Introduction

It has been very much perceived that the cancers of the oral cavity and the pharynx are a general medical issue and therefore, there is an extraordinary number of fatality and individuals experiencing sicknesses or inability in numerous countries. It is indeed found that these cancers are increasing rapidly in developing countries, and this is a matter of great concern.

Comparison of expression of myofibroblasts in normal oral mucosa, oral epithelial dysplasia, and oral squamous cell carcinoma using α-SMA and vimentin: An immunohistochemical study

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Abstract

Background: Myofibroblasts are thought to play critical roles in inflammation, growth, repair, premalignancy, and malignancy. This study was done to evaluate, compare and co-relate the progressive increase in the immunohistochemical expression of myofibroblasts in normal oral mucosa, epithelial dysplasia, and oral squamous cell carcinoma (OSCC). To compare and co-relate the expression of myofibroblasts in normal oral mucosa, epithelial dysplasia, and oral squamous cell carcinoma. To co-relate the progressive increase in myofibroblasts expression in normal oral mucosa, epithelial dysplasia, and oral squamous cell carcinoma. Materials and Method: Forty-nine paraffin-embedded tissue blocks with 7 cases of normal oral mucosa, 21 cases of epithelial dysplasia, and 21 diagnosed cases of OSCCs were studied. The samples were subjected to heat-induced antigen retrieval methods followed by staining using primary mouse monoclonal antibodies against α-smooth muscle actin (SMA) and vimentin. Staining index of all the sections was calculated. Statistical analysis was performed using the Kruskal–Wallis test, Mann–Whitney U test, and Chi-square test. Values of P less than or equal to 0.05 (P ≤ 0.05) were considered statistically significant. Results: Statistically significant staining index was obtained by α-SMA and vimentin between normal oral mucosa, epithelial dysplasia, and OSCC. Conclusion: Myofibroblast may play a role only during initial tumorigenesis that is the conversion of severe dysplasia into OSCC.

Keywords: α-SMA, immunohistochemistry, myofibroblasts, vimentin

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The occurrence of oral cancer is getting higher in most countries, particularly in developing countries. In India, it positions number one among men and third among women.\[3\]

Despite therapeutic advances, survival rates for patients with oral squamous cell carcinoma (OSCC) remain at approximately 50% and have not improved over several decades. Improving detection, diagnosis, and treatment of precancerous changes and early asymptomatic cancers is imperative to increase survival and improve functional outcomes for persons at risk to develop oral cancer. Oral carcinogenesis proceeds through a stepwise accumulation of genetic damage over time. Genetic mutations often produce early phenotypic changes that may present as clinically apparent, recognizable lesions. Oral lesions that have been identified clinically as having potential for malignant conversion include leukoplakia, erythroplakia, lichen planus, and submucous fibrosis.\[3\]

As there is a transformation of normal epithelium to precancerous and to squamous cell carcinoma, the stroma likewise changes from normal to primed to activated or tumor-associated known as the stromal reaction.\[4\] In the past, the stroma was regarded as a support tissue for cancer cells, the outcomes obtained in various scientific studies indicated that it can control the processes of tumor invasion and metastasis.\[5\]

One of the stromal responses is the presence of specific fibroblasts called myofibroblasts.\[4\] Myofibroblasts have smooth muscle-like features due to the presence of contractile apparatus and were first seen by “Gibbiani” in granulation tissue during wound healing under an electron microscope.\[6,7\]

These cells play a key role in physiologic procedures like wound healing and pathologic conditions such as reactive lesions, benign tumors, locally aggressive tumors, and malignancies affecting the oral cavity. Myofibroblasts, that are found in normal skin tissues, pulmonary septa, and periodontal ligaments, are distinctive and attributable to their location, they are known as juxtaparenchymal cells.\[5,8,9\] Some authors also named myofibroblasts as cancer-associated fibroblasts.\[10\]

Because of their contractile features and capability to produce extracellular matrix components, cytokines, proteases, and proangiogenic factors, myofibroblasts have been involved in the progression of many tumors, including OSCC. Therefore, myofibroblasts might be utilized as a stromal marker for the progression of oral premalignancy into malignancy.\[4\]

In this study, we intend to evaluate the immunohistochemical expression of myofibroblasts using alpha-smooth muscle actin (α-SMA) and vimentin in formalin-fixed paraffin-embedded sections of normal oral mucosa, epithelial dysplasia, and OSCC and to find out the correlation between the expression of myofibroblasts within the three groups.

**Materials and Methods**

After obtaining permission from the institutional ethics committee, the study sample included 49 archival tissue specimens embedded in paraffin wax blocks of reported cases, which were previously diagnosed in the Department of Oral Pathology and Microbiology, Himachal Institute of Dental Sciences, Paonta Sahib.

Epithelial dysplasia was recorded as mild, moderate, and severe and the OSCC cases were histologically graded as well, moderately, and poorly differentiated. Inflammation was minimal in normal tissues and all were devoid of pathologic conditions. Samples were categorized as:

- Group I: consisted of 7 cases of normal oral mucosa.
- Group II: consisted of 21 cases of epithelial dysplasia.
- Group III: consisted of 21 cases of OSCC.

Endothelial cell lined blood vessels were used as an internal positive control for α-SMA and human tonsil were used as a positive control for vimentin.

Sections of 4-micron and 3-micron thickness were prepared from each paraffin block using semi-automatic microtome. Figures 1-3 shows the Immunohistochemical staining of myofibroblasts for α-SMA and vimentin in various grades of dysplasia and OSCC. The 4-micron sections were floated onto albumin- coated slides and stained according to routine H and E protocol. The 3-micron sections were floated on poly-L-lysine coated slides for immunohistochemical staining with α-SMA and vimentin.

**Immunohistochemical staining procedure**

Immunostaining for demonstration of Myofibroblasts was performed according to the manufacturer’s instructions, using...
primary mouse monoclonal antibodies against α-SMA (clone 1A4; Biogenex) and vimentin (clone V9; Biogenex). According to datasheet provided, both the antibodies stain cytoplasm only.

**Procedure**

The staining protocol was followed according to manufacturer’s instructions. Sections were allowed to dry at room temperature overnight and heated at 60°C on a slide warming table for 1 hour before staining. Sections were deparaffinized in two changes of xylene for 10 min each. Rehydration was done in 100% alcohol for 5 min, 85% alcohol for 5 min, and 70% alcohol for 5 min. Slides were rinsed in running tap water for 5 min. Antigen retrieval was performed by heat-induced epitope retrieval (HIER) technique using microwave (EZ retriever system, Biogenex). For this section were immersed in 10 mM sodium citrate buffer (pH 6.0) in the containers supplied with the retrieval system and two cycles were run: first cycle at 90°C for 10 minutes and second cycle at 95°C for 15 minutes. Slides were cooled to room temperature for 30 minutes and washed with phosphate-buffered saline (PBS) in three changes for 3 minutes each. Slides were then incubated with peroxide block (3% hydrogen peroxide) for 10 minutes in the humidifier chamber to block endogenous peroxidase activity. Slides were washed with three changes of PBS for 3 min each. Incubation with power block (for nonspecific background staining) was done for 15 minutes in the humidifier chamber. Sections were then incubated with mouse monoclonal antibody to α-SMA and vimentin, respectively for 1 hr at room temperature. Slides were washed with three changes of PBS for 3 min each. Slides were then incubated with a super enhancer for 25 minutes. Incubation with secondary antibody labeled with horseradish peroxidase was done for 30 min. Slides were again washed with three changes of PBS for 3 min each. Incubation with 3,3’- diaminobenzidine tetrahydrochloride (DAB) chromogen for 5-10 minutes. After this, slides were washed in running tap water for 2 minutes. All slides were then counterstained with Harris haematoxylin for 30 seconds. Slides were washed in running tap water for 5 minutes and dehydrated in 70% alcohol for 2 min, 80% alcohol for 2 min, and 100% alcohol for 2 min. For clearing, slides were placed in two changes of xylene for 2 min each. All slides were mounted in dibutyl phthalate polystyrene xylene (DPX). With every batch of immunohistochemistry (IHC) staining, positive control was used to standardize the procedure.

**Cell counting and scoring**

Histopathological pictures were captured using Labovision Freedom 5000 5 MP Wi-Fi Camera. To evaluate the level of α-SMA and vimentin expression the percentage of positive stained stromal spindle cells (at 400x in five microscopic fields) and the staining intensity were graded on a scale of 0–3 by three observers according to the method proposed by Tuxhorn JA et al.\[11]\)

**Percentage of myofibroblasts**

0: 0% positive cells.
1: 1–33% positive cells.
2: 34–66% positive cells.
3: 67–100% positive cells.

**Staining intensity**

0: no staining.
1: staining obvious only at 400x.
2: staining obvious at x100 but not at 40x.
3: staining obvious at 40x.

For each sample, the staining percentage and staining intensity scores were multiplied to give the staining index. Staining index was classified as:

**Figure 2:** Immunohistochemical staining for vimentin, (a) normal oral mucosa (4x) (b) mild dysplasia (4x); (c) moderate dysplasia (4x); (d) severe dysplasia (4x)

**Figure 3:** Immunohistochemical staining for vimentin, (a) well differentiated squamous cell carcinoma (4x); (b) moderately differentiated squamous cell carcinoma (4x); (c) poorly differentiated squamous cell carcinoma (4x);
Staining index
0: Zero.
1–2: Low.
3–4: Moderate.
6–9: High.

Statistical analysis
Statistical analysis was performed using the Kruskal–Wallis test, Mann–Whitney U test, and Chi-square test. Values of P less than or equal to 0.05 (P ≤ 0.05) were considered statistically significant.

Discussion
In our study, α-SMA positive myofibroblasts were not seen in normal oral mucosa and epithelial dysplasia [Graph 1]. The staining index was zero in all of these cases. These findings are in accordance with the previous studies done by Kellerman MG et al. (2008), Rodrigues PC et al. (2015), and Smitha A et al. (2019). Similar results have also been reported by various other authors. But in contrast to our study, small amount of myofibroblasts were reported in high-risk epithelial dysplasia by Chaudhary M et al. (2012), Kapse SC et al. (2013), Gupta K et al. (2015), and Khalid A et al. (2019).

The literature along with our study findings indicates that stromal myofibroblasts cannot be used as a marker to predict malignant transformation potential of epithelial dysplasia. This relative absence of myofibroblasts in the stroma of epithelial dysplasia may also reflect its low malignant transformation potential despite its high prevalence.

Five out of 7 cases of both well-differentiated squamous cell carcinoma (WDSCC) as well as moderately differentiated squamous cell carcinoma (MDSCC) showed α-SMA positive myofibroblasts and 6 out of 7 cases of poorly differentiated squamous cell carcinoma (PDSCC) showed α-SMA positive myofibroblasts [Graph 1]. Overall, out of total 21 cases of OSCC, 16 cases showed α-SMA positive myofibroblasts that means 71.9% cases are positive. Similar findings have also been reported by Rodrigues PC et al. (2015) (59.8%), Jayaraj G et al. (2015) (45.3%), Chaudhary M et al. (2012) (97.29%), Kapse SC et al. (2013) (70%), in OSCC though the percentage of positive cases differs. The staining index was zero in 5 cases, low in 4 cases, moderate in 8 cases, and high in 4 cases of OSCC [Table 1].

A statistically significant difference was observed between normal oral mucosa, epithelial dysplasia, and OSCC (P < 0.05) but the distribution of myofibroblasts was not significantly different between three histological grades of Squamous Cell Carcinoma (P = 0.145). Studies done by Kellerman MG et al. (2008) and Moghadam ES et al. (2009) also revealed no statistically significant difference in the mean number of myofibroblasts between well, moderately, and poorly differentiated OSCC and suggested that transdifferentiation of myofibroblasts is induced during the invasive stage of carcinomatous epithelium and further loss of tumoral differentiation does not affect the number of cells. In contrast to our study, Rodrigues PC et al. (2015) observed significantly higher expression of myofibroblasts in MDSCC and PDSCC as compared to WDSCC. The difference in positive findings can be due to tumor stroma because tumors lacking fibrous stroma were devoid of myofibroblasts. This has been postulated by Jayaraj J et al. (2015). Our findings along with previous studies indicated that myofibroblasts are not associated with the transformation of epithelial dysplasia into OSCC. Dysplasia represents an intermediate step, wherein the stroma is losing its control over epithelial morphogenesis. Once the dysplastic epithelium accumulates further mutations resulting in the

Table 1: Mean immunohistochemical staining index of myofibroblasts for α-SMA in normal oral mucosa and different grades of epithelial dysplasia and OSCC

| Groups          | Number of Cases (n) | Zero | Low | Moderate | High |
|-----------------|---------------------|-----|-----|----------|------|
| Normal Oral Mucosa | Mild                | 7   | 0   | 0        | 0    |
| Epithelial Dysplasia | Moderate          | 7   | 0   | 0        | 0    |
| OSCC            | WDSCC               | 7   | 2   | 3        | 0    |
|                 | MDSCC               | 7   | 2   | 2        | 1    |
|                 | PDSCC               | 7   | 1   | 0        | 3    |

WDSCC: well-differentiated squamous cell carcinoma; MDSCC: moderately differentiated squamous cell carcinoma; PDSCC: poorly differentiated squamous cell carcinoma; OSCC: oral squamous cell carcinoma
Invasion, the stroma responds by acting as a co-conspirator. Hence, myofibroblasts differentiation is an event that occurs in the carcinogenesis process.[3][4]

A major issue in our study is that there is no way of knowing whether the dysplasias would transform into OSCC or remain as dysplastic entities. But there seems to be a genetic alteration in the carcinomatous epithelium, which produces an inductive effect on the adjacent stroma to produce myofibroblasts. These mutated epithelial-stromal interactions may be responsible for the induction of initial carcinogenesis only. Additional investigations on these myofibroblasts and the related factors will help us to clarify how and to what extent these cells contribute to carcinogenesis.

In this study, myofibroblasts were found surrounding the tumor islands and cords in the stroma, and often in the deep invasive front of the tumors. Some tumors expressed only a few myofibroblasts in delicate rows surrounding and abutting the tumor islands, while others showed an abundance of myofibroblasts in the stroma, which were organized in a syncytium. In our study, we also found that some epithelial cells at the periphery of tumor islands have also been stained by α-SMA. This has not been reported by any of the studies till date. As we know that epithelial cells can undergo epithelial-mesenchymal transitions and transform into myofibroblasts.[8] So this may be the reason for the positive staining of these cells.

All the cases of normal oral mucosa, dysplasia, and OSCC showed a positive reaction of α-SMA around blood vessels, which was used as an internal positive control.

The other marker used in our study to observe myofibroblasts was vimentin. In our study, normal oral mucosa (n = 3/7, 42.85%), mild dysplasia (n = 4/7, 57.4%), moderate dysplasia (n = 3/7, 42.85%), severe dysplasia (n = 5/7, 71.42%), WDSCC (n = 6/7, 85.7%), MDSCC (n = 7/7, 100%), PDSCC (n = 7/7, 100%) showed positive spindle cell staining with vimentin [Graph 2]. Similar results were obtained by Kapse SC et al. (2013).[21] Sawant SS et al. (2014),[24] and Liu LK et al. (2010).[25] Vimentin, a general connective tissue marker, stained all the connective tissue components, which was in accordance with the study done by Smitha GP et al. (2016).[26] In our study, we concluded that vimentin cannot be used alone as a marker to observe myofibroblasts as it stains a lot of other connective tissue cells including fibroblasts, adipocytes, muscle cells, and endothelial cells. Till date only Moghadam ES et al. (2009)[17] and Kapse SC et al. (2013)[21] have compared the staining performance of vimentin and α-SMA to stain myofibroblasts. They also concluded that α-SMA stains myofibroblasts better than vimentin.

In our study, staining index of vimentin was higher in OSCC than epithelial dysplasia and normal oral mucosa. Statistically significant difference was found in staining index between normal oral mucosa, epithelial dysplasia, and OSCC (p = <0.05) but when staining index of WDSCC, MDSCC, and PDSCC was compared, no statistically significant results were obtained (P = 0.145); also there was no significant difference in the staining index of epithelial dysplasia and normal oral mucosa (P = 0.614).

Since statistically significant staining index was obtained by α-SMA and vimentin between normal oral mucosa, epithelial dysplasia, and OSCC, we can conclude that myofibroblast may play a role only during initial tumorigenesis that is the conversion of severe dysplasia into OSCC.

Liu LK et al. (2010)[25] studied the pattern of tumor invasion at normal tissue tumor interface using vimentin. They concluded that increased expression of vimentin was associated with decreased expression of e-cadherins and hence higher chances of metastasis and recurrences. Hence, myofibroblasts do play a key role in invasion, the stroma responds by acting as a co-conspirator.

Table 2: Mean immunohistochemical staining index of myofibroblasts for vimentin in normal oral mucosa and different grades of epithelial dysplasia and OSCC

| Groups               | Number of Cases (n) | Vimentin Staining Index |
|----------------------|---------------------|-------------------------|
|                      |                     | Zero | Low | Moderate | High |
| Normal Oral Mucosa   | 7                   | 4    | 3   | 0        | 0    |
| Mild                 | 7                   | 3    | 4   | 0        | 0    |
| Epithelial Dysplasia |                     |      |     |          |      |
| Moderate             | 7                   | 5    | 1   | 1        | 0    |
| Severe               | 7                   | 3    | 4   | 0        | 0    |
| WDSCC                | 7                   | 1    | 0   | 0        | 6    |
| OSCC                 |                     |      |     |          |      |
| MDSCC                | 7                   | 0    | 0   | 3        | 4    |
| PDSCC                | 7                   | 0    | 0   | 0        | 7    |

WDSCC: well-differentiated squamous cell carcinoma; MDSCC: moderately differentiated squamous cell carcinoma; PDSCC: poorly differentiated squamous cell carcinoma; OSCC: oral squamous cell carcinoma
also stated that it can provide us with an effective target for cancer therapy. In the future, it will be worth exploring the autocrine and paracrine interactions at the tumor invasion front but their identification is challenging.

In our study, we observed that vimentin has high sensitivity and poor specificity as stated by Painter JT et al. (2010). It can be used to differentiate epithelial cells from stromal cells but cannot be used to differentiate fibroblasts from myofibroblasts because vimentin stains a number of stromal cells as explained above but α-SMA is a better marker than vimentin as it stains myofibroblasts and smooth muscle fibers only. Tomasek JJ et al. (2002) also stated that α-SMA is the most reliable marker for differentiated myofibroblasts because of higher specificity.

The network pattern of myofibroblasts in OSCC represents higher invasive features and weaker prognosis. We can also say that due to more amount of myofibroblasts in network arrangement, neoplastic lesions show more rigorous invasive behavior in contrast to spindle pattern.

In future, if our findings are established by further examinations, therapeutic targeting of myofibroblasts, their byproducts, or factors responsible for their transdifferentiation from fibroblasts may be beneficial to OSCC patients and can probably be considered as a new auxiliary method that will cause fewer complications. However, further studies are recommended for more reliable achievements to be reached.

**Clinical significance**

Different surgical methods, radiotherapy, and chemotherapy, which are currently used in the treatment of squamous cell carcinoma cause some complications that have not improved the life expectancy of the patients in 25 years. If our findings are confirmed by future investigations, therapeutic targeting of myofibroblasts, their byproducts, or factors responsible for their transdifferentiation from fibroblasts may be beneficial to OSCC patients and can probably be considered as a new auxiliary method that will cause fewer complications.

**Limitations**

The limitations of this study take account of the fact that there was no way to determine whether the cases of oral epithelial dysplasia would have remained as dysplastic entities or whether they would have transformed into OSCC. However, further studies with an increased sample size are recommended for more reliable achievements to be reached.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Natarajan E. Contemporary concepts in the diagnosis of oral cancer and precancer. Dent Clin North Am 2011;55:63-88.
2. Fotedar V, Fotedar S, Gupta M, Manchanda K, Sharma M. Oral cancer knowledge, attitudes and practices: A survey of undergraduate medical students in Himachal Pradesh, India. J Clin Diagn Res 2015;9:5-8.
3. Gillenwater A, Papadimitrakopoulou V, Kortum RR. Oral premalignancy: New methods of detection and treatment. Curr Oncol Rep 2006;8:146-54.
4. Lucio PSC, Cavalcanti AL, Alves PM, Godoy GP, Nonaka CF. Myofibroblasts and their relationship with oral squamous cell carcinoma. Braz J Otorhinolaryngol 2013;79:112-8.
5. Pinisetty S, Manyam R, Suresh B. Myofibroblasts in oral lesions: A review. J Oral Maxillofac Pathol 2014;18:52-7.
6. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown AR. Myofibroblasts and mechano regulation of connective Tissue remodelling. Nat Rev Mol Cell Biol 2002;3:349-63.
7. Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 1971;27:549-50.
8. Shirol PD, Shirol DD. Myofibroblasts in health and disease. Int J Oral Maxillofac Pathol 2012;3:23-7.
9. Bagul N, Ganjre A, Goryawala SN, Kathariya R, Dusane S. Dynamic role of myofibroblasts in oral lesions. World J Clin Oncol 2015;6:264-71.
10. Bello IO, Vered M, Dayan D, Dobriyan A, Yahalom R, Alagen K, et al. Cancer-associated fibroblasts, a parameter of the tumor microenvironment, overcomes carcinoma-associated parameters in the prognosis of patients with mobile tongue cancer. Oral Oncol 2011;47:33-8.

**Conclusions**

Benefitting from the advantage of myofibroblasts in physiologic processes and blocking the processes leading to the causation and progression of OSCC is the need of the hour. Additional knowledge and clinical studies involving this unique cell may provide us with an effective target for cancer therapy. In the future, it will be worth exploring the autocrine and paracrine effects of transformed myofibroblasts and stromal epithelium interaction as a potential therapeutic approach against cancer progression via drug interaction.
11. Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. Reactive stroma in human prostate cancer: Induction of myofibroblast phenotype and extracellular matrix remodeling. Clin Cancer Res 2002;8:2912-23.

12. Kellermann MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, et al. Mutual paracrine effects of oral squamous cell carcinoma cells and normal oral fibroblasts: Induction of fibroblast to myofibroblast transdifferentiation and modulation of tumor cell proliferation. Oral Oncol 2008;44:509-17.

13. Rodrigues PC, Miguel MCD, Aquino SND, Fonseca FP, Silva ARS, Leme AFP, et al. Stromal myofibroblasts in potentially malignant and malignant lesions of the oral cavity. Oncol Lett 2015;9:667-70.

14. Smitha A, Rao K, Umadevi HS, Smitha T, Sheethal HS, Vidya MA. Immunohistochemical study of α-smooth muscle actin expression in oral leukoplakia and oral squamous cell carcinoma. J Oral Maxillofac Pathol 2019;23:59-64.

15. Jayaraj G, Sherlin HJ, Ramani P, Premkumar P, Natesan A. Stromal myofibroblasts in oral squamous cell carcinoma and potentially malignant disorders. Indian J Cancer 2015;52:87-92.

16. Kellerman MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, et al. Myofibroblasts in the stroma of oral squamous cell carcinoma are associated with poor prognosis. Histopathology 2007;51:849-53.

17. Moghadam ES, Khalili M, Tigrany F, Aaladi M. Evaluation of myofibroblasts in oral epithelial dysplasia and squamous cell carcinoma. J Oral Pathol Med 2019;48:ZC52-7.

18. De-Assis EM, Pimenta LGCS, Costa-e-Silva E, Souza PEA, Horta MCR. Stromal myofibroblasts in oral leukoplakia and oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2012;17:e733-8.

19. Majeed AH, Yass NS, Sarkis SA. Immunohistochemical distribution of myofibroblasts in oral squamous cell carcinoma, verrucous carcinoma and oral epithelial dysplasia. Iraqi Dent J 2014;36:20-4.

20. Chaudhary M, Gadhai AR, Vidhale G, Mankar MP, Gondivkar SM, Gawande M, et al. Comparison of myofibroblasts expression in oral squamous cell carcinoma, verrucous carcinoma, high risk epithelial dysplasia, low risk epithelial dysplasia and normal oral mucosa. Head Neck Pathol 2012;6:305-13.

21. Kapse SC, Rathod N, Baad R, Mandlik J, Sharma AS, Bommanavar S. Quantitative assessment of myofibroblast in severe dysplasia, microinvasion and oral squamous cell carcinoma: An immunohistochemical study. J Contemp Dent Pract 2013;14:34-8.

22. Gupta K, Metgud R, Gupta J. Evaluation of stromal myofibroblasts in oral leukoplakia, oral submucous fibrosis, and oral squamous cell carcinoma: An immunohistochemical study. J Cancer Res Ther 2015;11:893-8.

23. Khalid A, Siddiqui S, Faizi N, Hassan J, Nehal N, Siddiqui A. Role of stromal myofibroblasts in the progression of oral lesions from dysplasia to invasive carcinoma. Indian J Med Paediatr Oncol 2019;40:536-41.

24. Sawant SS, Vaidya MM, Chaukar DA. Clinical significance of aberrant Vimentin expression in oral premalignant lesions and carcinomas. Oral Dis 2014;20:453-63.

25. Liu LK, Jiang XY, Zhou X, Wang DM, Song XL, Jiang HB. Upregulation of vimentin and aberrant expression of E-cadherin/b-catenin complex in oral squamous cell carcinomas: Correlation with the clinicopathological features and patient outcome. Mod Pathol 2010;23:213-24.

26. Smitha GP, Sheno YS, Narayan TV, Jayaram R. Comparison of myofibroblasts between solid/multicystic ameloblastoma and unicystic ameloblastoma: An immunohistochemical analysis. J Clin Diagn Res 2016;10:ZC52-7.

27. Painter JT, Clayton NP, Herbert RA. Useful immunohistochemical markers of tumor differentiation. Toxicol Pathol 2010;38:131-41.