential oral markers of autoimmune salivary gland dysfunction in primary Sjogren's syndrome. BMC Clin Pathol 5(1):4. https:// doi. org/ 10. 1186/ 1472- 6890-5-4

22. Humphrey SP, Williamson RT (2001) A review of saliva: normal composition, flow, and function. J Prosthet Dent 85(2):162–169. https://doi.org/ 10. 1067/ mpr. 2001. 113778

23. Mese H, Matsuo R (2007) Salivary secretion, taste and hyposalivation. J Oral Rehabil 34(10):711–723. https://d oi.org/1 0 .1111 /j. 1365- 2842. 2007. 01794.x

24. Kalk WW, Vissink A, Spijkervet FK, Bootsma H, Kallenberg CG, Nieuw Amerongen AV (2001) Sialometry and sialochemistry: diagnostic tools for Sjogren's syndrome. Ann Rheum Dis 60(12):1110–1116. https:// doi.org/10.1136/ard. 60.12. 1110

25. Marton K, Boros I, Fejerdy P, Madlena M (2004) Evaluation of unstimulated flow rates of whole and palatal saliva in healthy patients wearing complete dentures and in patients with Sjogren's syndrome. J Prosthet Dent 91(6):577–581. https:// doi.org/ 10. 1016/j. prosd ent. 2004. 03. 031

26. Marton K, Boros I, Varga G, Zelles T, Fejerdy P, Zeher M et al (2006) Evaluation of palatal saliva flow rate and oral manifestations in patients with Sjogren's syndrome. Oral Dis 12(5):480–486. https:// doi. org/ 10. 1111/j. 1601- 0825. 2005. 01224.x

27 . Osailan SM, Pramanik R, Shirlaw P, Proctor GB, Challacombe SJ (2012) Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. Oral Surg Oral Med Oral Pathol Oral Radiol 114(5):597–603. https://doi. org/10.1016/j. oooo.2012.05. 009

28. Ergun S, Cekici A, Topcuoglu N, Migliari DA, Kulekci G, Tanyeri H et al (2010) Oral status and Candida colonization in patients with Sjogren's syndrome. Med Oral Patol Oral Cir Bucal 15(2):e310–e315. https:// doi.org/ 10. 4317/ medor al. 15. e310

29. Rusthen S, Young A, Herlofson BB, Aqrawi LA, Rykke M, Hove LH et al (2017) Oral disorders, saliva secretion, and oral health related quality of life in patients with primary Sjogren's syndrome. Eur J Oral Sci 125(4):265–271. https://doi.org/1 0 .1111 /e os.1235 8

30. Culp DJ, Stewart C, Wallet SM (2019) Oral epithelial membrane associated mucins and transcriptional changes with Sjogren's syndrome. Oral Dis 25(5):1325–1334. https://doi.org/10. 1111/odi.13098

31. Chaudhury NM, Shirlaw P, Pramanik R, Carpenter GH, Proctor GB (2015) Changes in saliva rheological properties and mucin glycosylation in dry mouth. J Dent Res 94(12):1660–1667. https://doi. org/ 10. 1177/ 00220 34515 609070

32. Generali E, Costanzo A, Mainetti C, Selmi C (2017) Cutaneous and mucosal manifestations of Sjögren's syndrome. Clin Rev Allergy Immunol 53(3):357–370. https://doi.org/10.1007/ s12016- 017- 8639-y

33. Samarawickrama DY (2002) Saliva substitutes: how effective and safe are they? Oral Dis 8(4):177–179. https:// doi.org/ 10. 1034/j. 1601- 0825.2002. 02848.x

34. Shamel M, Al-Ankily MM, Bakr MM (2019) Influence of different types of whitening toothpastes on the tooth color, enamel surface roughness and enamel morphology of human teeth. F1000Res 8:1764. https:// doi. org/ 10. 12688/ f1000 resea rch. 20811.1

35. Gómez-Moreno G, Guardia J, Aguilar-Salvatierra A, CabreraAyala M, Maté-Sánchez de-Val JE, Calvo-Guirado JL, (2013) Effectiveness of malic acid 1% in patients with xerostomia induced by antihypertensive drugs. Med Oral Patol Oral Cir Bucal. 18(1):49–55. https://doi.org/10.4317/ medor al. 18206

36. Ying Joanna ND, Thomson WM (2015) Dry mouth - an overview. Singapore Dent J 36:12–17. https://doi.org/1 0.1016 /j.s dj.2014.1 2. 001

37. Porter SR, Scully C, Hegarty AM (2004) An update of the etiology and management of xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 97(1):28–46. https:// doi.org/1 0.1016/j. tripl eo. 2003. 07. 010

38. Tanasiewicz M, Hildebrandt T, Obersztyn I (2016) Xerostomia of various etiologies: a review of the literature. Adv Clin Exp Med. 25(1):199–206. https://doi. org/ 10. 17219/ acem/ 29375

DISCUSSION AND SUMMARY

10

General discussion

GENERAL DISCUSSION

Various aetiologies cause dry mouth. Therefore, its diagnosis is complex. Despite the availability of a broad spectrum of both objective as well as subjective diagnostic tools, their total effectiveness seems insufficient and limited in discriminating between the various causes of dry mouth. In addition, the factors that affect the choice of dry-mouth interventions by patients are not fully understood. Altogether, this makes a proper diagnosis, and subsequent therapeutic advice very challenging.

The research presented in this thesis aimed to improve the assessment of dry mouth by introducing a new method for measuring the perceived dryness at various intra-oral locations. As the palatal surface plays an important role in the perception of dry mouth, the effect of the palatal surface area on the salivary distribution was investigated in particular. Also, the use of dry-mouth interventions amongst various dry-mouth patients was investigated to better understand which factors affect the choice and use of these interventions.

In brief, this thesis concluded that the newly developed questionnaire, the Regional Oral Dryness Inventory (RODI), can quantify the severity of perceived dryness at various locations in the mouth. Next, by using the RODI we were able to identify differences in patterns of perceived intra-oral dryness among different patient groups. For example, Sjögren's Syndrome (SS) patients experienced the posterior palate as most dry, while patients suffering from dry mouth due to medication experienced the anterior tongue as most dry. As for the intra-oral surface area, quantifying this surface area was possible with both cone-beam computed tomography (CBCT) as well as using an intra-oral scanner in combination with digital analysis. There was no association found between the palatal surface size and the salivary film thickness covering the palate. Another interesting finding was that the use of various dry-mouth interventions was associated with the perceived overall oral dryness or with specific pattens of intra-oral mouth dryness, indicating that severity and location of mouth dryness seem to affect the choice of dry-mouth intervention.

Developments in dry-mouth screening

The Xerostomia Inventory (XI) is an internationally validated questionnaire, used to measure the severity of mouth dryness in general, but it is not well suited to discriminate between various dry-mouth patients. However, with the help of the RODI, it is now possible to differentiate in the perceived dryness at various intra-oral locations and find patient-specific patterns as well. For example, SS patients experienced the posterior palate as most dry and had

higher RODI scores (severe dryness) for all intra-oral locations (**Chapter 3**). In comparison, medication induced dry-mouth patients experienced the anterior tongue as most dry and had lower RODI scores (less severe dryness) for all intra-oral locations (**Chapter 3**). These results can help dental clinicians to screen patients, especially those with high susceptibility for a dry mouth.

Alternatively, the combination of the RODI and the XI can also help to create a better overview of the dry-mouth complaints of individual patients and it provides deeper insight about the location where these complaints occur. In addition, it is tempting to assume that the RODI can provide a suggestion about the possible aetiology of dry mouth, however this needs to be established and validated in future studies.

The RODI may also be a helpful tool for general practitioners (GPs) to screen patients in order to get a better understanding where dry-mouth problems manifest in the mouth. Namely, a substantial number of patients will consult their GP instead of the dentist when having problems with their mouth [1]. So, informing GPs about dry mouth can significantly contribute to broaden the scope of dry mouth, and related, remedies.

In the previous mentioned research (**Chapter 3**) we explored the effect of the number of medications used by dry-mouth patients on intra-oral dryness. However, the roles of specific types of medication on intra-oral dryness was not addressed in this thesis. As medications differ in their xerogenic potential, this effect of specific types of medication needs to be addressed in future studies.

The RODI scores of particular intra-oral locations had a strong association with the total XI scores in dry-mouth patients (**Chapter 3**). For the floor of the mouth and for the anterior and posterior tongue especially, these correlations were strong (**Chapter 3**). These correlations indicate that patients which experience a very dry mouth in general (as indicated by high XI scores) will also experience more severe oral dryness at these three intra-oral locations (high RODI scores at these locations). These results indicate that the floor of the mouth and the anterior and posterior tongue play important roles in the perception of dry mouth. So, these findings emphasize the need to measure perceived intra-oral dryness with the RODI questionnaire. It can be useful in regular dental clinics to screen (new) dental patients for high susceptibility to oral dryness. Completing the RODI questionnaire is brief and easy, and it can be done in the dental waiting room or at home. If high RODI scores are obtained for specific regions (score ≥ 3), further dry-mouth diagnostics may be implemented. In some cases, when the patient has more complex dry-mouth problems, the dentist can even decide to refer a patient to a specialized saliva clinic.

For a valid diagnosis of oral dryness, a combination of several objective and subjective diagnostic tools is warranted. However, in regular dental clinics, individual tools like the XI, Clinical Oral Dryness Score and RODI are currently rarely used, which may lead to a delay in diagnosis and incomplete or ineffective care. For this reason, most of the patients with dry-mouth problems are nowadays referred to specialized saliva clinics for diagnosis and further help. These saliva clinics are therefore overloaded with huge numbers of referred dry-mouth patients. In this light, and because of the high prevalence of xerostomia and hyposalivation, we envisage that there is a need for rapid and easy to use dry-mouth diagnostics for general dental clinics.

A digital tool that combines current and new dry-mouth methods would likely make knowledge, specialty care and improved dry-mouth diagnostics within reach for a broader public. For this reason, we envisage a digital dry-mouth screening tool and/or diagnostic tool that consists of two parts. A first part should consist of a general part which registers relevant aspects of general health including health status, age, and sex, combined with questionnaires about dry mouth, stress, and psychological status. This part could be filled in by the patient, possibly at home. The second part should cover a clinical part that consists of extra-oral and intra-oral examination and/or saliva measurements which should be performed by an oral health professional. After both parts are filled in, all the information could be combined and presented in a schematic overview indicating the risk for dry mouth and the need of patients to be referred to a specialized saliva clinic (Figure 1 for a potential representation of the results). In this overview, suggestions about the possible dry-mouth interventions could also be processed. This tool would provide a clear overview of all available information and it prevents that information is missing that could influence the diagnosis or therapy. It would enable the clinician to present the collected information in a comprehensive way to the patient. When such a tool is implemented in general dental practice, a large number of patients could then be helped directly by their own dentist, without the necessity of referral to a specialised dry-mouth clinic.

Figure 1: Suggestion of a visual presentation of dry mouth risk factors in two different patients using a digital tool.

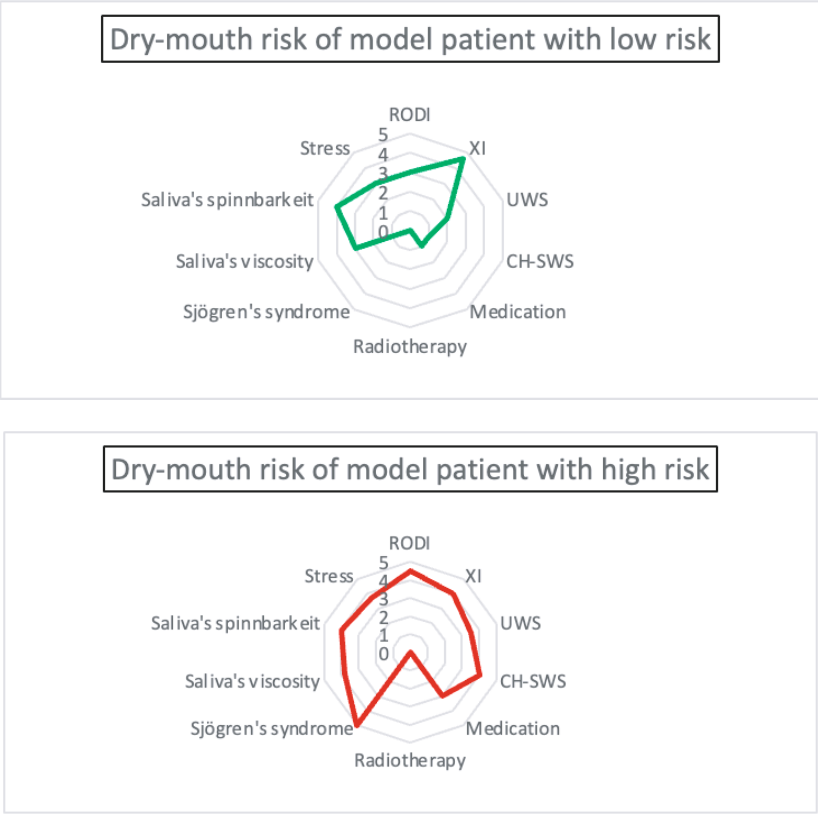
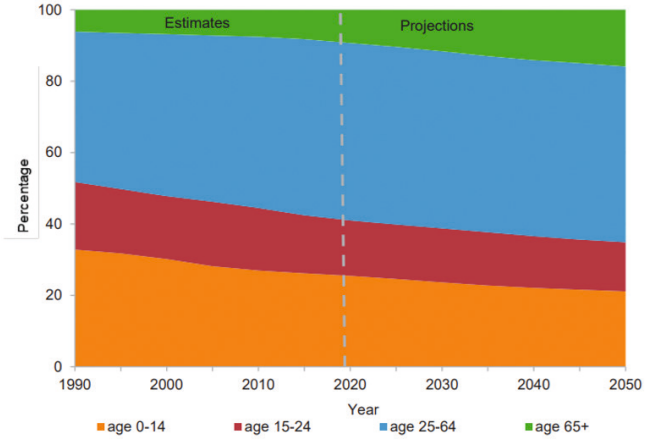


Figure 2: Global population by various age groups, 1990-2050 (percentage) [6].



In the event of broad use of such a tool for dry-mouth screening and/or diagnosis, an important prerequisite is that relevant dental education should be provided to dental students about saliva and dry-mouth-related topics. In general, the current European dental schools have included the anatomy of the salivary glands, the functions of saliva and causes of dry mouth in their dental curriculum [2], but the importance of dry-mouth diagnosis and interventions are less frequently covered. Almost half of the dental schools spend only 10 hours or less on saliva-related topics during their entire curriculum [2]. This indicates that it is important to extend the saliva-related education in the curricula of dental schools and emphasize its importance, for the benefit of current and future dry-mouth patients.

The prevalence of xerostomia is currently estimated to be approximately 20%, with higher prevalence in females (up to 30%) and in older adults (up to 50%) [3-5]. However, the number of people suffering from xerostomia will probably increase in the upcoming years due to ageing of the worldwide population (Figure 2, prognosis of the number of individuals ≥65 years in upcoming years) [6]. Therefore, the need for improved diagnostics in regular dental clinics, and better dry mouth education, is emerging.

Salivary film and MUC5B

In this thesis, the volume of the salivary film was determined at various intra-oral locations. At first, for each region of the oral mucosa a standardized filter paper (SialoPaper strips) was placed to sample the saliva fluid on the surface of interest. After a given period of time, when the saliva was immersed in the filter, the volume was be determined using the change in conductance in a Periotron [7]. However, more recently, it was shown that Schirmer test strips, traditionally used in the diagnosis of xerophthalmia, can be a helpful instrument in intra-oral dryness assessment as well [8, 9]. The Schirmer test strip consists of a 35-mm long prefabricated absorbent filter paper strip with millimeter scaling. While the test strip is collecting fluid, the advancing fluid front is visualized by the blue coloration of the indicator due to the pH of saliva, and can be read from the millimeter scale [9]. This will enable easy measurement of saliva volumes at various intra-oral locations without the need of a Periotron device. Schirmer test strips can also easily be used in saliva clinics, because no specialized equipment is needed.

In this thesis, the levels of MUC5B were measured at various intra-oral regions in healthy volunteers (**Chapter 6**). The salivary glycoprotein MUC5B plays a versatile role in maintaining oral health. It contributes to lubrication, pellicle formation, antimicrobial defense, and water retention, and its glycans are an

important nutrient for oral bacteria [10]. A recent systematic review reported that the MUC5B level in whole saliva is only significantly lower in patients who have been irradiated in the head and neck area [10]. In medication-induced dry-mouth patients and in SS patients, the MUC5B levels are not significantly lower, but the MUC5B glycosylation is significantly impaired in these patients [10]. Impaired glycosylation could negatively affect water binding, lubrication and thus salivary spinnbarkeit. Interestingly, in our studies using healthy volunteers, the MUC5B levels showed considerable variation among different intra-oral locations, with the anterior tongue having the highest levels (**Chapter 6**). Further studies on MUC5B levels at different intra-oral locations in patients with different causes of oral dryness, and investigation of the glycosylation of MUC5B at these locations, is crucial to increase our understanding about mouth dryness.

Dry-mouth interventions

There are multiple interventions to relieve mouth dryness. In this study, we investigated the effect of home care dry-mouth interventions on the perceived oral dryness and/or salivary secretion.

It was concluded that perceived dryness and intra-oral dryness in particular were associated with the use of specific dry-mouth interventions (**Chapters 7 and 8**). In medication-induced dry-mouth patients, locally applied interventions, for example "using a mouth gel", were associated with dryness of the anterior tongue in particular (**Chapter 7**). In SS patients "drinking water'', "rinsing of the mouth", and "drinking small volumes" were associated with the RODI scores of the posterior palate, and the anterior and posterior tongue, respectively. Also, the "use of a mouth gel'' was associated with the RODI scores of the inside cheeks (**Chapter 8**). These findings can be used to give a more tailored therapeutic advice for dry-mouth patients. For example, based on the obtained findings, clinicians should suggest use of a mouth gel if the anterior tongue is experienced as (severe) dry in medication induced dry-mouth patients. For SS patients by contrast, the use of a mouth gel is based on dryness of the inside cheeks. These interesting findings underline that it is also very important to further investigate the efficacy of the available dry-mouth interventions, and their frequency of use. The perceived effectiveness of the dry-mouth interventions should also be evaluated; for example, by asking the patients to rate their effectiveness on a Likert scale. As the effectiveness of dry-mouth interventions might depend on the degree to which the salivary glands are still sensitive to stimulation, data on stimulated salivary secretion of the patients should also be collected [11].

Another important aspect of dry-mouth interventions is the frequent discontinuation of the use of saliva substitutes by patients after a short period of time, mainly due to an unpleasant taste and sticky consistency. This problem is very essential and impactful in SS patients [12, 13]. In this light, investigating the preferences of dry-mouth patients before developing saliva substitutes is crucial and this information could improve the quality and efficacy of dry-mouth inventions. The present thesis only describes the preferences of SS patients (**Chapter 9**). Further investigating the preferences of other dry-mouth patient groups is also important. Especially so far the preferences of patients suffering from oral dryness due to the use of xerogenic medications or polypharmacy, as they comprise by far the largest number of patients suffering from a dry mouth.

As mentioned above, the RODI can be helpful in informing the selection of appropriate dry-mouth interventions, but it remains essential that future studies evaluate the efficacy of upcoming new dry-mouth interventions. Investigating the perceived overall mouth dryness before and after the use of a new intervention is very common in clinical studies. The added value of measuring the intra-oral dryness before, during and after the use of a specific dry mouth intervention is that it can provide detailed information about the effect on a specific intra-oral region. Current diagnostic tools may fail to detect a local improvement in oral dryness, as they focus on the overall mouth dryness. However, some interventions may not have a huge impact on the overall mouth dryness, but could possibly improve dryness at specific intra-oral locations, such as the palate or the tongue. Thus, the RODI questionnaire can have added value in evaluating the efficacy of new dry-mouth interventions in combination with measuring the overall dry mouth and objective measurement of the saliva secretion rate. It is also important to note that suggested new interventions may lack any beneficial effect on dry mouth in well-designed studies, but, even then, it remains important that clinicians and patients are informed about the findings.

Summarizing future plans and perspectives

This thesis presented that the RODI questionnaire could be helpful in various aspects of dry-mouth screening and therapy, especially in combination with the XI. To broaden its scope, it is envisaged that the RODI questionnaire should be validated in other languages.

The RODI could be used to help with the screening of medication induced dry-mouth patients; it could be possible to investigate the relationship

between specific types of medication and perceived intra-oral dryness in specific regions of the mouth. Potentially, this could help dentists and general practitioners to considered whether it is possible to substitute a medication by a similar one with fewer oral side-effects.

Not only should the perceived intra-oral dryness be studied in more detail, but it is also very important to investigate the salivary film and MUC5B levels in dry-mouth patients. In particular, the possible relationship between the perceived intra-oral dryness and the salivary film (thickness) at various intra-oral locations should be explored thoroughly. Such a study would improve our understanding of the distribution of saliva in the oral cavity and dry-mouth perception in patients with a dry mouth. An important question that could be answered is whether the salivary film (thickness), MUC5B-levels and MUC5B-glycosilation affect the perceived dryness at specific intra-oral locations.

In this thesis, novel insights about factors affecting the use of dry-mouth interventions are presented. However, additional studies are needed; for example, to further explore the use of dry-mouth interventions among various patient groups. New research could also focus on the efficacy of current and new interventions. Besides, the preferences for new saliva substitutes by medication induced dry-mouth patients should be explored further. This information might contribute to more effective treatment of dry-mouth patients in the future. Preferably, all knowledge about dry-mouth and diagnostic tools will become digitally available for clinicians. A digital application could systematically combine information from all applied diagnostic tools, and advice about treatment options based on the information provided. This will simplify dry-mouth diagnosis and could result in more effective dry-mouth interventions.

REFERENCE LIST

1. Cope AL, Wood F, Francis NA, Chestnutt IG. Patients' reasons for consulting a GP when experiencing a dental problem: a qualitative study. Br J Gen Pract. 2018;68(677):e877-e83.
2. Al-Khakany H, Brand HS. Teaching of saliva-related topics at European dental schools. J Dent Res 2018;97(Spec Iss B):2721.
3. Napeñas JJ, Brennan MT, Fox PC. Diagnosis and treatment of xerostomia (dry mouth). Odontology. 2009;97(2):76-83.
4. Millsop JW, Wang EA, Fazel N. Etiology, evaluation, and management of xerostomia. Clin Dermatol. 2017;35(5):468-76.
5. Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R. Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev. 2011;10.1002/14651858.CD008934.pub2(12):Cd008934.
6. United Nations, Department of Economic and Social Affairs, Population Division. World Population Ageing 2019: Highlights. (ST/ESA/SERA/430). 2019.
7. Villa A, Connell CL, Abati S. Diagnosis and management of xerostomia and hyposalivation. Ther Clin Risk Manag. 2015;11:45-51.
8. Saleh J, Figueiredo MA, Cherubini K, Salum FG. Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol. 2015;60(2):242-55.
9. Schoppmeier CM, Helpap J, Hagemeier A, Wicht MJ, Barbe AG. Using the modified Schirmer test for dry mouth assessment: A cross-sectional study. Eur J Oral Sci. 2022;130(4):e12880.
10. Faruque M, Wanschers M, Ligtenberg AJ, Laine ML, Bikker FJ. A review on the role of salivary MUC5B in oral health. J Oral Biosci. 2022;10.1016/j.job.2022.09.005.
11. Purdie MJ, Carpenter MD, Noll JL, Stephens CL, Taylor YJ, Hammitt KM, *et al.* Patient satisfaction and impact of salivary flow rate on effectiveness of xerostomia products. Oral Surg Oral Med Oral Pathol Oral Radiol. 2022;10.1016/j.oooo.2022.08.017.
12. Brand HS, Ouzzine R, Bots CP. Sticky saliva products. Br Dent J. 2013;214(3):95.
13. Brand HS, Ouzzine R, Bots CP. Speelselsubstituten: voor verbetering vatbaar!. Ned Tijdschr Tandheelkd. 2013;120(1):4.

11

Summary

SUMMARY

In this thesis, I performed research to improve the the current, available diagnostic tools for dry mouth by developing a new method for measuring the perceived dryness at specific various intra-oral locations. More specifically, we focussed on the role of specific intra-oral surface areas, and the salivary distribution over these areas. Furthermore, to understand which factors affect the use of dry-mouth interventions, the use of these interventions by various dry-mouth patients was investigated.

Chapter 2 described the Regional Oral Dryness Inventory (RODI), a newly developed questionnaire which quantifies the severity of dryness at various locations in the mouth. It was found that there is a significant difference in dry-mouth feeling between different intra-oral locations. The most severe oral dryness was perceived at the posterior palate and the least dry location was experienced at the floor of the mouth. We envisaged that the RODI might help to discriminate among different causes of oral dryness in patients, which is explored in **Chapter 3.** It was concluded that the RODI questionnaire was indeed able to identify differences in perceived intra-oral dryness of different patient groups. For example, both healthy volunteers as well as Sjögren's syndrome (SS) patients experienced the posterior palate as most dry. In turn, patients suffering from dry mouth due to medication experienced the anterior tongue as most dry. Besides, it was found that SS patients, including those using ≥4 medications had the highest RODI scores for all intra-oral regions, in contrast to healthy controls and dry-mouth patients using up to 4 medications. These findings suggest that the RODI questionnaire might be a useful diagnostic tool for dry-mouth diagnostics, because it can help distinguish between possible causes of oral dryness in patients.

It was postulated that the dimensions of the intra-oral surface area, especially the palatal surface area, affect the distribution of saliva over various mucosal surfaces and thereby could influence the dry mouth feeling at various intra-oral regions. This topic was explored in **Chapter 4** where the intra-oral surface areas were quantified using cone-beam computed tomography (CBCT) in combination with digital analysis. Additionally, the potential correlations between intra-oral surface areas and facial anthropomorphic measurements were investigated. At one hand, this study presented a reproducible technique for the determination of intra-oral surface areas in human cadavers. On the other hand, this study found a moderate, but statistically significant,

correlation between the palatal surface area and the length of the head. Besides, a correlation was found between the surface area of the tongue and the depth of the head. In this light, it could be envisaged that individual intra-oral surface areas can be estimated by measuring facial features, which is more convenient for the patient. Inspired by these findings, in **Chapter 5** the relation between the palatal surface area, measured using an intra-oral scanner, and anthropometric measurements was validated in living subjects. This study concluded that only in females the mandibular length and palatal width correlated with the palatal surface area.
Next, we aimed to determine the salivary film thickness and MUC5B levels at various intra-oral locations in healthy volunteers (**Chapter 6)**. MUC5B is a large salivary glycoprotein with a wide variety of hydrophilic carbohydrate side chains important for moistening, visco-elasticity and lubrication. Furthermore, measurements of the palatal surface area were executed to explore the potential relationship between the palatal surface area, the palatal salivary film thickness and MUC5B levels. It was found that the salivary film and MUC5B levels were unequally distributed over the intra-oral surface. The anterior tongue had the thickest salivary film and contained the highest levels of MUC5B, whereas the anterior palate had the thinnest salivary film with the lowest MUC5B levels. There was no association found between the palatal surface area and the salivary film thickness of the palate.

Various interventions are available to relieve oral dryness. The factors that could affect the choice and use of these interventions were studied in several chapters. In **Chapter 7** the use of dry-mouth interventions in subgroups of patients with different causes of oral dryness was investigated. Additionally, the possible relation of the applied interventions with intra-oral dryness and salivary flow rate was explored. This study concluded that various dry-mouth patients used a wide range of interventions to relieve their oral dryness. The use of these dry-mouth interventions was significantly associated with the overall dry-mouth feeling (total Xerostomia Inventory score, XI) as well as with dry-mouth feeling at different intra-oral locations (RODI scores). In medication induced dry-mouth patients, the use of interventions aimed to relieve dryness of the entire mouth, such as “drinking water” and “rinsing of the mouth”, were significantly associated with the total XI score. However, in medication induced dry-mouth patients locally applied interventions, for example “using a mouth gel”, were significantly associated with dryness of the anterior tongue in particular.

In **Chapter 8** it was found that SS patients used a wide range of interventions to relieve their oral dryness. Especially "drinking water'' was a frequently used intervention. "Drinking water'', "rinsing of the mouth", and "drinking small volumes" had significant associations with the RODI scores of the posterior palate, and the anterior and posterior tongue, respectively. The "use of a mouth gel'' had a significant association with the RODI scores of the inside cheeks. These findings provide a deeper insight into the association between the use of dry-mouth interventions and oral dryness and help clinicians to give a more specific and a patient-tailored advice on interventions for the relief of oral dryness complaints.

In **Chapter 9**, the preferences of SS patients regarding various characteristics of new saliva substitutes were evaluated, including taste, consistency, and objections against certain ingredients. The conclusion was that, according to SS patients, the most ideal saliva substitute has a thin-watery consistency in a spray form with a neutral flavor and providing long alleviation of dry mouth complaints. Additionally, it should not contain artificial sweeteners or alcohol, and should not have a bitter taste or cause discoloration of the teeth.

12

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Dit proefschrift beschrijft onderzoek dat is verricht om de diagnostische methoden van droge mond te verbeteren. We hebben ons daarbij specifiek gericht op de rol van verschillende oppervlakten in de mond die mogelijk gerelateerd zijn aan het gevoel van een droge mond, alsmede de verdeling van speeksel over deze oppervlakten. Verder is het gebruik van droge-mondinterventies (remedies en therapieën) door verschillende patiëntgroepen met droge mond klachten onderzocht, om meer inzicht te verkrijgen welke factoren de keuze voor interventies beïnvloeden.

In **Hoofdstuk 2** beschrijven we een nieuw ontwikkelde vragenlijst, de 'Regional Oral Dryness Inventory' (RODI), die de ernst van monddroogte op verschillende plaatsen in de mond in kaart brengt. Er werd gevonden dat er een verschil is in het gevoel van een droge mond tussen verschillende locaties. In algemene zin werd het achterste deel van het verhemelte als het meest droog ervaren. Daarentegen, het minst droge gebied bleek de mondbodem.

Mogelijk kan de RODI helpen om onderscheid te maken tussen verschillende oorzaken van monddroogte bij patiënten, hetgeen is onderzocht in **Hoofdstuk 3**. Er werd geconcludeerd dat we met de RODI-vragenlijst inderdaad in staat waren om verschillen tussen patiëntengroepen vast te stellen. Bij zowel gezonde vrijwilligers als patiënten met het syndroom van Sjögren (SS) werd het achterste deel van het verhemelte als het meest droog ervaren. Patiënten met een droge mond ten gevolge van medicijnengebruik ervaarden op hun beurt het voorste deel van de tong als het meest droog. Bovendien bleek dat SS-patiënten, inclusief degenen die meer dan 4 verschillende soorten geneesmiddelen gebruikten, de hoogste RODI scores hadden voor alle intra-orale regio's, in tegenstelling tot gezonde vrijwilligers en patiënten met een droge mond die maximaal 3 soorten medicijnen gebruikten. Deze bevindingen suggereren dat de RODI-vragenlijst een nuttig diagnostisch hulpmiddel kan zijn voor diagnostiek van droge mond, omdat het kan helpen onderscheid te maken tussen mogelijke oorzaken van monddroogte bij patiënten en zodoende kan helpen voor de keuze van een passende therapie.

Er werd verondersteld dat de afmetingen van het intra-orale oppervlak, in het bijzonder van het verhemelte, betrokken zijn bij de verdeling van speeksel over de orale mucosa en daardoor het droge mondgevoel in verschillende intra-orale gebieden zouden kunnen beïnvloeden. In **Hoofdstuk 4** werden de intra-orale oppervlakten in menselijke kadavers gekwantificeerd met behulp van 'cone-beam computertomografie' (CBCT). Daarnaast werden mogelijke correlaties onderzocht tussen de aldus gemeten intra-orale oppervlakten en

gezichts- en hoofdmetingen. Er werd gevonden dat er een lichte correlatie bestaat tussen het oppervlak van het verhemelte en de lengte van het hoofd. Daarnaast werd een correlatie gevonden tussen het oppervlak van de tong en de diepte van het hoofd. Op basis hiervan werd verondersteld dat het mogelijk zou kunnen zijn om bepaalde intra-orale oppervlakten bij individuen in te schatten door gelaatsafmetingen te bepalen, wat weinig belastend is voor de patiënt. Geïnspireerd door deze bevindingen werd in **Hoofdstuk 5** de relatie tussen het oppervlak van het verhemelte, gemeten met een intra-orale scanner, en metingen van het gezicht en hoofd onderzocht bij levende proefpersonen. Deze studie concludeerde dat alleen bij vrouwen de lengte van de onderkaak en breedte van het verhemelte correleerden met het oppervlak van het verhemelte. Zodoende lijkt inderdaad dat er een mogelijkheid is om door het bepalen van gezichtsafmetingen een (deel) van het mondoppervlak te bepalen. De toepassing in de praktijk lijkt echter beperkt.

Vervolgens wilden we de speekselfilmdikte en MUC5B-niveaus bij gezonde vrijwilligers bepalen op verschillende intra-orale locaties. MUC5B is een groot glycoproteïne in speeksel met een enorme verscheidenheid aan hydrofiele koolhydraatzijketens die water binden, het speeksel visco-elastische eigenschappen geven en betrokken zijn bij de smering tussen oppervlakten. Kortom, MUC5B speelt een sleutelrol in de bevochtiging van de mond. Verder werd het oppervlak van het verhemelte gemeten om de mogelijke relatie met de lokale speekselfilmdikte en MUC5B-niveaus te onderzoeken (**Hoofdstuk 6**). Er werd gevonden dat de speekselfilm en MUC5B-niveaus ongelijk verdeeld waren over het intra-orale oppervlak. Het voorste deel van de tong had de dikste speekselfilm en bevatte de hoogste niveaus van MUC5B, terwijl het voorste deel van het verhemelte de dunste speekselfilm had met de laagste MUC5B-niveaus.

Er zijn verschillende manieren (interventies) om monddroogte te verlichten. Factoren die van invloed kunnen zijn op de keuze en het gebruik van deze interventies werden bestudeerd. In **Hoofdstuk 7** werd het gebruik van droge-mondinterventies onderzocht bij subgroepen van patiënten met verschillende oorzaken van monddroogheid. Daarnaast werd de mogelijke relatie tussen toegepaste interventies met intra-orale droogheid en speekselproductie onderzocht. Deze studie concludeerde dat patiënten met een droge mond een breed scala aan interventies gebruikten om hun droge mond te verlichten. Het gebruik van deze droge-mondinterventies was significant geassocieerd met de algemene monddroogte (gemeten met de algemene droge mond vragenlijst; de Xerostomia Inventory, XI) en het droge-mondgevoel op verschillende plaatsen in de mond (gemeten met de RODI). Bij medicatie-

geïnduceerde droge mondklachten bleken interventies toegepast op het verlichten van de droogte van de gehele mond, zoals “water drinken” en “spoelen van de mond”, significant geassocieerd met de totale XI score. Bij deze patiënten waren lokaal toegepaste interventies, zoals bijvoorbeeld “gebruik van een mondgel”, daarentegen significant geassocieerd met droogheid van met name de voorzijde van de tong. In **Hoofdstuk 8** werd geconcludeerd dat SS-patiënten een breed scala aan interventies gebruikten om hun droge mond te verlichten. Met name ‘water drinken’ was een veelgebruikte interventie. ‘Water drinken’’, ‘mond spoelen’ en ‘kleine hoeveelheden drinken’ hadden significante associaties met respectievelijk de RODI scores van het achterste gehemelte, het voorste deel van de tong en het achterste deel van de tong. Het “gebruik van een mondgel” had een significante associatie met de RODI scores van de binnenkant van de wangen. Deze bevindingen geven een nader inzicht in het verband tussen monddroogte en het gebruik van droge-mondinterventies, en kunnen clinici helpen om een meer specifiek, op de individuele patiënt toegesneden advies te geven over interventies ter verlichting van monddroogte klachten.

In **Hoofdstuk 9** werden de voorkeuren van SS-patiënten met betrekking tot nieuw te ontwikkelen speekselsubstituten geïnventariseerd, qua smaak, consistentie en bezwaren tegen specifieke ingrediënten. Volgens SS-patiënten heeft het ideale speekselsubstituut een dun-waterige consistentie in een sprayvorm, met een neutrale smaak en geeft langdurig verlichting van droge mondklachten. Bovendien mag het geen kunstmatige zoetstoffen of alcohol bevatten, geen bittere smaak hebben of verkleuring van de tanden veroorzaken.

APPENDICES

CONTRIBUTIONS OF THE AUTHORS

Chapter 2

Regional differences in perceived oral dryness as determined with a newly developed questionnaire, the Regional Oral Dryness Inventory:
All authors (Z. Assy, D.H.J. Jager, E. Mashhour, F.J. Bikker, H.S. Brand) contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Z. Assy and E. Mashhour. The first draft of the manuscript was written by Z. Assy, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Chapter 3

Differences in perceived intra-oral dryness in various dry-mouth patients as determined using the Regional Oral Dryness Inventory:
All authors (Z. Assy, C.P. Bots, H.Z. Arisoy, S.S. Gülveren, F.J. Bikker, H.S. Brand) contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by Z. Assy, H.Z. Arisoy and S.S. Gülveren. The first draft of the manuscript was written by Z. Assy, and all the authors commented on successive versions of the manuscript. All the authors read and approved the definitive manuscript.

Chapter 4

Determination of intra-oral surface areas by cone-beam computed tomography analysis and their relation with anthrometric measurements of the head:
Z. Assy contributed to conception, design, data acquisition and interpretation, performed all statistical analyses, drafted and critically revised the manuscript; C. Klop contributed to conception, data acquisition and interpretation and critically revised the manuscript; H.S. Brand contributed to conception, design, contributed to analysis and critically revised the manuscript; R.C. Hoogeveen contributed to conception, design and critically revised the manuscript; J.H. Koolstra contributed to conception, design and critically revised the manuscript; F.J. Bikker contributed to conception, design, data interpretation, drafted and critically revised the manuscript.

Chapter 5

Correlations of palatal surface area with anthropometric dimensions of head and face:
All authors (Z. Assy, D.H.J. Jager, H.S. Brand, F.J. Bikker) contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by Z. Assy. The first draft of the manuscript was written by Z. Assy, and all authors commented on successive versions of the manuscript. All authors read and approved the definitive manuscript.

Chapter 6

Salivary Film Thickness and MUC5B Levels at Various Intra-Oral Surfaces:
All authors (Z. Assy, D.H.J. Jager, H.S. Brand, F.J. Bikker) contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by Z. Assy. The first draft of the manuscript was written by Z. Assy, and all authors commented on successive versions of the manuscript. All authors read and approved the definitive manuscript.

Chapter 7

The relationship between the severity of oral dryness and the use of dry-mouth interventions by various subgroups of dry-mouth patients:
All authors (Z. Assy, H.S. Brand, C.P. Bots, F.J. Bikker) contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by Z. Assy. The first draft of the manuscript was written by Z. Assy, and all authors commented on successive versions of the manuscript. All authors read and approved the final manuscript.

Chapter 8

The association between oral dryness and use of dry-mouth interventions in Sjögren's syndrome patients
All authors (Z. Assy, F.J. Bikker, O. Picauly, H.S. Brand) contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by Z. Assy and O. Picauly. The first draft of the manuscript was written by Z. Assy, and all authors commented on successive versions of the manuscript. All authors read and approved the definitive manuscript.

Chapter 9

Preferences of Sjögren's syndrome patients regarding potential new saliva substitutes

All the authors (Z. Assy, F.J. Bikker, E. Mashhour, M. Asadi, H.S. Brand) contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by Z. Assy, E. Mashhour, and M. Asadi. The first draft of the manuscript was written by Z. Assy, and all the authors commented on successive versions of the manuscript. All the authors read and approved the definitive manuscript

ACKNOWLEDGEMENTS

Doing this PhD trajectory is a privileged opportunities in my life. Throughout this journey I have learned a lot about saliva, dry mouth and scientific research techniques. However, most importantly were the people I met during this journey. I am really grateful for everything you did to enrich my journey and all the things you are still doing for me! There are no words to describe my gratitude to you all, thank you so much!

I am honoured to thank some people personally for their significant and positive effect during my PhD trajectory.

Prof. dr. Bikker, dear Floris (known as professor saliva, or in Dutch as 'professor spuug'). There are very few professors like you; you are full of knowledge, always helpful, always kind and respectful, and you always challenge me and other department members to be excellent scientific researchers. You really have it all! The most important thing I learnt from you is your advice about scientific writing: 'heden verse eieren te koop'. Each time I write a manuscript I think about this saying, and I am always trying to implement it as much I can. Your positive vibes are contagious, and they not only have a positive effect on me, but also the whole Biochemistry crew. For this reason, Oral Biochemistry is one of the best departments for research. Thank you for everything and especially your confidence in me!

Dr. Brand, dear Henk. Doing research with you is a very worthful experience! You have a lot of expertise in multiple scientific-related fields and that makes you a wonderful supervisor. Especially your statistical knowledge is something I am really jealous about. I am still trying to be as statistically as good as you are. You helped me to overcome my challenges and not just seeing them as difficulties that were facing my path. In the beginning of my PhD trajectory writing a rebuttal letter was stressful, but with your advice I know how to approach these letters with significantly less stress and anger. Besides these academic skills, you are helpful and friendly. I know you recently reached a milestone, as you now have >200 publications. However, I think the number of 250 is even more special. So, keep the hard work so you can reach this 250 even before your retirement. Thank you so much!

Dr. Jager, dear Derk-Jan. I really enjoyed joining you during your saliva clinic. Each time I was fascinated by your knowledge about the various dry-mouth aetiologies and dry-mouth interventions. For this reason, I learned a lot after visiting your saliva clinic, especially the way you approach and inform your patients. Your support revising my manuscripts was much appreciated especially given your lack of time and plentiful responsibilities. Your feedback

on my manuscripts had a clinical perspective and that greatly improved the clinical value of these manuscripts. I really appreciate your trust in including me in the Salivary Research group. I am very grateful for everything!

Dr. Bots, dear Caper. Unfortunately, I did not see you that much during my PhD trajectory. However, your feedback was helpful and improved the manuscripts even more. Reading your positive feedback made me happy. Thank you so much!

Committee members

I would like to thank the committee members for their time and effort reading and reviewing my PhD thesis. I am really grateful, thank you so much **Prof. dr. M.L. Laine, Prof. dr. A. Vissink, Prof. dr. T. Forouzanfar, Prof. dr. W.M. Thomson, Prof. dr. B. O'Connell**!

Oral biochemistry team

We really have a wonderful team that always try to help and improve each other. All my gratitude goes to **Ir. Kamran Nazmi**, who helped me a lot with my laboratory activities, especially with the ELISA technique. I was privileged to work with someone with such unbelievable knowledge of the lab. There is 'De Aardige Pers', but **Kamran** you are 'De Slimme Pers'.

Thank you, Dr. Toon Ligtenberg, for all help during my PhD trajectory; your door was always open for my questions. Practicing my presentations with you and the feedback you shared helped me to improve the quality of these presentations. Thank you so much, **Toon**!

The 'icing on the cake' of the Oral Biochemistry is **Dr. Wendy Kaman**! You totally flourished the department; your laugh, funny stories and positive vibes were making our days even more beautiful. You helped me a lot with feedback and questions about my presentations. I really enjoy sitting near you in the same office now. Thank you for everything you did for me, **Wendy**!

All my love and gratitude go to my fellow PhD candidates in department of Oral Biochemistry: **Saskia**, **Mouri**, **and Cuicui**. You are not just colleagues, but also lovely and kind friends. The most important thing I gained is having you as friends in my life. You listen to me, and you gave me always helpful advices. **Ladies**, you are my support; a million thanks ladies for all the beautiful memories together!

Dear **Hanan**, I wished I could have seen you more frequently. You are so funny, and I enjoy all our talks together.

Dear **Annina** although we have only known each other for a short period of time, I really enjoy your company! I am glad we share the same thoughts about certain topics.

Dr. Farhad Rezaee I really enjoyed the talks with you, especially our political talks! You are 'De Aardige Pers' of our department.

Family and friends

I would like to thank the most important person; my mum, **Alia Hussein**. Words cannot describe my gratitude for your help, support and effort during my PhD trajectory and during my whole life. You listened to all my presentations multiple times, and you never complained about hearing the same presentation multiple times. You are really a Super-Mum! I cannot reward you for being such a wonderful mum, thank million times!

Also, my lovely **family** in particular my uncles, aunts and cousins thank you for our support in this process. Thank you, so much dear family, for everything you did or said to help me! Because we are such a big family, I did not include all family member names, though one special person should be mentioned. My dearest uncle **Mahdi Al-Mussawi**, we all really miss you! Although you are not with us anymore; you will be always remembered and cherished in our thought.

I would like to thank **all my friends** for their support and help during the past years! A special thank for my two lovely paranymphs:

Dear **Sherien,** you supported me and my mum in difficult times. We are grateful for all your help and support. You are more than a wonderful friend; you are really a family member to us. All my love to you and **Nour**.

Dear **Eliška**, since secondary school we shared wonderful memories together. Although we do not speak or see each other frequently, still you are a special person to me. I really want to share this once-in-a-lifetime experience with you. Hopefully we can enjoy a lifelong friendship together!

Thank you so much **Sherien** and **Eliška** for everything!!

ACTA PhD Council (APC)

Dear **Danuta, Mouri, Thiprawee, Yuqing**; you were not just fellow members of the APC, but you are now good friends. The effort and time you put into the APC meetings and activities had a far-reaching positive effect on the PhD community. You are nice and lovely ladies, thank you so much for everything!!

Cariology team

All the members of the Cariology department; you are amazing colleagues! Thank you for your understanding and support.

ABOUT THE AUTHOR

Zainab Assy was born on 22nd of August 1989 in Bagdad, Iraq. After finishing her secondary education (VWO) at the Oostvaarders College in Almere in 2009, she started studying Dentistry at the Academic Centre of Dentistry Amsterdam (ACTA). Her bachelor and master thesis had both saliva-related topics. Her Bachelor thesis was entitled the 'the effect of fruit chewing gums on salivary pH', while her Master thesis systematically discussed the effect of acupuncture on xerostomia and hyposalivation. In July 2016, she received her master's degree in Dentistry. In the same year, she started working as a dentist-teacher at the department of Cariology (ACTA) and also in dental practice as a dentist. Subsequently, she adjusted her Master thesis into a scientific article under the supervision of Dr. Henk Brand. Her enthusiasm for saliva-related research topics led to a successful nomination by Prof. Floris Bikker and Dr. Henk Brand (from the department of Oral Biochemistry, ACTA) in their research grant funded by the Nederlands Tijdschrift voor Tandheelkunde (NTVT) in 2018. Consequently, Zainab started her PhD trajectory entitled 'Diagnosis of dry-mouth symptoms' at the Department of Oral Biochemistry (ACTA) under the supervision of Prof. Floris Bikker (ACTA), Dr. Henk Brand (ACTA), and Dr. Derk-Jan Jager (Department of Oral and Maxillofacial Surgery / Oral Pathology, Amsterdam UMC) in January 2019. During her PhD trajectory she conducted her research whilst continuing to work as a dentist-teacher. Zainab currently continues her saliva-related research in the Department of Oral Biochemistry (ACTA).

LIST OF PUBLICATIONS

Assy Z, Brand HS. A systematic review of the effects of acupuncture on xerostomia and hyposalivation. BMC Complement Altern Med. 2018 Feb 13;18(1):57. doi: 10.1186/s12906-018-2124-x. PMID: 29439690; PMCID: PMC5811978.

Assy Z, Klop C, Brand HS, Hoogeveen RC, Koolstra JH, Bikker FJ. Determination of intra-oral surface areas by cone-beam computed tomography analysis and their relation with anthrometric measurements of the head. Surg Radiol Anat. 2020 Sep;42(9):1063-1071. doi: 10.1007/s00276-020-02530-7. Epub 2020 Jul 11. PMID: 32653942; PMCID: PMC7363725.

Assy Z, Brand HS, Ligtenberg AJM. De relatie tussen xerostomie en speekselsecretie bij jongvolwassenen. Ned Tijdschr Tandheelkd. 2020 Oct;127(10):573-580. Dutch. doi: 10.5177/ntvt.2020.10.19121. PMID: 33156300.

Assy Z, Jager DHJ, Mashhour E, Bikker FJ, Brand HS. Regional differences in perceived oral dryness as determined with a newly developed questionnaire, the Regional Oral Dryness Inventory. Clin Oral Investig. 2020 Nov;24(11):4051-4060. doi: 10.1007/s00784-020-03276-7. Epub 2020 May 7. PMID: 32382921; PMCID: PMC7544722.

Assy Z, Jager DHJ, Mashhour E, Bikker FJ, Brand HS. Intra-orale verschillen in ervaren monddroogte, bepaald door middel van de Regional Oral Dryness Inventory. Ned Tijdschr Tandheelkd. 2020 Nov;127(11):635-638. Dutch. doi: 10.5177/ntvt.2020.11.20074. PMID: 33252605.

Assy Z, Brand HS, Bikker FJ. Helpt acupunctuur bij een droge mond? Ned Tijdschr Tandheelkd. 2021 Jun;128(6):307-309. Dutch. doi:10.5177/ntvt.2021.06.21038. PMID: 34096929.

Assy Z, Bots CP, Arisoy HZ, Gülveren SS, Bikker FJ, Brand HS. Correction to: Differences in perceived intra-oral dryness in various dry-mouth patients as determined using the Regional Oral Dryness Inventory. Clin Oral Investig. 2021 Nov;25(11):6475. doi: 10.1007/s00784-021-04200-3. Erratum for: Clin Oral Investig. 2021 Jun;25(6):4031-4043. PMID: 34591182; PMCID: PMC9172830.

Assy Z, Bikker FJ, Picauly O, Brand HS. The association between oral dryness and use of dry-mouth interventions in Sjögren's syndrome patients. Clin Oral Investig. 2022 Feb;26(2):1465-1475. doi: 10.1007/s00784-021-04120-2. Epub 2021 Aug 10. PMID: 34374853; PMCID: PMC8816756.

Assy Z, Brand HS, Bots CP, Bikker FJ. The relationship between the severity of oral dryness and the use of dry-mouth interventions by various subgroups of dry-mouth patients. Clin Oral Investig. 2022 Mar;26(3):3097-3108. doi: 10.1007/s00784-021-04292-x. Epub 2022 Jan 10. PMID: 35006295.

Assy Z, Jager DHJ, Brand HS, Bikker FJ. Salivary film thickness and MUC5B levels at various intra-oral surfaces. Clin Oral Investig. 2023 Feb;27(2):859-869.

Assy Z, Jager DHJ, Brand HS, Bikker FJ. Correlations of palatal surface area with anthropometric dimensions of the head and face. Surg Radiol Anat. 2022 Sep;44(9):1261-1267. doi: 10.1007/s00276-022-03008-4. Epub 2022 Sep 2. PMID: 36056237; PMCID: PMC9492607.

Assy Z, Bikker FJ, Mashhour E, Asadi M, Brand HS. Preferences of Sjögren's syndrome patients regarding potential new saliva substitutes. Clin Oral Investig. 2022 Oct;26(10):6245-6252. doi: 10.1007/s00784-022-04576-w. Epub 2022 Jun 11. PMID: 35688954; PMCID: PMC9525427.