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Associations between xerostomia, histopathological alterations, and autonomic innervation of labial salivary glands in men in late midlife

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ABSTRACT

Objective: One aim of the present study was to investigate whether symptoms of oral dryness (xerostomia) during daytime, assessed in a study group of middle-aged male positive and negative outliers in cognition scores, were associated with age-related degenerative changes in human labial salivary glands and with quantitative measures of the glandular autonomic innervation. Another aim was to study the relation between the autonomic innervation and loss of secretory acinar cells in these glands.
Methods: Labial salivary gland biopsies were taken from the lower lip from 190 men, born in 1953 and members of the Danish Metropolit birth cohort, who were examined for age-related changes in cognitive function and dental health as part of the Copenhagen University Center for Healthy Aging clinical neuroscience project. The glands were routinely processed and semi-quantitatively analyzed for inflammation, acinar atrophy, fibrosis, and adipocyte infiltration. Sections of labial salivary gland tissue were stained with the panneuronal marker PGP 9.5. In a subsample of 51 participants, the autonomic innervation of the glands was analyzed quantitatively by use of stereology.
Results: Labial salivary gland tissue samples from 33% of all participants displayed moderate to severe acinar atrophy and fibrosis (31%). Xerostomia was not significantly associated with structural changes of labial salivary glands, but in the subsample it was inversely related to the total nerve length in the glandular connective tissue. Acinar atrophy and fibrosis were negatively correlated with the parenchymal innervation and positively related to diffuse inflammation.
Conclusions: The results from the present study indicate that aspects of the autonomic innervation of labial salivary glands may play a role in the occurrence of xerostomia which in the present study group was not significantly associated with degenerative changes in these glands. The findings further indicate that the integrity of labial salivary gland acini is related to the parenchymal autonomic innervation, whereas inflammatory processes may compromise it by alternative mechanisms.

1. Introduction

Subjective symptoms of oral dryness, designated as xerostomia, may arise from significantly reduced saliva production, hyposalivation or salivary compositional changes that lead to altered rheological and moisturizing properties of saliva. Moreover, xerostomia is often a side effect to medical treatment or may be caused by certain diseases including the autoimmune disorder Sjögren's syndrome, exocrine dysfunction, neurological disorders or diabetes mellitus (Fox et al., 1985; Jensen et al., 2010; Navazesh and Ship, 1983; Pedersen et al., 2002; Smidt et al., 2011). Age has also been shown to be a contributing factor, since a marked increase in the frequency of xerostomia has been observed between the years of 50 to 65 (Johansson et al., 2009) and the prevalence is high among elderly persons (Smidt et al., 2011; Sreebny, 2000). Persons who suffer from persistent xerostomia are at risk of impaired oral and dental health, altered taste sensation, difficulties in

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swallowing and speech, and in the long run, the condition may lead to a significantly impaired quality of life (Fox et al., 1985; Navazesh and Ship, 1983; Pedersen et al., 2002).

Labial salivary glands belong to the group of minor salivary glands, which together with the sublingual glands are responsible for the largest mucin-release (about 70%) into saliva (Milne and Dawes, 1973). Mucins are large glycoproteins with lubricating, viscoelastic, adhesive and antimicrobial properties, which coat all oral tissues and protect against desiccation and injuries (Tabak et al., 1982). A lack of these substantial components in saliva may be considered as one possible cause of xerostomia (Tabak, 1995; Tabak et al., 1982), since symptoms of oral dryness are abundant among persons who display low saliva production from mucin secreting glands, such as the sublingual and submandibular glands (Fox et al., 1987). Labial salivary glands receive primarily parasympathetic innervation (Rossoni et al., 1979) and as demonstrated by a morphofunctional study, mucous acinar cells release mucins in response to cholinergic stimulation (Riva et al., 2002). The important role of parasympathetic cholinergic stimulation in mucous secretion has also been shown in a study on rat sublingual glands, which resemble human minor and sublingual glands (Culp et al., 1991). Thus, it is possible that parasympathetic dysfunction may play a role in the etiology of xerostomia that is related to insufficient amounts of mucins in saliva. Human labial salivary glands are known to undergo age-related structural changes (De Wilde et al., 1986; Drummond and Chisholm, 1984; Scott, 1980; Syrjänen, 1984; Vered et al., 2000), which include atrophy of the parenchymal secretory acinar cells, fibrosis, diffuse inflammation or focal lymphocytic as well as to a certain extent fat infiltration. The autonomic nerves, which innervate salivary glands, exert influence on the integrity and maintenance of the glandular parenchyma (Proctor and Carpenter, 2007). However, not much is known about the extent to which the glandular innervation or parenchymal integrity of minor, mucous secreting glands, relates to the presence of xerostomia.

In a parallel investigation we have observed that the point-prevalence of daytime xerostomia was 22% in a study group of middle-aged outliers in cognition scores and that it was not significantly associated with lowered unstimulated whole saliva flow rates (Sørensen et al., unpublished results). The aims of the present study were therefore to investigate in the same study group whether the occurrence of xerostomia was associated with age-related degenerative changes in human labial salivary glands and to explore a potential role of the glandular autonomic innervation in the etiology of xerostomia. A further aim was to investigate the interrelation between autonomic nerves and acinar atrophy and fibrosis in labial salivary glands.

2. Materials and methods

2.1. Study participants

The present study is part of the Copenhagen University Center for Healthy Aging clinical neuroscience project (Hansen et al., 2014), and was performed on 195 volunteer male participants, all born in 1953 and recruited from the Metropolit Cohort of the Copenhagen Aging and Midlife Biobank (CAMB) project (Osler et al., 2006). The participants had been originally selected as two groups of male, middle-aged positive and negative outliers in cognition scores, based on results from two previous cognitive assessments as described elsewhere (Hansen et al., 2014; Osler et al., 2012). They took part in a parallel clinical dental examination, during which the dental health status was registered, saliva flow rates were measured, and symptoms of oral dryness (xerostomia) were assessed by a questionnaire. The present part of the study deals with the occurrence of xerostomia during daytime, including morning, which was evaluated by use of 4 scores, ranging from no oral dryness (score 0) to severe daytime xerostomia (score 3). Data from 5 men were not included due to dropout (n = 1), retrospectively not fulfilling inclusion criteria (n = 1) and not consenting to have taken a labial salivary gland biopsy (n = 3). Exclusion criteria comprised diagnosed neurodegenerative and psychiatric disorders as well as brain lesions and abusive alcohol and drug consumption. The participants were both in writing and orally informed, and gave written consent to take part in this study. The study protocol was approved by the local Danish Ethical Committees (no. H-3-2010-016).

2.2. Labial salivary gland biopsy and tissue preparation for immunohistochemistry

As part of the parallel clinical dental examination, labial salivary gland biopsies were taken under regional anesthesia (30 mg/ml Citanest® Dental Octapressin®, Dentsply Pharmaceutical, Surrey, England) from the submucosa of the lower lip as previously described (Pedersen et al., 1999). The biopsies were fixed in 10% neutral buffered formalin (pH 7.3, 24 h 20 °C), dehydrated in graded alcohols and embedded in paraffin wax. Sections of 2 μm were cut by use of a microtome (Leica, Germany) and stained with hematoxylin and eosin for routine histopathological examination. The degree of the different histopathological changes was evaluated semiquantitatively by graded scores; 0 (none), 1 (slight), 2 (moderate) and 3 (severe). The presence of focal sialadenitis was determined by calculating the focus score defined as the number of lymphocytic foci per 4 mm^2 of tissue following the lines laid down by Daniels (1984). A lymphocytic focus refers to a well defined, closely packed focal aggregation of at least 50 inflammatory cells, mainly lymphocytes (Daniels, 1984). Whole labial salivary glands, which were used for stereological analyses were cut into serial sections of alternating 7 and 50 μm thickness. Out of these, 7 μm thin sections were stained with hematoxylin and eosin for routine histopathological examination. Sections of labial salivary gland tissue used for the innervation study were mounted on glass slides (Superfrost®) and stored at 4 °C until use for immunohistochemistry.

2.3. Pretreatment and immunostaining procedure

The tissue sections were deparaffinized in Tissue-Tek® Tissue-Clear® (Sakura Finetek Europe) and rehydrated in a series of descending alcohol. They were transferred to Tris-EDTA-buffer, pH 9.0 and pretreated for antigen retrieval by microwave irradiation at 100 °C (Samsung, at 600 W for 5 min and 300 W for 15 min). The tissue sections were cooled to room temperature and washed in Tris-buffered saline (TBS). For the following immunostaining procedure the Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse kit (K5007, Dako, Denmark) was used according to the manufacturer's instructions. All steps were performed at room temperature. Endogenous peroxidase was blocked (S2023, Dako, Denmark), followed by rinse in TBS. The sections were incubated for 60 min with a primary antibody against the neuronal marker protein gene product 9.5 (PGP 9.5) (polyclonal rabbit anti-PGP 9.5, code number Z5116, Dako, Denmark), diluted 1:700 in antibody diluent (S2022, Dako, Denmark). The sections were rinsed in TBS and incubated for 30 min with secondary antibody, conjugated with horse radish peroxidase labeled polymer, followed by rinse in TBS. Bound antigen was visualized with the substrate-chromogen 3,3′-diaminobenzidine (DAB, diluted 1:50). The tissue sections were counterstained with Carazzi's hematoxylin (Ampliqon, Denmark) and mounted in Pertex® mounting medium (HistoLab, Sweden).

2.4. Stereological analysis of serial sections

The following parameters were estimated in each labial salivary gland: The total volumes of the entire gland, the parenchyma (including the interlobar connective tissue), and the remaining connective tissue, including fat tissue; the length density (length per volume) of PGP 9.5-positive bundles of nerve fibers in the entire gland, in the parenchyma, and in the connective tissue, as well as the volume fraction of parenchyma relative to the total gland volume; the total lengths of nerve

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