学 位 論 文 の 要 旨

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学 位 論 文 名　Immunohistochemical Expression of Filaggrin Is Decreased in Proton Pump Inhibitor Non-responders Compared With Proton Pump Inhibitor Responders of Eosinophilic Esophagitis

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論 文 内 容 の 要 旨

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic allergic inflammatory disease in which many eosinophils infiltrate into the epithelial layer of esophagus with an increasing number of patients all over the world. However, the mechanisms of barrier deficiencies of the squamous epithelium leading to immune responses in EoE are still incompletely understood.

The genes encoding protein molecules such as filaggrin, loricrin and involucrin that are expressed in the squamous epithelium are clustered in the chromosome 1q21. These proteins are essential for epidermal differentiation and constitute epidermal differentiation complex (EDC). Many allergic skin diseases are known to be related to decreased expression of filaggrin. Recently, it has also been reported that downregulation of EDC genes contributes to the mechanism of development of EoE

Clinically, EoE is divided into proton pump inhibitor-non-responders (PPI-NR) and PPI-responders (PPI-R). Our main aims were to investigate the differences of expression of EDC proteins and desmoglein that are considered to play important roles in formation of the epidermal skin barrier between these two conditions and to seek the usefulness of the differences in pathological diagnosis. Conventional histopathological findings and allergic background were also compared between PPI-NR and PPI-R.

MATERIALS AND METHODS

Twenty-nine PPI-NR, PPI-R and 35 reflux esophagitis (RE) patients were enrolled after searching the database of Shimane University Hospital between 2005 and 2018. The patients were defined as PPI-R when their intraepithelial eosinophilic infiltration decreased to less than 15/HPF and the symptoms were completely relieved after 2 months of PPI therapy. If the symptoms were not relieved by the same therapy, they were defined as PPI-NR. After clinical information including the patients' age, gender and allergic background and histopathological findings were reviewed, immunohistochemical expression of EDC proteins (filaggrin, loricrin, and involucrin) and desmoglein in all three groups were examined and semi-quantitatively scored by combining scores of proportions of positive cells and staining intensity: the proportion score was determined in each of the four categories as 0 (no staining), 1 (up to 33%), 2 (34 to 66%) and 3 (67% or more) and the staining intensity was also scored as 0 (no staining), 1 (weak positivity) and 2 (strong positivity). The combined staining scores were calculated and employed for statistical analysis. For statistical analysis, Bonferroni's multiple comparison test was performed, based on the data obtained by one-way analysis of variance (ANOVA) to determine which of the three between-group differences were statistically significant. Statistical significance was defined as $p<0.05$. All the analyses were carried out using SPSS (version 23.0, IBM SPSS Inc., Chicago, IL). The study protocol was approved by the Ethics Committee of Shimane University (approval number: 20190715-1).

RESULTS AND DISCUSSION

Regarding allergic conditions including asthma, food allergy, atopic dermatitis, rhinitis and hay fever, the prevalence of asthma was significantly higher in PPI-NR than in PPI-R. The other four allergic conditions showed no differences between the two groups. Histopathological findings we examined were the numbers of eosinophils, lymphocytes, neutrophils and mast cells and epithelial inflammatory changes represented by basal cell hyperplasia and spongiosis. They all did not exhibit statistical difference between PPI-NR and PPI-R. However, as for immunohistochemical findings, immunostaining score of filaggrin in PPI-NR was significantly lower than in PPI-R, while the expressions of involucrin, loricrin and desmoglein demonstrated no differences. Utilizing the expression of filaggrin, we set the staining score cut-off to 2. Sensitivity, specificity, positive predictive value and negative predictive value of the score less than two, which means 0, for PPI-NR were 79%, 68%, 62%, and 83%, respectively. Since PPI-NR had a stronger association with asthma than PPI-R, positive predictive value increased to 80% when asthmatic background of EoE was taken into account (PPI-NR with asthma: 15 cases, PPI-R with asthma: 8

cases), though negative predictive value decreased to 63%. Sensitivity and specificity remained unchanged.

It has been reported with molecular biological and immunohistochemical approaches that the expression of filaggrin is reduced in EoE. However, comparison of expression of EDC proteins between PPI-NR and PPI-R has not yet been reported. In the present study, we have demonstrated for the first time that the immunohistochemical expression of filaggrin in PPI-NR is significantly lower than in PPI-R.

In clinical practice, the patients of EoE are often treated with PPI, but it is now well known that the drug is ineffective for about half of them. According to our results, it is speculated that an influx of larger number of allergens into the epithelium in PPI-NR due to decreased expression of filaggrin than in PPI-R could overcome either anti-inflammatory or acid blocking effect of PPI treatment. In contrast, since PPI-R retain more filaggrin than PPI-NR, PPI administration can improve the barrier function of the epithelium more readily, resulting in remission of EoE. The difference of filaggrin expression between PPI-NR and PPI-R may have an impact on making clinical decisions. When the cutoff of 2 was chosen for immunostaining score of filaggrin, sensitivity, specificity, positive predictive value and negative predictive value for PPI-NR were relatively high. Although these values were not yet sufficient to separate the two conditions accurately, pathologists with the immunostaining results could helpclinicians to decide when to start topical steroid treatment or vonoprazan, a novel potassium-competitive acid blocker for EoE patients, before irreversible changes occur in the subepithelial tissue.

CONCLUSION

The present results suggest a role of reduced filaggrin expression in the difference of effectiveness of PPI treatment between PPI-NR and PPI-R. Moreover, immunohistochemical determination of filaggrin expression in EoE patients could be informative in clinical decision of how to treat the patients.