Evaluation of the Efficacy of Loricrin as a Diagnostic Marker in Patients with Oral Submucous Fibrosis

Niva Mahapatra, Kailash C. Dash, Lipsa Bhuyan, Abiksheet Panda, Shyam S. Behura, Pallavi Mishra

Department of Oral and Maxillofacial Pathology, Kalinga Institute of Dental Sciences, Kalinga Institute of Industrial Technology, Deemed to Be University, Bhubaneswar, Odisha, India

Introduction: Loricrin is usually observed in abundance in keratinizing epithelium in response to mechanical stress, which may be associated with development and malignant transformations in conditions such as oral submucous fibrosis (OSMF). Therefore, understanding of various molecular mechanisms associated with difference in gene expressions between OSMF and that of normal oral tissue is important. Aim: The aim of this study was to evaluate of the efficacy of loricrin as a diagnostic marker in patients with OSMF. Materials and Methods: Fifty formalin-fixed paraffin-embedded tissue blocks were obtained from the archives of the department. The study sample was grouped into two groups of normal mucosa (group I; n = 20) and OSMF (group II; n = 30) specimens. The study tissues were immunohistochemically stained with loricrin antibody and were further graded on basis of staining intensity. Results: Loricrin immunostaining was observed significantly more in OSMF cases and even in stratum granulosum in comparison to normal mucosa. Conclusion: Loricrin can act as an early indicator and a prognostic marker for detection of deleterious changes within epithelium in OSMF.

Keywords: Areca nut, immunohistochemistry, loricrin, normal mucosa, oral submucous fibrosis

Received: 30-01-2020. Revised: 04-02-2020. Accepted: 02-03-2020. Published: 28-08-2020.

INTRODUCTION

Oral submucous fibrosis (OSMF), one of the potentially malignant disorders, by definition is a chronic insidious disease affecting any part of the oral cavity and sometimes the pharynx, occasionally, preceded by and/or associated with vesicle formation and is always associated with a juxta-epithelial inflammatory reaction followed by progressive hyalinization of the lamina propria.[1,2] Association with the habit of chewing areca nut is one of the key etiological agents leading to development of OSMF. Though multifactorial model has been proposed which causes both direct and indirect effects and also mediates the immune system,[1,4] epidemiologically, over a period of 17 years, the risk of its conversion to oral squamous cell carcinoma is 7.6%.[9]

Various studies are being directed to understand the molecular mechanisms related with development and malignant transformations in OSMF. Therefore, understanding of various molecular mechanisms associated with difference in gene expressions between OSMF and that of normal oral tissue is important. Many molecular biomarkers that can act as potential diagnostic markers are known and many are under research. Under these, certain proteins are also known these days to be useful as potential markers for improved outcome of the patients.

Address for correspondence: Dr. Kailash Chandra Dash, Department of Oral and Maxillofacial Pathology, Kalinga Institute of Dental Sciences, Kalinga Institute of Industrial Technology, Deemed to Be University, Bhubaneswar, Odisha, India. E-mail: kcdash1986@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Mahapatra N, Dash KC, Bhuyan L, Panda A, Behura SS, Mishra P. Evaluation of the efficacy of loricrin as a diagnostic marker in patients with oral submucous fibrosis. J Pharm Bioall Sci 2020;12:S264-7.
One of such proteins is loricrin, which is a key component of the cytokeratin envelop of human epidermis and constitutes about 85% of a fully differentiated keratinocyte. They belong to a multigene family with greater than 30 intermediate filament genes contributing to the cytoskeletal barrier of the epithelial cells.[6-9] By immunohistochemical (IHC) methods, expression of loricrin has been detected in stratum granulosum within keratinized epithelium subjected to trauma or stress.[7] This fact has led to an assumption that loricrin may have role in barrier function of such lesions.

Therefore, this study was undertaken with an aim to evaluate the efficacy of loricrin as a diagnostic marker in patients with OSMF.

**Materials and Methods**

This study is a retrospective, cross-sectional IHC study that was carried out in the Department of Oral and Maxillofacial Pathology, Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha. A total sample comprising 50 formalin-fixed paraffin-embedded tissue blocks was selected from the archives of the department. The study sample was divided into two major groups comprising normal mucosa (group I; \( n = 20 \)) and diagnosed cases of OSMF (group II; \( n = 30 \)) specimens. Complete data of the patients including their history were obtained from the departmental records. Ethical clearance for the conduct of the study was obtained from the institutional ethical committee.

Two sections each were obtained from selected tissue blocks and were further stained using routine hematoxylin and eosin (H and E) staining and IHC staining. For immunohistochemistry, loricrin polyclonal antibody (Abcam Allied Scientific, Cambridge, MA) was used as a primary antibody. The antibody was obtained in a concentrated form and had to be diluted in a concentration of ratio 1:500 and was then incubated for an hour at room temperature.

**IHC assessment**

In normal mucosa, loricrin immunostaining was restricted to stratum granulosum and stratum corneum of stratified squamous epithelium in keratinized mucosa. Absence of any staining was observed in non-keratinizing epithelium. Within the cells, it is expressed together in cytoplasm and nucleoplasm. Each positive slide was scored according to the intensity of staining as follows: 0 or negative staining was given in absence of any stain, (+) was given as mild staining intensity, (++) was given as moderate staining intensity, and (+++) was intensively stained slide.

**Statistical analysis**

Data obtained were tabulated and analyzed using SPSS, version 17.0 (SPSS Inc, IBM, IL). Pearson's chi-square test was applied for group comparison. \( P \) value < 0.05 was taken as statistically significant. Kappa analysis was used for comparing loricrin staining among tissues.

**Results**

A total of 50 cases were included in the study, which were further grouped as group I with 20 cases of normal mucosa and group II with 30 cases of OSMF. Male population predominated, accounting for 76% of the study population whereas female population was just 24%. Thus, a male to female (M:F) ratio of 3.2:1 was obtained.

Staining positivity: Group I (normal mucosa) showed negative staining in 85% (\( n = 17 \) out of 20) of cases while it was mild positive in 15% (\( n = 3 \) out of 20) cases. Group II (study group/OSMF group) overall showed IHC positivity in 67.5% (\( n = 27 \) out of 40) cases whereas a negative staining was observed in 32.5% (\( n = 13 \) out of 40) cases. This variation in results was statistically also significant (\( P < 0.05 \)) [Graph 1].

Staining intensity: Distribution of staining intensity among both the groups was graded as no staining, mild, moderate, and intense staining. Group I (normal mucosa) showed negative staining in 85% (\( n = 17 \) out of 20) of cases whereas it was of mild intensity in 15% (\( n = 3 \) out of 20) cases. Within study group (OSMF cases), negative staining intensity was observed in 32.5% (\( n = 13 \) out of 40) cases. Mild staining was observed in...
17.5% (n = 7) of the cases whereas moderate staining intensity was observed in 30% (n = 12) and intense staining intensity was observed in 20% (n = 8) cases. On comparison, the difference among groups was statistically also significant (<0.05) [Graph 2].

**Staining index among different strata of epithelium**

Further we analyzed the staining index in all three strata’s of epithelium.

Within stratum spinosum: Normal mucosa did not show any staining in stratum spinosum. In OSMF, stratum spinosum did not show any staining in 80% (n = 38) of cases, whereas mild staining was observed in 5% (n = 2) of cases, moderate staining was observed in 7.5% (n = 3) of cases, and intense staining was observed in 7.5% (n = 3) of cases. The difference in results obtained was statistically not significant (P > 0.05).

Stratum granulosum: Normal mucosa showed no staining in 85% (n = 17) cases whereas mild staining was seen in 25% (n = 3) cases. Whereas in OSMF, stratum granulosum did not show any staining in 40% (n = 18) cases, while mild staining was observed in 15% (n = 6) cases, moderate staining was observed in 27.5% (n = 11) cases, and intense staining was observed in 12.5% (n = 5) cases. The difference in results obtained was statistically not significant (P > 0.05).

Stratum corneum: Normal mucosa showed no staining in 100% of cases. Whereas in OSMF, stratum corneum did not show any staining in 87.5% (n = 35) cases, while both mild staining and moderate staining were observed in 15% (n = 2 cases each) cases each, and intense staining in 2.5% (n = 1) of the cases. The difference in results obtained was statistically not significant (P > 0.05).

**Discussion**

Enhanced expression of loricrin is usually absent in non-keratinizing mucosa, but if its expression is observed in such mucosa (like in OSMF), it implies that the mucosa is under stress and exposed to harmful agents. Enhanced loricrin expression is meant to provide a protective barrier against traumatic stimuli. Its expression in keratinizing epithelia also depicts mechanical stress, such as human foreskin epidermis.

The pathogenesis behind this increased expression has been explained by previous authors, stating that the differentiation of keratinocytes in cases of OSMF is due to exposure of oral mucosa to harmful stimuli of both chemical and mechanical nature due to usage of slaked lime and areca nut chewing. Continuous microtrauma due to areca nut chewing affects the signaling mechanisms within the epithelium, which in turn disrupts the protection barrier. This is further accelerated by increased calcium concentration due to slaked lime (calcium hydroxide). Enhanced loricrin expression is hereby a compensatory mechanism by the oral mucosa.

In our study, a total sample of 50 cases was included of which 20 cases were of normal mucosa and 30 cases were of OSMF. We observed that male patients outnumbered female patients with an M:F ratio of 3:2:1.

In this study, staining positivity of loricrin in normal mucosa was 15% whereas in OSMF staining positivity was 67.5%. This was statistically also significant (P < 0.05). In similarity to our study, Li et al.[16] reported 63.6% loricrin positivity in OSMF cases. Similarly, Nithya et al.[14] also reported 66.7% loricrin positivity in OSMF cases in their study.

Staining intensity in this study showed that normal mucosa showed negative staining in 85% of cases, whereas mild intensity was seen in 15% cases and that also within stratum granulosum and no staining in stratum spinosum. In accordance to our study, Katou et al.,[17] Li et al.,[16] and Nithya et al.[14] reported similar findings.

Further, within study group (OSMF cases) negative staining intensity was observed in 32.5%. Mild staining intensity was observed in 17.5% of the cases whereas moderate staining intensity was observed in 30% and intense staining intensity was seen in 20% (n = 8) cases. On comparison, the difference among groups was also significant. Stratum spinosum showed mild staining in 5% cases, moderate staining in 7.5% cases, and intense staining in 7.5% cases as well. Stratum granulosum showed mild staining in 15% cases, moderate staining in 27.5% cases, and intense staining in 12.5% cases [Table 1].

This showed that positive mild staining was seen in stratum spinosum in comparison to normal mucosa, and in stratum granulosum predominantly moderate type of staining was observed in comparison to normal mucosa. Thus, increased expression in superficial layers of stratified epithelium was seen in OSMF in comparison to normal mucosa. These findings are in concordance with the findings of Li et al.[16] and Nithya et al.[14]

| Groups                      | Positivity (%) | Negativity (%) |
|-----------------------------|----------------|---------------|
| Group I (n = 20; normal mucosa) | 15             | 85            |
| Group II (n = 30; OSMF)     | 67.5           | 32.5          |

Table 1: Immunostaining positivity of loricrin within study groups

Mahapatra, et al.: Loricrin as a diagnostic marker in patients with OSMF
Nithya et al.\[14\] reported that usually expression of loricrin is restricted to the stratum granulosum within keratinized epithelium and the epidermis and it is usually absent within non-keratinized epithelium. Their study demonstrated positive staining in stratum spinosum of OSMF cases and justified their findings by saying that the reasons could be the following: first, due to epithelial atrophy there is decrease in cell density; second, due to microtrauma; and finally due to decrease in retinoic acid receptors.

Another study by Ishida et al.\[18\] also reported that loricrin IHC expression in OSMF was seen in the superficial layers of stratum spinosum. Li et al.\[16\] conducted a microarray analysis and observed that loricrin was one of the three top upregulated genes in their study.

**CONCLUSION**

Thus, from our study we can conclude that loricrin can act as an early indicator and a prognostic marker for detection of deleterious changes within epithelium due to continuous physical and chemical trauma. But we suggest further more detailed studies with increased sample size to validate the results of our study.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007;36:575-80.

2. Rajendran R. Oral submucous fibrosis: etiology, pathogenesis, and future research. Bull World Health Organ 1994;72:985-96.

3. Bari S, Metgud R, Vyas Z, Tak A. An update on studies on etiological factors, disease progression, and malignant transformation in oral submucous fibrosis. J Cancer Res Ther 2017;13:399-405.

4. Meghji S, Warnakulasuriya S. Oral submucous fibrosis: an expert symposium. Oral Dis 1997;3:276.

5. Sharma R, Raj SS, Miahra G, Reddy YG, Shenava S, Narang P. Prevalence of oral submucous fibrosis in patients visiting dental college in rural area of Jaipur, Rajasthan. J Indian Acad Oral Med Radiol 2012;24:1-4.

6. Wakamatsu K, Ogita H, Okabe N, Irie K, Tanaka-Okamoto M, Ishizaki H, et al. Up-regulation of loricrin expression by cell adhesion molecule nectin-1 through rap1-ERK signaling in keratinocytes. J Biol Chem 2007;282:18173-81.

7. Nithya S, Radhika T, Jeyd N. Loricrin—an overview. J Oral Maxillofac Pathol 2015;19:64-8.

8. Odani T, Ito D, Li MH, Kawamata Ai, Isobe T, Iwase M, et al. Gene expression profiles of oral leukoplakia and carcinoma: genome-wide comparison analysis using oligonucleotide microarray technology. Int J Oncoi 2006;28:619-24.

9. Presland RB, Jurevic RJ. Making sense of the epithelial barrier: what molecular biology and genetics tell us about the functions of oral mucosal and epidermal tissues. J Dent Educ 2002;66:564-74.

10. Elias PM. Stratum corneum defensive functions: an integrated view. J Invest Dermat 2005;125:183-200.

11. Hohl D, Mehrel T, Lichit U, Turner ML, Roop DR, Steinert PM. Characterization of human loricrin. Structure and function of a new class of epidermal cell envelope proteins. J Biol Chem 1991;266:6626-36.

12. Yoneda K, Hohl D, McBride OW, Wang M, Cehrs KU, Idler WW, et al. The human loricrin gene. J Biol Chem 1992;267:18060-6.

13. Candi E, Melino G, Mei G, Tarcesa E, Chung SI, Marekov LN, et al. Biochemical, structural, and transglutaminase substrate properties of human loricrin, the major epidermal cornified cell envelope protein. J Biol Chem 1995;270:26382-90.

14. Nithya S, Joshua E, Kannan R, Thavarajah R, Rao UK. Loricrin expression and its implication in oral submucous fibrosis, hyperkeratosis and normal mucosa with association to habits—an immunohistochemical study. J Oral Biol Craniofac Res 2019;9:226-31.

15. Trivedy CR, Craig G, Warnakulasuriya S. The oral health consequences of chewing areca nut. Addict Biol 2002;7:115-25.

16. Li N, Jian XC, Xu CJ. Expression of loricrin and cytochrome P450 3A5 in oral submucous fibrosis and their significance. J Stomatol 2009;27:29-33.

17. Katou F, Shirai N, Kamakura S, Tagami H, Nagura H, Motegki K. Differential expression of cornified cell envelope precursors in normal skin, intra orally transplanted skin and normal oral mucosa. British J Dermatol 2003;148:898-905.

18. Ishida YA, Takahashi H, Iizuka H. Loricrin and human skin diseases: molecular basis of loricrin keratodermas. Histol Histopathol 1998;13:819-26.