

Expression of β -catenin in oral leukoplakia and oral submucous fibrosis: An immunohistochemical study

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Abstract

Background: Oral potentially malignant disorders have increased propensity to turn malignant than its apparently normal counterparts. Histopathological examination, although gold standard, needs adjunct technique to give accurate diagnosis. Immunohistochemistry has proved to be a promising adjunct to aid in the diagnosis so far. The quest for a definitive marker is still on. Beta-catenin (β -catenin), a structural protein has been evaluated to identify its likely role in malignant transformation of potentially malignant lesions and possibly designate it as one of the identifiable signature molecules in the transformation.

Aim and Objective: To evaluate and estimate the expression of β -catenin in different grades of dysplasia, oral submucous fibrosis (OSMF) and normal mucosa and compare the same.

Methodology: A total number of 40 cases including different grades of dysplasia, OSMF and normal mucosa were immunohistochemically stained, location and intensity of its expression were evaluated for β -catenin. The results were statistically analyzed using the one-way analysis of variance and Chi-square test.

Results: The expression of β -Catenin in the cytoplasm as well as in the nucleus increased from mild-to-moderate dysplasia to OSMF and to severe epithelial dysplasia in an increasing order. The expression is seen to translocate from membranous to cytoplasm to nucleus indicating a proliferative potential in these group of lesions.

Conclusion: β -catenin is a promising marker which indicates the malignant transformation potential in the higher grades of dysplasia and OSMF.

Keywords: Beta-catenin, immunohistochemistry, oral potentially malignant disorders, oral submucous fibrosis, transformation

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Submitted: 01-Feb-2020, **Revised:** 30-Nov-2020, **Accepted:** 19-Jan-2021, **Published:** 14-May-2021

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth leading cancer by the incidence worldwide. Most OSCCs arise in the epithelial lining of the oral cavity, oropharynx, larynx and

hypopharynx, with a percentage of 5-year survival for patients with OSCC varying from 40% to 50% morbidity rate causing functional disability and associated esthetic implications.^[1]

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How to cite this article: Chowdhury P, Nagamalini BR, Singh J, Ashwini BK, Sharada, Swaminathan U. Expression of β -catenin in oral leukoplakia and oral submucous fibrosis: An immunohistochemical study. J Oral Maxillofac Pathol 2021;25:124-30.

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10.4103/jomfp.JOMFP_41_20

OSCC is commonly preceded by a range of tissue and cellular alterations consistent with carcinoma, yet restricted to the surface epithelial layer, termed oral epithelial dysplasia. These changes often manifest in a clinical mucosal lesion.^[2] A creditable number of these lesions are thought to be associated with or preceded by precancerous lesions and conditions, now designated as potentially malignant lesions by the WHO recommendation.

The term implies that this is a family of morphological altered lesions some of which may have an increased potential for malignant transformation. These lesions not only predict the likely site of malignancy but also indicate an increased risk of future malignancies elsewhere in clinically normal appearing oral mucosa.

Recognised lesions in the group include leukoplakia, erythroplakia, palatal lesion of reverse cigar smoking, oral lichen planus, oral submucous fibrosis (OSMF) and discoid lupus erythematosus. A higher risk is observed in dysplasia and OSMF. Identifying these lesions has gained more importance nowadays. A disturbing new trend has been observed over the past few years with a greater percentage of younger age groups becoming susceptible to oral potentially malignant disorders (OPMDs) mainly attributed to increase in substance abuse among the youth.^[3]

Although not always clinically obvious, many of these lesions most likely show tell-tale histopathological signs of dysplasia which helps in their recognition and evaluation.

Histopathological assessment alone, however, is not sufficient to provide an accurate assessment of malignant transformation risk thus necessitating use of varied molecular parameters.

Studying the altered expression of various signalling factors and other structural and functional protein through immunohistochemistry is one such well-recognized technique.

In this study, the expression of beta-catenin (β -catenin), a structural protein has been evaluated to identify its likely role in malignant transformation of potentially malignant lesions and possibly designate it as one of the identifiable signature molecules in the transformation.

β -catenin, is a multitasking and evolutionarily conserved molecule, belonging to the armadillo family of proteins and is found at multiple subcellular localizations, including junctions where it contributes to the stabilization of cell-cell contacts and cytoplasm and plays a crucial role in

a multitude of homeostatic and developmental process. It is an integral part of Wnt pathway which plays a role in directing cell proliferation, cell polarity and cell fate determination during embryonic development and tissue homeostasis. A mutation in the Wnt pathway is often linked to human birth defects, cancer and other diseases.^[4]

Thus, evaluating the expression of beta catenin in OPMDs might provide insight in the working of the transformation pathway as well as establish beta catenin as a more likely and easily identifiable bio marker.

Objectives of the study

1. To immunohistochemically stain selected cases of different grades of oral dysplasia's, OSMF and normal mucosa with β -catenin antibody
2. To evaluate the β -catenin expressions at different locations (membrane, cytoplasm and nuclear) in various strata of the epithelium in oral dysplasia's, OSMF and normal mucosa and statistically analyzed the result.

METHODOLOGY

A retrospective, case–control study was conducted including a total of 40 cases, 10 of mild dysplasia, 5 each of moderate and severe dysplasia, 10 cases of OSMF and 10 clinically normal tissues. These cases were retrieved from the archives of Department of Oral Pathology and Microbiology, AECS Maaruti College of Dental Sciences and Research Center, Bangalore.

The cases were categorized as following: G1 = mild dysplasia, G2 = moderate dysplasia, G3 = severe dysplasia, G4 = OSMF, and G5 = normal mucosa. All the 40 specimens were subjected to immunohistochemical staining for β -catenin antibody, evaluated microscopically for its expression.

Hematoxylin and eosin staining

The formalin-fixed paraffin-embedded tissue sections were heated on slide warmer at 60°C for 5 min. The slides were then dewaxed in two changes of xylene, each for 5 min duration. The tissues were then hydrated through decreasing grades of ethyl alcohol (100%, 90% and 70%) and were washed under running water for 5 min. Stained in Harris hematoxylin for 3 min. The slides were then dipped in acid alcohol and placed in alkali water for 1 min. The sections were washed well in running tap water for 10–15 min or until “blueing.” Thereafter, they were stained in 1% Eosin-Y for 10 min. The slides were washed in running water, dried and then dehydrated through increasing grades

ethyl alcohol (70%, 90% and 100%) and cleared using Xylene and mounted with DPX.^[5,6]The stained slides were then viewed under the research microscope.

Immunohistochemical staining

About 3 μ m, thick sections were obtained and mounted on the Aminopropyltriethoxysilane coated slides. The slides were de-paraffinized, tissues hydrated, and then subjected to antigen retrieval. The antigen retrieval was carried out in the pressure cooker. The tissues were then incubated with peroxide block for 20 min at the room temperature to block endogenous peroxide activity. The sections were subsequently incubated for 15 min with protein block to eliminate background staining. The sections so treated were then incubated with primary antibody i.e., mouse β -Catenin (PathnSitu Biotechnologies PVT. LTD. India). Washed in phosphate buffer, the slides were incubated with target binder for 30 min. Subsequently, the slides were incubated with PathnSitu polymer for 30 min. Subsequently, the slides were incubated with fresh DAB chromogen for 3 min. After which, the slides were washed in water to stop the chromogen reaction and remove the excess DAB and counter stained with Mayer’s hematoxylin provided with the kit for 30 s. The slides were then dehydrated, cleared and mounted with DPX. With each set of slides stained with mouse anti β -Catenin, one positive control slide of breast carcinoma was also stained. The immunostained slides were observed for positivity under $\times 4X/10X/40X$ under light microscope.

Interpretation of staining

All the 10 cases of mild dysplasia, 5 cases of moderate dysplasia, 5 severe dysplasia and 10 OSMF showed overall positivity (i.e., 100% of the total cases) for the expression for the antibody, varying in intensity and location [Table 1]. β -catenin expression was assessed in terms of location-membranous, cytoplasmic, nuclear, and also the extension up to which layer was estimated. The results were tabulated as given below and statistically analyzed using the one-way analysis of variance and Chi-square test. Photomicrographs were recorded from 3 representative areas under high power objective ($\times 40$) in an orderly manner.

RESULTS

It was seen that OSMF showed highest mean cytoplasmic positivity ranging from 10% to 80% [Figure 1e], followed by severe dysplasia ranging from 18% to 55% [Figure 1d], then moderate dysplasia with 11%–45% [Figure 1c] and finally mild dysplasia ranging from 10% to 30% [Figure 1b, Table 2 and Graph 1]. In OSMF as well as in severe dysplasia, the

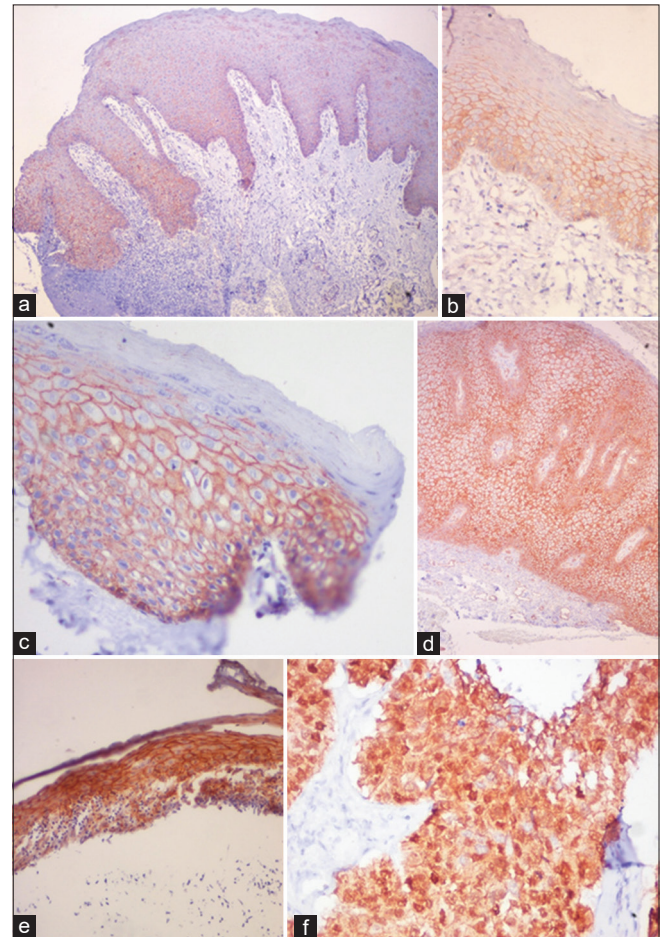


Figure 1: (a) Normal oral mucosa showing membranous β -Catenin positivity (b) Mild epithelial dysplasia showing membranous and cytoplasmic β -Catenin positivity (c) Moderate epithelial dysplasia showing membranous, nuclear and cytoplasmic β -Catenin positivity (d) Severe epithelial dysplasia showing membranous, nuclear and cytoplasmic β -Catenin positivity (e) oral submucous fibrosis showing membranous, nuclear and cytoplasmic β -Catenin positivity (f) Control: Breast cancer tissue as positive control

Table 1: No. of positive cells according to location

Group	Stratum extension	Location					
		Membranous		Cytoplasm		Nucleus	
		NO of cases positive	Per 100 cells in each case	Number of cases positive (%)	Per 100 cells in each case (%)	Number of cases positive (%)	Per 100 cells in each case (%)
G1	Granulosum	10/10	100	100	10-30	40	1-3
G2	Granulosum	10/10	100	100	11-45	60	2-9
G3	Superficial	5/5	100	100	18-55	100	5-13
G4	Superficial	5/5	100	100	10-80	70	1-7
G5	Granulosum	10/10	100	-	-	-	-

cytoplasmic positivity extended up to stratum superficiale and up to stratum granulosum in case of moderate and mild dysplasia [Figure 1]. There was a statistically significant increase in the expression in severe dysplasia and OSMF from mild dysplasia. OSMF also showed higher expression than moderate dysplasia. Normal mucosa did not show cytoplasmic staining [Table 2, Graph 1 and Figure 1].

Nuclear positivity was observed to be the highest in severe dysplasia ranging from 5% to 13% extending up to the superficial layer; followed by moderate dysplasia ranging from 2% to 9% extending up to stratum granulosum, then OSMF ranging from 1% to 7% extending up to stratum granulosum and least in mild dysplasia with 1%–3% extending up to stratum granulosum [Table 3, Graph 2 and Figure 1a-e]. This difference was statistically significant between mild dysplasia and severe dysplasia as well as OSMF. Statistically significant difference was also observed between moderate dysplasia and severe dysplasia as well as between severe dysplasia and OSMF.

Membranous positivity was present in all the cases. It extended up to stratum granulosum in mild dysplasia and

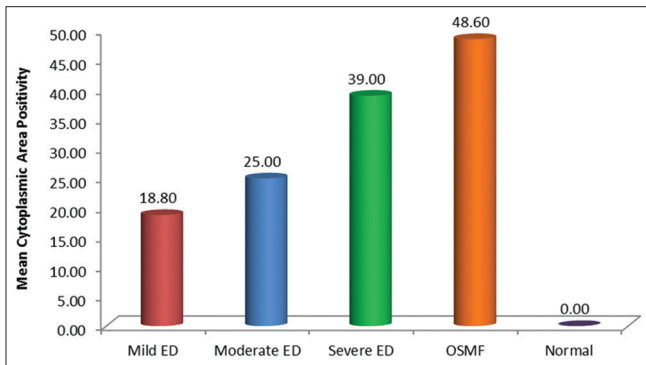
moderate dysplasia but till stratum superficiale in severe dysplasia as well as in OSMF [Table 3, Graph 2 and Figure 1a-e].

Hence, to sum up multiple comparison analysis conducted to assess the difference in β -catenin expression between the groups showed statistically significant increase with increasing grades of dysplasia and in OSMF, especially in terms of cytoplasmic and nuclear positivity.

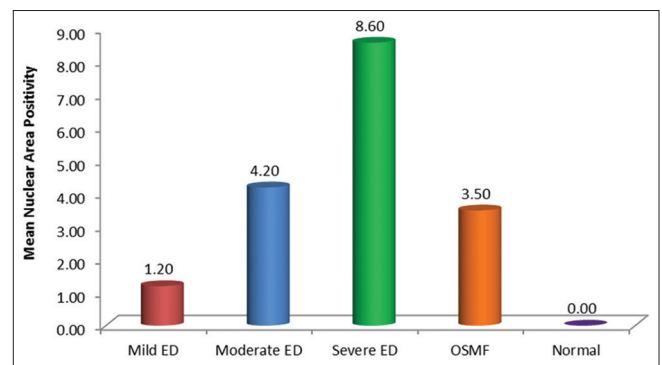
Therefore, it can be concluded from the above-observed result that with the increasing in grades of dysplasia the β -catenin expression is seen to translocate from membranous to cytoplasm to nucleus indicating a proliferative potential in these group of lesions.

DISCUSSION

Oral cancer is the sixth-most common cancer worldwide, the higher incidence attributed to cultural, ethnic, geographic factors, and the popularity of addictive habits and can arise from mucosal lining of the floor of the mouth, cheek, gingiva, lips, or palate.



Graph 1: Comparison of mean cytoplasmic area marked by Beta catenin in different study groups



Graph 2: Comparison of mean nuclear area marked by Beta catenin in different study groups

Table 2: Comparison of mean between different groups based on cytoplasmic area marked by β -Catenin in different layers using one-way ANOVA test

Groups	Positivity extension	n	Mean	SD	Minimum	Maximum	F	P
Mild ED	Granulosum	10	18.80	8.05	10	30	22.725	<0.001*
Moderate ED	Granulosum	5	25.00	14.97	11	45		
Severe ED	Superficiale	5	39.00	13.36	18	55		
OSMF	Superficiale	10	48.60	17.90	10	80		
Normal	Granulosum	10	0.00	0.00	0	0		

*Statistically significant. ED: Epithelial dysplasia, OSMF: Oral submucous fibrosis, SD: Standard deviation

Table 3: Comparison of mean nuclear area marked by β -Catenin in different study groups using one-way ANOVA test

Groups	Positivity extension	n	Mean	SD	Minimum	Maximum	F	P
Mild ED	Granulosum	10	1.20	0.63	1	3	18.719	<0.001*
Moderate ED	Granulosum	5	4.20	2.95	2	9		
Severe ED	Superficiale	5	8.60	3.05	5	13		
OSMF	Superficiale	10	3.50	2.55	1	7		
Normal	Granulosum	10	0.00	0.00	0	0		

*Statistically significant. ED: Epithelial dysplasia, OSMF: Oral submucous fibrosis, SD: Standard deviation

Table 4: A systematic review of various studies done in Oral potentially malignant disorders and Oral squamous cell carcinoma

Name	Years	Study design	Tissue	Sample size	Relevance
Williams et al. ^[19]	1998	Immunocytochemical expression of cadherins and catenins	OPML and OSCC	n= 12	The membranous expression of β -catenin was reduced both in severe dysplasia and carcinoma <i>in situ</i> , a late event associated with invasion
Bankfalvi et al. ^[20]	2002	Immunohistochemical expression of β -catenin, CD 44, E cadherin expression	OSCC	n=93	Loss of E-cad/ β -Catenin was observed in the invasive tumour front and also in the cases of metastases and recurrences thus indicating that there is some perturbed expression of adhesion molecules during the step-wise course of oral-carcinogenesis and tumour progression
Ishida et al. ^[15]	2007	Expression of β -catenin	Normal mucosa and leukoplakia	n= 12	Nuclear expression of β -catenin is considered to be involved in the progression of dysplasia
Lee et al. ^[21]	2010	β -catenin expression	Normal oral mucosa and areca nut chewing-associated OSCC	n=50	β -catenin expression is significantly upregulated in areca quid chewing-associated OSCC
Chaw et al. ^[16]	2012	β -catenin expression	Normal mucosa and leukoplakia	n= 100	β -catenin expression was significantly reduced in moderate-severe dysplasia as compared with normal mucosa
Kaur et al. ^[22]	2013	β -catenin and E cadherin expression	Normal mucosa, hyperplasia, dysplasia and OSCC	n= 191	Loss of membranous expression of E-cadherin and β -catenin proves that these are early events in oral tumorigenesis, occurring in pre- neoplastic stages
Zaid ^[17]	2014	β -catenin and E cadherin expression	Normal oral mucosa and OSCC	n=33	The aberrant expression of β -catenin are significant factors in predicting the histological grade in patients with OSCC
Reyes et al. ^[18]	2015	Immunohistochemical expression of β -catenin	Grades of oral epithelial dysplasia	n=35	Increased presence of β -catenin in severe and moderate dysplasia when compared to mild dysplasia suggests a role of this protein in the progression of dysplasia, thus making it a probable marker in the detection of Oral dysplasia
Zhou et al. ^[23]	2015	Immunohistochemical expression of SFRP 1 and 5 and β -catenin	Normal oral epithelium, OSMF and OSCC	n= 115	Reduced cytoplasmic/nuclear β -catenin expression, which is correlated with higher tumour grade and stage of OSCC

OSMF: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma

Though known to arise *de novo*, a large portion of these lesions are thought to be preceded by precancerous or potential cancerous lesions or conditions. The lesions referred to as OPMD precursors include leukoplakia, erythroplakia, oral sub-mucous fibrosis, lichen planus, actinic keratosis, discoid lupus erythematosus and palatal lesions among reverse smokers, constitute OPMDs.^[7] The prevalence of OPMDs worldwide varies between 1% and 5%.^[8]

However, the transformation potential of each of these lesions varies, is dependent on multiple co factors, with few of them showing unpredictable behavior. Under favorable conditions, many are considered reversible. The potential for malignant transformation among these conditions varies considerably, ranging from <1% to as high as 36%. It is presumed that mild dysplasia will progress to severe dysplasia and then to carcinoma.^[9] Further, it is often influenced by the postdiagnosis cessation or continuity of the high-risk behaviors such as tobacco and alcohol use, as well as clinical intervention.^[10]

Since prevention is anytime better than cure, an early treatment reduces the morbidity and mortality, so it is always a favorable choice.

Hence, early recognition of such a condition becomes important. Over the period, various modes and techniques of identification of these lesions, especially those with higher transformation potential have been investigated with differing results. Since the basis of such a tissue alteration is abnormal genetic modulation and signalling, identification of altered protein expression through immunohistochemistry is easy and highly followed. Accordingly, study of immunohistochemical expression of proliferative, adhesive, metastatic, oncogenic and other group of markers in potentially malignant lesion is a popular means of identifying high risk lesions.

In our study, an attempt is made to study the expression of the β -catenin in leukoplakia with various grades of dysplasia and OSMF of the oral cavity as compared to the normal oral mucosa to evaluate the role of altered beta catenin activation in carcinogenesis.

β -catenin is a proliferative marker belonging to the armadillo proteins, a central molecule in Wnt signaling pathway. β -catenin originally identified in the cell adherens junctions, functions to bridge the cytoplasmic domain of cadherins to α -catenin and the actin cytoskeleton. It is a primary component of the cell membrane and plays crucial roles in the regulation of processes such as cell survival, migration and polarity,

cell proliferation, cell fate specification, body axis patterning and self-renewal in stem cells. Under resting condition, the cytoplasmic β -catenin is bound to its destruction complex. Phosphorylated beta catenin is recognized by ubiquitin ligase beta-TrCP and undergoes ubiquitylation and degradation. Therefore, the cytoplasmic level of β -catenin is kept low in the absence of Wnt/FZD signaling. Therefore, the cytoplasmic level of β -catenin is kept low in the absence of Wnt/FZD signalling.^[11] If β -catenin is not present in the nucleus, the Lymphoid enhancer-binding factor/T-cell factor (LEF/TCFs) cannot activate the target genes and acts when stabilized and translocated into the nucleus where it is able to associate with TCF/LEF-1 to form a functional transcription factor that mediates the transactivation of target genes involved in cancer progression.^[12] As a result, mutations in the Wnt pathway are often linked to human birth defects, cancer and other diseases.^[12]

When compared to normal oral mucosa, membranous β catenin expression was significantly reduced in moderate and severe dysplasia cases, accompanied by a change in the localization of β catenin expression in the cytoplasm and/or nuclei with increased staining intensity.^[13]

An association is already established between β -catenin and other tumors like actinic cheilitis, OSCC, and lichen planus.^[14] Studies have also been done to establish its role in oral lesions previously with varied results.

The study by Ishida *et al.* in 2007 showed that the normal oral epithelium and oral leukoplakia without dysplasia showed β -catenin expression only in the cell membrane and with its translocation to the nucleus in cases with dysplasia eliciting its role in the progression of dysplasia in oral leukoplakia [Table 4].^[15]

Chaw *et al.* in June 2012 found out that as there is an increase in the histopathological grading there is a shift in the epithelial-catenin localization from membranous to the cytoplasm or nucleus. Thus, concluding that β -catenin via Wnt pathway dysregulation is a potential marker for the malignant transformation [Table 4].^[16]

Zaid in 2014 showed that the expression of β -catenin and E-cadherin were independent prognostic factors for histological grades of OSCC. E-cadherin was closely linked to β -catenin expression in OSCC and to tumor differentiation, which reflects a structural association and the role of both in tumor progression [Table 4].^[17]

The study in 2015 by Reyes *et al.* showed increased presence of beta catenin in severe and moderate dysplasia

compared to mild dysplasia; however, the expression of nuclear β -catenin decreased after starting the invasive neoplastic process [Table 4]. This suggests a role for this protein in the progression of dysplasia and early malignant transformation to OSCC, confirming immunodetection of β -catenin could be a possible immune marker in the detection of oral dysplasia.^[18]

Our study results fall on the same lines with an increase in overall expression of beta catenin with increasing grades of dysplasia as well as in OSMF.

The β -catenin positivity was seen in normal mucosa cases confining only to the cell membrane. However, cytoplasmic positivity was noticed in mild and moderate dysplasia in an increasing order but restricting till the stratum granulosum. The OSMF and moderate and severe dysplasia showed the β -catenin positivity in cytoplasm as well as in the nucleus. Severe dysplasia showed the highest mean nuclear positivity, followed by OSMF and moderate dysplasia being the least amongst these three. Statistically significant difference in cytoplasmic positivity was seen between mild and severe dysplasia; mild and OSMF and between moderate and OSMF. Whereas pertaining to the nuclear positivity, statistical significance was seen in mild and severe dysplasia as well as between mild dysplasia and OSMF; also, was seen between moderate and severe dysplasia as well as between severe dysplasia and OSMF cases. Wherever statistically significant was not seen, it was due restriction of our study to only limited number of cases.

CONCLUSION

The expression of β -Catenin in the cytoplasm as well as in the nucleus increased from mild to moderate dysplasia to OSMF and to severe epithelial dysplasia in an increasing order. It can be concluded that as the severity of the dysplastic features increases, there is an increase in the translocation of β -catenin from its primary site, i.e., from the cell membrane toward the cytoplasm to finally reach the nucleus triggering its proliferation. Such a shift observed at higher stratum level in severe dysplasia and OSMF in our study may also indicate increasing dedifferentiation occurring in these cases, thus indicating the cancerous potential in the higher grades of dysplasia and OSMF.

However, since our study group had a limited number of test samples a study on a broader number range would add substantial credit to the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. *Oral Maxillofac Surg Clin North Am.* 2014 May;26(2):123-41. doi: 10.1016/j.coms.2014.01.001.
- Singh J, Swaminathan U, Sharada P, Alur JB, Chowdhury P, Mrinal U, *et al.* Estimation of expression of beta-human chorionic gonadotropin levels through progression of disease from normal to epithelial dysplasia to malignancy. *J Oral Maxillofac Pathol* 2019;23:108-13.
- Hosagadde S, Dabholkar J, Virmani N. A clinicopathological study of oral potentially malignant disorders. *J Head Neck Physicians Surg* 2016; 4:29-34
- Bryan T. MacDonald, Keiko Tamai, and Xi He. Wnt / β -catenin signaling components, mechanisms, and diseases. *Dev Cell* 2009; 17(1): 9–26.
- Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. *Methods Mol Biol* 2014;1180:31-43.
- Bancroft JD, Layton C. The hematoxylin and eosin. In: Suvarna SK, Layton C, Bancroft JD, editors. *Theory Practice of Histological Techniques*. 7th ed., Ch. 10 and 11. Philadelphia: Churchill Livingstone of El Sevier; 2013. p. 179-220.
- 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.
- Mortazavi H, Baharvand M, Mehdipour M. Oral potentially malignant disorders: An overview of more than 20 entities. *J Dent Res Dent Clin Dent Prospects* 2014;8:6-14.
- Speight PM. Update on oral epithelial dysplasia and progression to cancer. *Head Neck Pathol* 2007;1:61-6.
- Arduino PG, Bagan J, El-Naggar AK, Carrozzo M. Urban legends series: Oral leukoplakia. *Oral Dis* 2013;19:642-59.
- Silva FB, de Castro CD, Von Zeidler SL, de Sousa SC, Batista AC, Yamamoto-Silva FP, *et al.* Altered β -catenin expression in oral mucosal dysplasia: A comparative study. *J Appl Oral Sci* 2015;23(5).
- Clevers H. Wnt/ β -Catenin Signaling in Development and Disease. *Cell* 2006;127:469-80.
- Komiya Y, Habas R. Wnt signal transduction pathways. *Organogenesis*. 2008;4:68-75. doi: 10.4161/org.4.2.5851.
- Bryan T. MacDonald, Keiko Tamai, and Xi He. Wnt / β -catenin signaling components, mechanisms, and diseases. *Dev Cell* 2009;17:9–26.
- Ishida K, Ito S, Wada N, Deguchi H, Hata T, Hosoda M, *et al.* Nuclear localization of beta-catenin involved in precancerous change in oral leukoplakia. *Mol Cancer* 2007;6:62.
- Chaw SY, Abdul Majeed A, Dalley AJ, Chan A, Stein S, Farah CS. Epithelial to mesenchymal transition (EMT) biomarkers—E-cadherin, beta-catenin, APC and Vimentin—in oral squamous cell carcinogenesis and transformation. *Oral Oncol* 2012;48:997-1006.
- Zaid KW. Immunohistochemical assessment of E-cadherin and β -catenin in the histological differentiations of oral squamous cell carcinoma. *Asian Pac J Cancer Prev* 2014;15:8847-53.
- Reyes M, Alcayaga GR, Maturana A, Aitken JP, Rojas C, Ortega AV, *et al.* Increased nuclear β -catenin expression in oral potentially malignant lesions: A marker of epithelial dysplasia. *Med Oral Patol Oral Cir Bucal* 2015;20:E540-6.
- Williams HK, Sanders DS, Jankowski JA, Landini G, Brown AM. Expression of cadherins and catenins in oral epithelial dysplasia and squamous cell carcinoma. *J Oral Pathol Med* 1998;27:308-17.
- Bankfalvi A, Krassort M, Buchwalow IB, Vegh A, Felszeghy E, Piffko J. Gains and losses of adhesion molecules (CD44, E-cadherin, and β -catenin) during oral carcinogenesis and tumor progression. *J Pathol* 2002;198:343-51.
- Lee SS, Tsai CH, Tsai LL, Chou MC, Chou MY, Chang YC, *et al.* B-catenin expression in areca quid chewing- associated oral squamous cell carcinomas and upregulated by arecoline in human oral epithelial cells. *J Formosan Med Assoc* 2012;111:194-200.
- Kaur J, Sawhney M, Datta Gupta S, Shukla NK, Srivastava A, Walfish PG, *et al.* Clinical significance of altered expression of β -catenin and E-cadherin in oral dysplasia and cancer: Potential link with ALCAM expression. *PLoS One* 2013;8:E67361, 1-12.
- Zhou S, Chen L, Mashrah M, Zhu Y, Liu J, Yang X, *et al.* Deregulation of secreted frizzled-related proteins is associated with aberrant β -catenin activation in the carcinogenesis of oral submucous fibrosis. *Oncotargets Ther* 2015;8:2923-31.