

Variations in the Expression and Distribution Pattern of AQP5 in Acinar Cells of Patients with Sialadenosis

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Abstract. *Previously, we pointed out on a possible role of aquaporin 5 (AQP5) in the development of sialadenosis. The goal of the present study was to further assess the association of AQP5 in the development of this salivary gland disease. The acinar diameter and mean surface area appeared elevated in sialadenosis tissues, which is a typical observation in this disease. AQP5 expression was evaluated by immunohistochemistry using tissue samples derived from salivary glands of patients with confirmed sialadenosis either as a primary diagnosis or as a secondary diagnosis within the framework of other salivary gland diseases. Normal salivary gland tissue served as a control. In sialadenosis tissues, the AQP5 signal at the apical plasma membrane of acinar cells frequently appeared stronger compared with that in normal salivary glands. In addition, the distribution of AQP5 at the apical region seemed to differ between normal and sialadenosis tissues, where AQP5 frequently was diffusely distributed near or at the apical plasma membrane of the acinar cells in contrast to normal controls where the AQP5 signal was strictly confined to the apical plasma membrane. These observations suggest that sialadenosis is associated with a different AQP5 expression and distribution pattern in salivary acinar cells.*

Sialadenosis is defined as a recurrent, non-inflammatory, non-neoplastic, soft, often painless, usually bilateral swelling

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of the major salivary glands particularly of parotid glands (1). Histopathological examination typically shows no signs of inflammation but reveals enlarged acinar cells, reaching sizes of up to 100 µm in diameter (1). The exact underlying pathomechanisms leading to sialadenosis are still unknown, although it is frequently found associated with other diseases, such as diabetes mellitus, diabetes insipidus and alcoholism. We have previously reported to sialadenosis of the major salivary glands in a patient with central diabetes insipidus treated with antidiuretic hormone (ADH) (2). Observations in salivary gland tissue samples of this patient indicated a possible involvement of aquaporin 5 (AQP5) in the development of sialadenosis. AQP5 is a close homologue of renal AQP2, which is regulated by ADH and is a key player in the pathogenesis of diabetes insipidus (3). Aquaporins are a group of water-channel proteins that are involved in rapid transport of water across cell membranes. They were discovered by Agre and co-workers in the late 1980s (4, 5). So far, more than 10 different aquaporins have been characterized, mainly in epithelial cells and in secretory cells involved in water balance. Several studies reported the expression of different aquaporins in human salivary glands (6, 7). AQP5 is mainly found at the apical plasma membrane of salivary gland acinar cells (7). As well as water secretion for saliva production, AQP5 also appears to be implicated in cell volume regulation (8, 9). The present study aimed to further assess the potential role of AQP5 in patients with sialadenosis.

Materials and Methods

Tissue samples. Parotid gland tissues from nine patients with confirmed sialadenosis, either as a primary diagnosis or as a secondary diagnosis within the framework of other salivary gland diseases, all treated at the Department of Otolaryngology, University

Table I. Characteristics of patients and tissue specimens derived from salivary glands demonstrating sialadenosis as a primary or secondary diagnosis.

#	Diagnosis	Sialadenosis		Age (years)	Gender
		Primary diagnosis	Secondary diagnosis		
1	Sialadenosis	+	-	70	Female
2	Sialadenosis	+	-	58	Male
3	Sialadenosis	+	-	88	Female
4	Sialadenosis	+	-	53	Male
5	Sialadenosis	+	-	32	Female
6	Squamous cell carcinoma, sialadenosis of adjacent salivary gland tissue	-	+	60	Male
7	Oropharyngeal cancer, sialadenosis of adjacent salivary gland tissue	-	+	50	Male
8	Pleomorphic adenoma, sialadenosis of adjacent salivary gland tissue	-	+	62	Male
9	Cystadenolymphoma, sialadenosis of adjacent salivary gland tissue	-	+	51	Male

Hospital Marburg, Germany, were analyzed in this study (Table I). The study was approved by the local Ethics Committee at the School of Medicine, Philipps University Marburg, Germany (#149/07). In all patients, history was recorded particularly considering the current medication and level of alcohol intake, as well as the presence of metabolic or endocrine disorders. In five patients, sialadenosis was the primary diagnosis; they presented with clinical asymptomatic unilateral or bilateral swellings of the parotid gland. The spectrum of additional diseases included hypertension, heart failure, alcoholism, malnutrition in ulcerative colitis and bulimia. In four patients, sialadenosis was considered as a secondary diagnosis found concurrent with salivary gland diseases (Table I). Healthy human salivary gland tissue served as a control. The tissue was routinely-fixed in buffered formalin and embedded in paraffin. Sections with a thickness of 4-6 µm were generated with a microtome (Leica, Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) and used for immunohistochemical analyses.

Immunohistochemistry. Immunohistochemical analysis was performed according to a standardized protocol using purified goat polyclonal antibodies directed against a peptide mapping to the carboxy terminus of AQP5 of human origin (sc-9890; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). As a secondary antibody, purified polyclonal immunoglobulins raised against goat IgG (Santa Cruz Biotechnology, Inc.) were used. Having removed the paraffin and rehydrated the sections, endogenous peroxidases were blocked with methanol containing 0.1% (v/v) H₂O₂ for 30 min. For antigen retrieval, the sections were heated in sodium citrate buffer (pH=6) in a microwave oven for 15 min at 600 W. Non-specific binding was prevented by incubating the sections in 3% BSA in PBS. The sections were incubated overnight at 4°C with the primary antibody (dilution 1:10) in Dako Antibody Diluent (Dako, North America, Inc., Carpinteria, CA, USA). Sections were washed thrice in PBS incubated with a species-specific secondary antibody for 45 min at 37°C, washed several times, and incubated for 30 min with the ABC reagent (Dako). Diaminobenzidine (Dako) was used for visualization of the signal. Specimens were evaluated by light microscopy (Olympus, Hamburg, Germany), taking into account signal strength and localization of AQP5.

Measurement of acinar diameter. Additionally, the average acinar diameter and acinar surface area of sialadenosis and control tissues

were examined by digitized image analysis using an Olympus Microscope AX70 (Olympus) and the MCID Elite software (Inter Focus Group, Linton, UK). The diameter and surface area of at least six acini per tissue sample was measured. From these values, the average value per patient was calculated and the mean and standard deviation were assessed for each of the two groups. ANOVA was used for statistical comparison.

Results

The acinus diameter (Figure 1A) and mean surface area (Figure 1B) were found to be increased in salivary gland tissues from patients when compared to healthy controls, which is a characteristic finding in sialadenosis (1). Since the differences did not reach significance in the ANOVA test (diameter: $p=0.090$, and surface area: $p=0.100$), which partly could be due to the limited number of samples, this observation has to be viewed as a trend. Tissues from almost the entire group of patients exhibited an AQP5 signal at the apical region of acinar cells, which differed in strength. In sialadenosis tissues, the AQP5 signal typically appeared stronger than in normal salivary glands (Figure 2), although in one case (Figure 2, #6), AQP5 was barely detectable. In addition to these observations, the distribution of AQP5 at the apical cell surface was frequently different between normal and sialadenosis tissues, with AQP5 being diffusely-distributed near or at the apical plasma membrane of the acinar cells in the latter, whereas in controls, it was typically more tightly-confined to the apical plasma membrane (Figure 2).

Discussion

The observations of this study point to an increased expression of AQP5, which is in line with the initial report (2), as well as an abnormal distribution of AQP5. Sialadenosis is associated with a number of diseases, in particular endocrine, metabolic and dystrophic disorders. According to Donath and Seifert, the disease is divided into

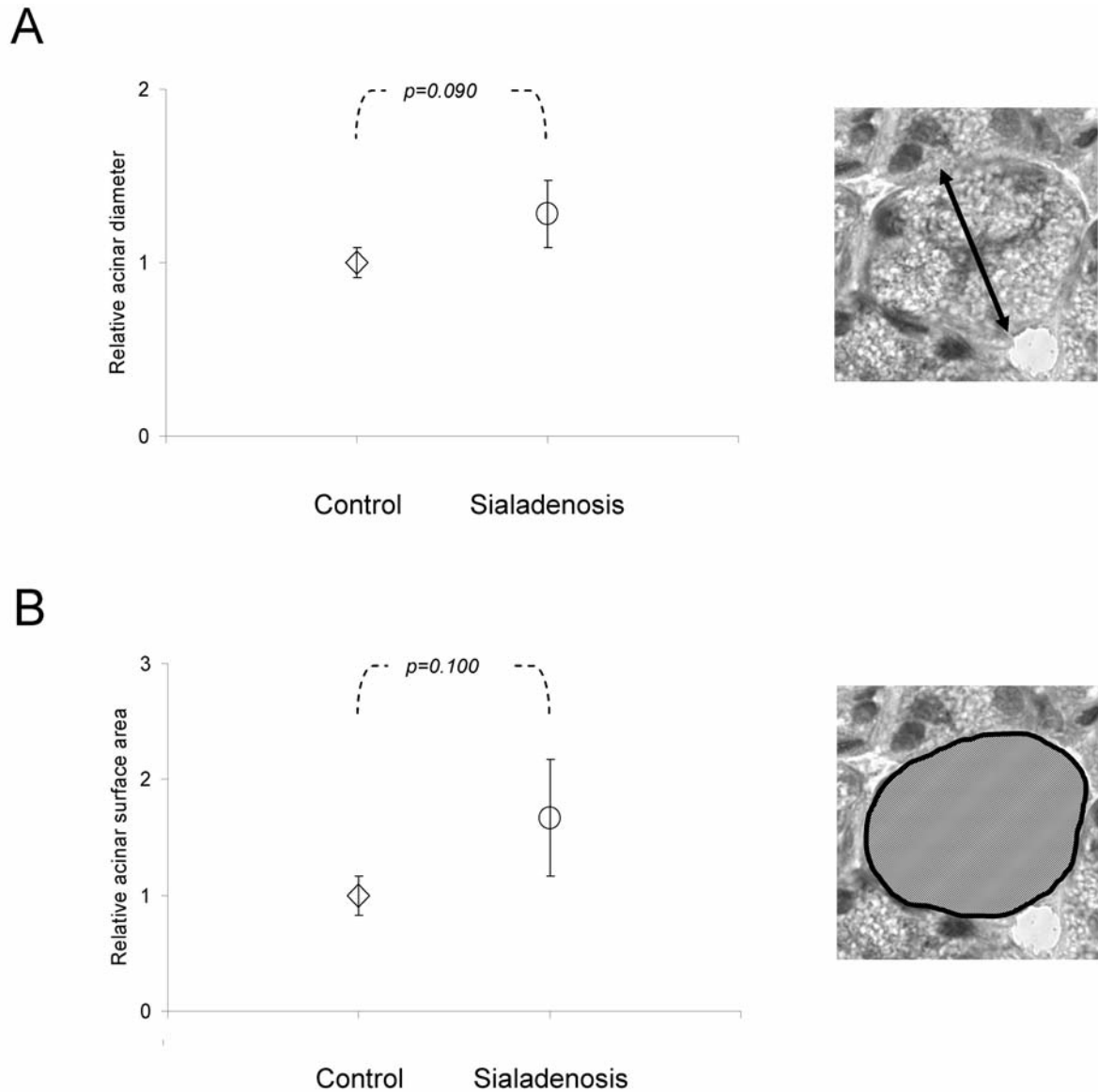


Figure 1. Measurement of acinar diameter and surface area. Mean acinar diameter (A) and surface area (B) are increased in patients with sialadenosis. Differences did not reach significance.

light, dark and mixed-grain sialadenosis based on electron microscopic observations (1). In their study, neural degeneration, observed within salivary gland tissues of patients with sialadenosis, was also implicated as a potential contributing factor to the development of this salivary gland disease. AQP5 water channels, which are found predominantly at the apical plasma membrane of both serous and mucous acinar cells, have been described to be the only salivary aquaporin playing a clear functional role in salivary secretion (7, 10, 11). Studies with *Aqp5*-knockout mice demonstrated that reduced hypertonic, viscous saliva is produced after stimulation with pilocarpine. This suggests

that AQP5 plays a key role in salivary fluid secretion (8). AQP2, which was isolated in the collecting tubules of the kidney, is subject to a regulatory effect by the hormone ADH. In the presence of ADH, AQP2 is inserted into the apical membrane of the collecting duct, which leads to water retention (3). In our initial report, we observed sialadenosis of the salivary glands in a patient with central diabetes insipidus treated with ADH. In this case report, increased AQP5 labelling was observed in the apical region of acinar cells in the diseased salivary gland in comparison to normal salivary gland tissue (2). This finding suggested a possible pathophysiological role of AQP5 in the development of

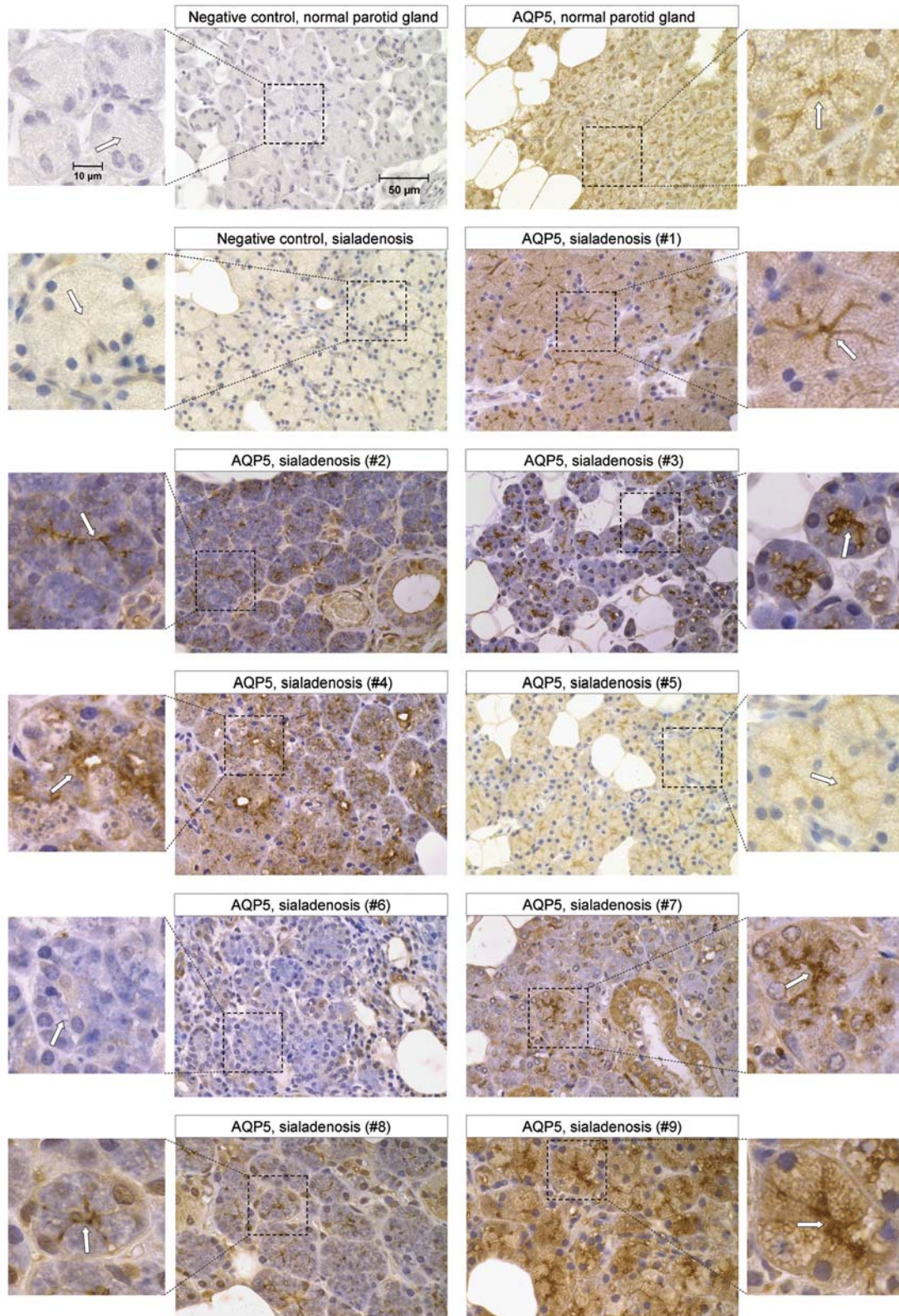


Figure 2. Changes in the expression and distribution pattern of aquaporin 5 (AQP5) in sialadenosis tissues. Differences in the AQP5 distribution pattern were frequently observed in parotid tissues from patients with sialadenosis (#1-5 and 7-9) (see also Table I) compared with normal control tissues. No apical staining of AQP5 can be seen in the negative control of normal and sialadenosis tissues. Arrows indicate the apical region of acinar cells. Depicted in the first image are bars for size comparison.

sialadenosis. In the present study, strong AQP5 labelling was frequently detected in acinar cells of patients with sialadenosis while it was only moderate in healthy controls. This observation further supports a role of AQP5 in the pathogenesis of sialadenosis. In addition to the different signal strength, a different distribution of the AQP5 signal between sialadenosis and normal parotid tissue was noted. In healthy salivary glands, localization of AQP5 appeared particularly restricted to the apical membrane of acinar cells which confirmed previous studies (11, 12). In sialadenosis tissue, the distribution of AQP5 in the apical region of the acinar cells seemed less restricted, indicating that the localization of this aquaporin could be different in acinar cells from patients with sialadenosis compared with normal acinar cells. This diffuse distribution could reflect an increased localization of AQP5 in apical intracytoplasmic vesicles. In animal experiments, translocation of AQP5 from intracytoplasmic vesicles into the apical plasma membrane after stimulation with isoproterenol points to the previously suspected role of the autonomic nervous system as a pathophysiological factor in sialadenosis (1, 7, 13).

Moreover, the abnormal distribution of AQP5 likely contributes to changes in cell volume regulation of acinar cells, which is a typical morphological feature in sialadenosis. Krane *et al.* showed that in *Aqp5*-knockout mice the capacity for cell volume regulation is significantly reduced (9). Accordingly, one could imagine that an increased expression of AQP5 is associated with an increase in volume of acinar cells. In the present study, an increase in acinar cell volume in sialadenosis tissue compared to normal parotid gland tissue was observed.

This study points to differences in the expression levels and subcellular distribution of AQP5 in salivary glands from patients suffering from sialadenosis compared to healthy controls. Since sialadenosis is a relatively rare disease, it would be necessary to consider multicenter studies in the future to statistically demonstrate the association of AQP5 with the development of sialadenosis. Further studies, *e.g.* on isolated acinar cells, should be helpful to more-accurately quantify differences in AQP5 levels between salivary gland tissues. Moreover, correlation of differences in the AQP5 distribution with subcellular structures of acinar cells could potentially aid in the understanding of the underlying pathomechanisms leading to sialadenosis.

Competing Interests

The Authors declare that they have no competing interests.

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