学 位 論 文 の 要 旨

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学 位 論 文 名　Expression of Aquaporin 3 and 5 Has Potential as a Marker for Distinguish Patients with Dry Mouth and Sjögren's Syndrome

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論 文 内 容 の 要 旨

INTRODUCTION

Xerostomia is the subjective sensation of mouth dryness as oppose to hyposalivation that refers to objective decrease in salivary secretion. As xerostomia has various symptoms that are detected by different examination methods, standardized methods for diagnosis and treatment have not been established. One of the most common salivary gland impairment diseases is Sjögren's syndrome (SS). It is not always easy to distinguish SS from other xerostomia.

Aquaporin (AQP) is a family of water-specific membrane channel proteins found in almost all internal organs. Some investigators have demonstrated that changes in the localization pattern of AQP5 cause the salivation disorder of SS patients. However, the expression, localization, and role of AQP3 and -5 in the salivary gland have been controversial.

We hypothesize that expression of AQP3 and -5 has potential as a marker to distinguish patients with dry mouth and SS.

MATERIALS AND METHODS

Participants characteristics

From September 2011 through March 2014 at the Department of Oral and Maxillofacial Surgery, Shimane University Hospital, samples were collected from twenty five patients with a chief complaint of dry mouth (DM). All patients underwent labial minor salivary gland biopsy (DM, n = 9; SS, n = 16; control, n = 8). According to the revised Japanese criteria of SS, if a focus score (the number of lymphocytes containing at least 50 in a 4-mm^2 glandular section, by hematoxylin and eosin staining) greater than 1 and at least one other criteria were positive, it was diagnosed as SS. Nine patients who did not fit the diagnostic criteria of SS and had included

hyposalivation and xerostomia were diagnosed DM, eight patients who had cystectomy of the lower lip mucus retention cyst without radiotherapy for head and neck, complaints or symptoms of DM, hyposalivation, and medication were used as normal control.

Oral examination

All patients were interviewed about their past medical history and subjective oral symptoms.

Salivary flow volume

Gum tests and saxon tests were performed for all patients. Hyposalivation was defined as a flow rate with ≤ 10 mL in 10 min by gum test or with ≤ 2 g in 2 min by Saxon test.

AQP3 and -5 mRNA expression levels using qRT-PCR

A total of 33 frozen samples were analyzed for AQP3 and -5 transcript expression by qRT-PCR. The relative levels of AQP3 and -5 mRNA were determined using beta-actin as the internal control.

Immunohistochemical staining and image analysis

All sections were examined using a standard light microscope with a ×40 objective lens. The following antibodies were used: rabbit polyclonal anti-C–terminus of human AQP3, rabbit monoclonal anti-C–terminus of human AQP5, and secondary antibody. Four square shape area (3×3 mm) including the apical, basolateral, and ductal cell membranes and cytoplasm of the labial minor salivary gland were randomly selected on the screen. The intensity of AQP3 and -5 immunoreactivity was then evaluated using Image J software by analyzing the brightness of each pixel in RGB images, with high values indicating weak intensity and low values indicating strong intensity. The mean intensity of the four square shape area was caluculated.

Statistical analysis

Results were compared among the groups using the Wilcoxon rank sum test or Kruskal-Wallis test (when the number of groups was respectively 2 or ≥3) for continuous variables, and Fisher's exact test for categorical variables. To investigate whether the combination of expression levels of AQP3 and -5 in certain regions distinguished the three groups, we performed analysis by conditional inference tree. A *p*-value of less than or equal to 0.05 was considered statistically significant. All statistical analyses were performed using SAS (Version 9.3, Cary, NC, USA) and R (Version 3.2.2, R Foundation, Vienna, Austria) with "ctree" as library.

The study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects.

RESULTS AND DISCUSSION

Participant characteristics and oral examination findings

The participants comprised 33 cases (3 male, 5 female, 25.0 ± 25.2 years old), 9 with DM (3 male, 6 female, 67.1 ± 13.1 years old), and 16 with SS (16 female, 67.7 ± 11.8 years old). Significant differences for age and sex were found among groups ($p = 0.005$ and $p = 0.014$). A greater proportion of patients with DM and SS had oral dryness, dry eye and DM and tongue, compared with controls ($p < 0.005$ for all).

Salivary flow volume

Significant differences were found among groups for the Saxon test ($p = 0.004$). There was significant difference between control and SS among the three participant groups ($p = 0.005$).

Expression of AQP3 and -5 mRNA

There was no significant difference in mRNA expression of AQP3 or -5 among the three participant groups.

Immunohistochemical intensity of AQP3 and -5

The immunoreactive intensity of AQP3 in the apical cell membrane of acinar cells and the ductal cell membrane was significantly stronger in the SS group than in the control and DM groups ($p = 0.037$ and $p = 0.022$). Immunoreactive intensity of AQP5 in the apical cell membrane of acinar cells and the cytoplasm was significantly weaker in the DM group than in the control and SS groups ($p = 0.011$ and $p = 0.009$). Regarding the immunoreactive intensity of AQP3 in the apical cell membrane of acinar cells, there was significant difference between control and SS ($p = 0.005$). Regarding the immunoreactive intensity of AQP5 in the apical cell membrane, there was significant difference between control and DM, DM and SS ($p = 0.018$, $p = 0.005$). The immunoreactive intensity of AQP5 in the cytoplasm, there was significant difference between control and DM, DM and SS ($p = 0.018$, $p = 0.004$). Tree analysis showed that a combination of AQP3 ($p = 0.011$) and AQP5 ($p = 0.016$) in the apical cell membrane of acinar cells was able to distinguish the three participant groups.

Relationship between immunoreactive intensity and mRNA expression level of AQP3 and -5 among three groups

In our study, tree diagram analysis showed that high immunoreactive intensity of both AQP3 and -5 in the apical cell membrane of acinar cells may indicate SS, but expression level of AQP3 and -5 mRNA was not significantly difference among the three groups. Therefore, the distribution of AQP3 and -5 in the apical cell membrane of the minor salivary gland, not the expression level of AQP3 and -5, would have a significant effect on the diseases and may be a key factor to distinguish between SS and DM.

CONCLUSION

This preliminary study showed the expression of AQP3 and -5 has potential as a marker for distinguishing DM and SS.