Original Article

ENDOGLIN (CD105): A marker of tumour vasculature for assessment of malignant potential of oral submucous fibrosis

Subraj Shetty, Treville Pereira, Sandhya Tamgadge, Sandeep Kale, Pooja Kamat, Sourab Kumar

Department of Oral and Maxillofacial Pathology and Microbiology, D. Y. PATIL University, School of Dentistry, Navi Mumbai, Maharashtra, India

Abstract

Background: *Oral submucous fibrosis* (OSMF) is a precancerous condition associated with the use of betel/areca nut in various forms. It is characterized by restricted mouth opening, tongue protrusion and cheek rigidity. Oral submucous fibrosis, is primarily prevalent among the people of the Indian subcontinent and it has been reported that about one third of the OSMF patients develop squamous cell carcinoma.

Endoglin (CD-105) is a hypoxia induced protein and a potential marker for activated endothelial cells which signify tumorigenic neoangiogenesis.

Aim: To determine expression of CD105 and study relation of neoangiogenesis with the clinical staging and histopathological grading of oral submucous fibrosis.

Material and Method: Immunohistochemical expression of CD105 was evaluated on forty nine (49) paraffin-embedded tissue sections of diagnosed cases of OSF and seven (7) control samples of healthy volunteers.

Results: There were 13 cases in Stage A, 11 cases in Stage B, 13 cases in Stage C, and 12 cases in Stage D. There were 4 cases in Grade 1 (Very early), 19 cases in Grade 2 (Early), 19 cases in Grade 3 (Moderately advance) and 7 cases in Grade 4 (Advance).

Conclusion: The present study was first immunohistochemical study to demonstrate MVD, MVP and MVAP with CD105 expression in OSMF cases. However, well-designed studies with larger sample size is required to validate CD105 as a reliable biomarker for malignant transformation in future research.

Keywords: CD105 marker, neoangiogenesis, oral submucous fibrosis

Address for correspondence: Dr. Sandhya Tamgadge, Department of Oral and Maxillofacial Pathology and Microbiology, D. Y. PATIL University, School of Dentistry, Sector 7, Nerul, Navi Mumbai - 400 706, Maharashtra, India.
E-mail: sandhya.tamgadge@gmail.com

Submitted: 06-Mar-2024, **Revised:** 09-Nov-2024, **Accepted:** 11-Nov-2024, **Published:** 31-Dec-2024

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, progressive, potentially malignant disorder of the oral mucosa. It is associated with areca nut chewing habit prevalent in India and South East Asia. It is clinically characterized by burning sensation of the oral mucosa accompanied by pallor and progressive, irreversible fibrosis leading to difficulty in opening the mouth, speech and swallowing.[1,2]

Characteristic histopathological features of this disease include epithelial atrophy with loss of rete ridges, reduced

Access this article online

Quick Response Code:

Website: https://journals.lww.com/JPAT/

DOI: 10.4103/jomfp.jomfp_67_24

How to cite this article: Shetty S, Pereira T, Tamgadge S, Kale S, Kamat P, Kumar S. ENDOGLIN (CD105): A marker of tumour vasculature for assessment of malignant potential of oral submucous fibrosis. J Oral Maxillofac Pathol 2024;28:589-95.

vascularity, chronic inflammatory infiltrate and hyalinization of the submucosal tissue.[2,3]

Oral submucous fibrosis (OSMF) has been reported with a prevalence rate of 0.4% in Indian rural population. This condition affects approximately 0.5% (5 million people) of the population in the Indian subcontinent.

This severely impairs eating and oral hygiene care. The epithelium overlying the fibrous condensation becomes atrophic in 90% of cases and is the site of malignant transformation in 4.5% of patients. Malignant transformation rates as high as 7.6% have been reported from the Indian subcontinent over a 17-year period.[4,5]

Now globally accepted as an Indian disease, OSMF has one of the highest rates of malignant transformation among potentially malignant oral lesions and conditions. The main reason for malignant transformation is attributed to the formation of new blood vessels from preexisting ones, known as angiogenesis. This forms an essential component for tumour formation.[2]

Angiogenesis is a multi-step process involved in the development of new blood vessels, by the division and migration of existing vasculature.[6] It is a phenomenon observed in several physiological and pathological events. Physiological events include embryogenesis, wound healing, lactation and cyclic changes associated with the female reproductive tract. Pathological angiogenesis occurs during the growth and metastasis of tumours, retinopathies, hemangiomas, fibrosis and rheumatoid arthritis.[7] Angiogenesis is a critical event in tumour growth and metastasis, mediated by several growth factors released by the tumour cells in the local environment.[8]

Markers such as CD31, CD34 and Von Willebrand factor are pan-endothelial markers that are expressed in normal vasculature and to some extent in tumour vasculature. CD105 which is also an endothelial cell marker expressed in all angiogenic tissues of tumours, but weakly or not at all with those of normal tissues, giving superiority of CD105 as a marker for tumour angiogenesis.[9]

CD105, also known as endoglin, is a receptor for transforming growth factor-beta (TGF-β) signalling, and plays an important role in angiogenesis and fibrogenesis. It is essential for endothelial cell proliferation, and promotes the activation phase of angiogenesis.[10] It has been shown that CD105 is hypoxia-inducible protein, and is preferentially expressed in the activated endothelial cells participating in neoangiogenesis.[11] Immunohistochemical studies have revealed that CD105 is strongly expressed in the blood vessels of tumour tissues.[12,13]

This inspired us to carry out the present study on mucosal vasculature by using immunohistochemical marker CD105 for demonstration of neoangiogenesis in OSMF.

Thus the present study was undertaken to determine and correlate the expression of CD105 in OSMF patients with clinical staging and histopathological grading of OSMF.

MATERIALS AND METHODS

The total number of samples in the present study comprised of 56 cases. The participants were categorized into the following groups, 49 cases of OSMF and 7 cases were selected as control group. Ethical clearance was obtained from the Faculty Research Committee of the Institution dated 30/07/2018.

All the study participants were given clear explanations about the objective of the study and a written informed consent was taken after a standard questionnaire interview was performed to obtain the history. Clinical photographs were taken. Each patient was subjected to biopsy procedure. Proper medication and postoperative instructions were given to the patients. It was ensured that adequately sterilized instruments were used for oral examination and sample collection to prevent cross-infection.

Inclusion criteria

- Previously untreated cases of OSFM diagnosed on clinical grounds and confirmed histologically were included in the study group.
- The control group cases were selected according to criteria such as, clinically healthy persons without the history of any oral habits like tobacco, pan, and alcohol consumption, smoking and normal appearing oral mucosa.

Exclusion criteria

- Patients already undergoing treatment for OSMF were not included.
- Patients with temporomandibular joint disorders sharing similar complaints of incomplete mouth opening were not included.
- Patients with bleeding disorders were not included.

For clinical staging of OSMF, criteria by Lai DR (1995)[14] was followed. Mouth opening of each patient was measured using a Vernier Calliper.

Biopsies were performed on the test (OSMF) subjects from buccal mucosa and tissues from control subject were obtained during surgical removal of impacted third molar. Tissue biopsy samples were obtained and immediately fixed in 10% neutral buffered formalin routinely processed for histology and embedded in paraffin wax. All these tissues were processed in automated tissue processing machine (YORCO) for paraffin embedding technique. Four micrometre thick sections are cut, deparaffinised, and stained with haematoxylin and eosin for histological examination. Three oral pathologists evaluated and interpreted the haematoxylin and eosin stained slides for confirmation of the diagnosis and to grade oral submucous fibrosis according to histopathological grading of fibrosis criteria given by Pindborg JJ and Sirsat SM (1966).[15]

Immunohistochemical staining technique was based on the labelled streptavidin biotin (LSAB) method. Endogenous peroxidase was blocked by first activating the section in 0.6% H_2O_2. The specimen was then incubated with primary antibody followed by sequential incubations with biotinylated link antibody and peroxidase labelled streptavidin staining was completed after incubation with substrate chromogen solution.

Interpretation of IHC staining

All morphological structures with a lumen surrounded by CD105-positive endothelial cells were considered as microvessels. The assessment was carried out at the level of endothelial cell lining the blood vessels by their brown cytoplasmic staining. Microvessel density was assessed in areas showing the highest density of staining (hot spots) as determined by an initial scan at X100 magnification [Figures 1 and 2].

Microvessels were counted at X400 magnification by means of computer-assisted image analysis using image Pro Software (Leica research microscope: (Model No. DM1000LED) Software - Lieca Image analysis software (Version. LAS V4.12) and the individual microvessel profiles were circled to prevent the duplication and omission of microvessel count. According to Lee *et al*.,[16] the vessel count was recorded from the 3 most vascular fields (X400 magnification) next to the epithelium; images were captured and quantified by means of computer assisted image analyser for mean vascular density (MVD), mean vascular perimeter (MVP) and mean vascular area percentage (MVAP).

Statistical analysis

Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD and results on categorical measurement were presented in number (%). Level of significance was fixed at $P = 0.05$ and any value less than or equal to 0.05 was considered to be statistically significant. Student '*t*' tests (two tailed, unpaired) was used to find the significance of study parameters on continuous scale between two groups. Analysis of Variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis). Further *post hoc* analysis was carried out if the values of ANOVA test were significant. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables, etc.

OBSERVATIONS AND RESULTS

The present study consisted of 49 OSMF cases (study group) and 7 normal cases (control group).

Of the 49 OSMF cases of the study group, 43 cases were males and 6 were females. The control group consisted of 7 females.

The age of the study group ranged from 17 to 56 years, with a mean of 31.33 years. The age of the normal healthy controls ranged from 21 to 45 years, with a mean of 30.43 years. Comparison of age among study group and control group using unpaired '*t*' test was done, but the difference was statistically insignificant [Table 1].

The clinical staging of the cases was done based on the extent of mouth opening, according to criteria suggested by Lai *et al*.[14] There were 13 cases in Stage A, 11 cases in Stage B, 13 cases in Stage C and 12 cases in Stage D.

Histopathological grades of the cases was done as per the criteria suggested by Pindborg and Sirsat.[15] There were 4 cases in Grade 1 (Very early), 19 cases in Grade 2 (Early), 19 cases in Grade 3 (Moderately advance) and 7 cases in Grade 4 (Advance).

The MVD in Stage A, Stage B, Stage C, Stage D and control was 13.12821, 11.333, 15.05128, 13.02778 and 9.666, respectively [Table 2].

The MVP in Stage A, Stage B, Stage C, Stage D and control was 68.76704 μm, 66.359 μm, 72.26962 μm, 74.91808 μm and 59.760 μm, respectively [Table 3].

The MVAP in Stage A, Stage B, Stage C, Stage D and control was 216.0301 μm^2, 279.714 μm^2, 290.3145 μm^2, 284.5898 μm^2 and 197.788μm^2, respectively [Table 4].

Table 1: Comparison of the MVD, MVP and MVAP values in terms of {Mean (SD)} among both the groups using unpaired '*t*' test

Variables	Group	*N*	Mean	Std. deviation	*t*	*P*	Significance
MVD	Cases	49	13.210	4.8665	1.741	0.087	Insignificant
	Controls	7	9.666	6.2538			
MVP	Cases	49	70.662	18.6037	1.426	0.160	Insignificant
	Controls	7	59.760	21.3183			
MVAP	Cases	49	266.824	160.6985	1.096	0.278	Insignificant
	Controls	7	197.788	110.8472			

Table 2: Comparison of MVD values in terms of {Mean (SD)} among different clinical stages and controls using 'ANOVA' test MVD

Group	*N*	Mean	Std. Deviation	*F*	*P*	Significance
Stage 1	13	13.128	5.5168	1.586	0.192	Insignificant
Stage 2	11	11.333	5.5377			
Stage 3	13	15.051	3.7659			
Stage 4	12	13.027	4.3842			
Controls	7	9.666	6.2538			
Total	56	12.767	5.1317			

Table 3: Comparison of MVP values in terms of {Mean (SD)} among different clinical stages and controls using 'ANOVA' test MVP

Group	*N*	Mean	Std. Deviation	*F*	*P*	Significance
Stage 1	13	68.767	16.2372	0.832	0.511	Insignificant
Stage 2	11	66.359	23.1940			
Stage 3	13	72.269	21.0090			
Stage 4	12	74.918	14.3697			
Controls	7	59.760	21.3183			

Table 4: Comparison of MVAP values in terms of {Mean (SD)} among different clinical Stages and controls using 'ANOVA' test MVAP

Group	*N*	Mean	Std. Deviation	*F*	*P*	Significance
Stage 1	13	216.030	64.9941	0.760	0.556	Insignificant
Stage 2	11	279.714	227.3468			
Stage 3	13	290.314	196.9086			
Stage 4	12	284.589	120.7516			
Controls	7	197.788	110.8472			

The comparison of MVD, MPV and MVAP values among different clinical stages and controls was done with ANOVA test, but the difference was statistically insignificant.

The MVD in Grade 1, Grade 2, Grade 3, Grade 4 and control was 16.66667, 12.543, 13.03509, 13.52381 and 9.666, respectively [Table 5].

The MVP in Grade 1, Grade 2, Grade 3, Grade 4 and control was 71.62217 μm, 67.087 μm, 73.87853 μm, 71.08519 μm and 59.760 μm, respectively [Table 6].

The MVAP in Grade 1, Grade 2, Grade 3, Grade 4 and control was 221.1453 μm^2, 241.592 μm^2, 305.2449 μm^2, 257.131 μm^2 and 197.788 μm^2, respectively [Table 7]. The comparison of MVD, MPV and MVAP values among different histopathological grades and controls was done with ANOVA test, but the difference was statistically insignificant.

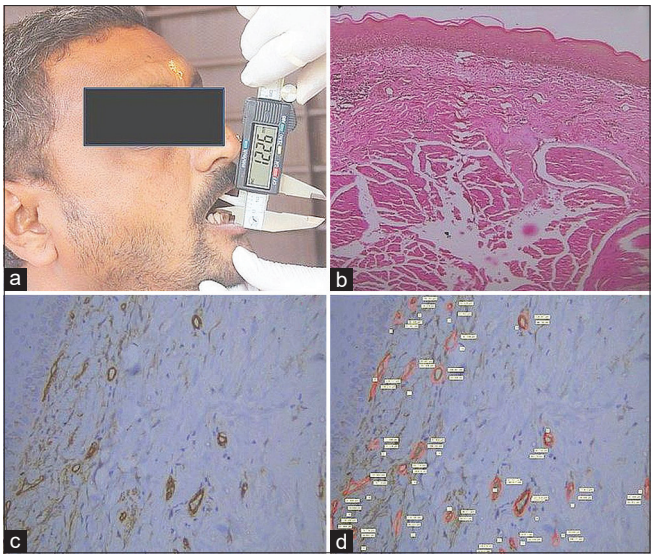

Figure 1: (a) Clinical photo of clinical stage d case. (b) Photomicrograph of H&E stained section of clinical stage d under higher magnification 10X'. (c) Photomicrograph of IHC stained section of clinical stage d case under higher magnification 40X. (d) Photomicrograph of IHC stained section of clinical stage D case under higher magnification 40X MVD, MVP and MVAP calibrations

DISCUSSION

Tumorigenesis is a multistep process and angiogenesis is one of it.[17,18] Thus angiogenesis is an important mechanism to sustain tumorigenic potential of neoplasia. Angiogenesis has been shown to play an important role in transition of normal tissues to preneoplastic state and eventually to full-blown cancer. Tumour progression is known to be accompanied by hypoxia.[19]

Hypoxia is also known to induce angiogenesis as tumours need a rich vascular supply in order for the cells to survive and grow but also to reach the circulation and metastasis. With insufficient supply of blood, tumour cells will undergo apoptosis/necrosis.[2,19-21]

A study by Desai *et al*.[2] has discussed extensively the role of vasculature in OSMF using CD34, but was unable to establish the role of vasculature in malignant transformation. Currently CD105 has been suggested as a powerful and specific marker for determining angiogenesis and its role in determining the prognosis of Oral squamous cell carcinoma.[12,13]

Table 5: Comparison of MVD values in terms of {Mean (SD)} among different Histopathological grades and controls using 'ANOVA' test MVD

Group	*N*	Mean	Std. deviation	*F*	*P*	Significance
Very early	4	16.666	3.3665	1.304	0.281	Insignificant
Early	19	12.543	6.0012			
Moderately advanced	19	13.035	3.3736			
Advanced	7	13.523	5.6596			
Controls	7	9.666	6.2538			

Table 6: Comparison of MVP values in terms of {Mean (SD)} among different Histopathological grades and controls using 'ANOVA' test MVP

Group	*n*	Mean	Std. deviation	*F*	*P*	Significance
Very early	4	71.622	11.7942	0.791	0.537	Insignificant
Early	19	67.087	18.5950			
Moderately advanced	19	73.878	21.0298			
Advanced	7	71.085	16.1582			
Controls	7	59.760	21.3183			

Table 7: Comparison of MVAP values in terms of {Mean (SD)} among different Histopathological grades and controls using 'ANOVA' test MVAP

Group	*N*	Mean	Std. deviation	*F*	*P*	Significance
Very early	4	221.145	61.2092	0.790	0.537	Insignificant
Early	19	241.592	170.1436			
Moderately advanced	19	305.244	175.0905			
Advanced	7	257.131	133.0825			
Controls	7	197.788	110.8472			

It has been suggested that CD105 is a hypoxia-induced angiogenic marker and is expressed mainly in solid tumours where there is hypoxia.[12,13] Hypoxia is a potent stimulator for CD105 gene expression in vascular endothelial cells and such an effect is potentiated in combination with TGF- β1. The upregulated CD105 appears to exert a self-protective role in endothelial cell (EC) under hypoxic stress. The possible reason for neoangiogenesis in OSMF could be that CD105 serves as a receptor protein for TGF-β which has bifunctional effects on endothelial cell proliferation and migration.[22]

In the present study, OSMF patients were clinically staged according to Lai D.R (1995) criteria into 4 groups; stage A (13 cases), stage B (11 cases), stage C (13 cases) and stage D (12 cases). Haider SM *et al.* (2000)[23] and Raina C *et al.* (2005)[24] observed that more than half of the patients were in clinical stage C and Hazarey VK *et al.* (2007)[25] also found almost half of the patients in their study were in clinical stage C.

The present study showed increase in MVD in OSMF cases (13.266 vessels) compared to normal (11.500 vessels), which was statistically insignificant. Rajendran *et al.*[26]

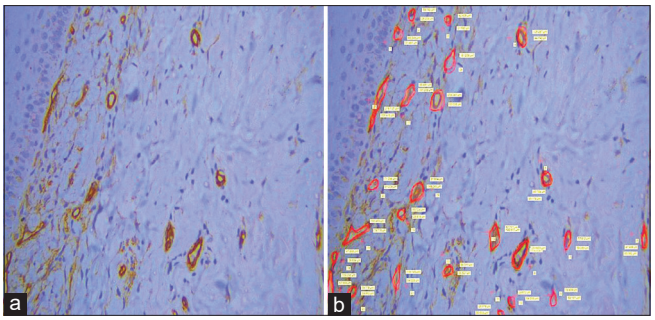

Figure 2: (a) Photomicrograph of IHC stained section of clinical stage D case under higher magnification 40X. (b) Photomicrograph of IHC stained section of clinical stage D case under higher magnification 40X MVD, MVP and MVAP calibrations

studied characterization and quantification in OSMF by using TGF-β expression, who showed that MVD was more or less the same in both the test and control groups. Whereas Murgod *et al.*[21] found that there was increase in MVD in early OSMF relative to advanced stage. Singh *et al.*[27] and Fang *et al.*[28] studied morphometric analysis in OSMF who found that MVD increased in early stages and decreased in advanced stages of OSMF. Sabarinath *et al.*[29] found increased MVD in moderately advanced OSMF, which is similar to our finding.[29] Gadbail AR *et al.*, suggested that assessment of tumour angiogenesis by using CD105 is not necessarily a characteristic of invasive tumours, but may occur near the initial event during disease progression from epithelial dysplasia to invasive squamous cell carcinoma in OSMF, and may be useful in predicting the clinical outcome of OSCC-OSMF.[30]

The present study has demonstrated increase in MVAP (263.053 μm^2) and MVP (70.033 μm) in OSMF as compared to controls which showed MVAP and MVP as 261.396 μm^2 and 59.379 μm, respectively, which was statistically insignificant. Our study showed increase in MVAP and MVP as the disease progressed, which was similar to findings of Rajendran *et al.*[26] Whereas, the findings of Desai *et al.*[2] (2010) showed decrease in MVAP and MVLD as the disease progressed. Probably this difference was due to study design which included classification system, sample size and distribution.

In the present study, we correlated the expression of CD105 in both clinical stages and histopathological grades and ANOVA test was applied to find out the significance but there was no significant correlation. These findings were similar to Pindborg J.J *et al.* (1964).[31] No significant correlation was obtained between clinical groups and histopathological grading in the patients of OSMF. The possibility of difference in the severity and extent of fibrosis in different regions of the oral mucosa

histopathologically and clinically could be because of the difference in location of fibrous bands and sight of biopsy.

In OSMF as the disease advances, the stroma becomes more and more hyalinized, the mediators of angiogenesis seem to diminish, and this will explain the decrease in MVD, thus tissue suffers resultant ischemia/hypoxia due to physical and biochemical effects of the process. Further the pathological mechanism takes place where tissue tries to cope up with hypoxia by actively promoting neo-vascularization as an adaptive response which is also known as angiogenic switch. An angiogenic switch seems to be triggered in moderately advanced OSMF due to the increased demand for blood to provide nutrition for the proliferating abnormal cells, and this will account for the increased MVD.[26] Our observation also demonstrated increased vascularity in moderately advanced OSMF, which might be associated with inflammation.

No significant correlation was obtained between clinical groups and histopathological grading in the patients of OSMF. The possibility of difference in the severity and extent of fibrosis in different regions of the oral mucosa histopathologically and clinically could be because of the difference in location of fibrous bands and sight of biopsy.

Highlights

The study provides valuable insights into the expression of CD105 in OSF, demonstrating a correlation between neoangiogenesis and both clinical staging and histopathological grading. These findings may contribute to a better understanding of the malignant potential of oral submucous fibrosis and could potentially serve as a diagnostic marker for assessing its severity.

SUMMARY AND CONCLUSION

Absence of prospective data on transformed cases of OSF is a major limitation of the present study. Hence, prospective studies on OSF using CD105, and providing results on malignant transformation cases will help in substantiating the hypothesis proposed in the present paper.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kumar KK, Saraswathi TR, Ranganathan K, Devi MU, Elizabeth J. Oral submucous fibrosis: A clinico-histopathological study in Chennai. Indian J Dent Res 2007;18:106-11.
2. Desai RS, Mamatha GS, Khatri MJ, Shetty SJ. Immunohistochemical expression of CD34 for characterization and quantification of mucosal vasculature and its probable role in malignant transformation of atrophic epithelium in oral submucous fibrosis. Oral Oncol 2010;46:553-8.
3. Rajendran R. Oral submucous fibrosis: Etiology, pathogenesis, and future research. Bull World Health Organ 1994;72:985-96.
4. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rate in oral submucous fibrosis over a 17-year period. Community Dent Oral Epidemiol 1985;13:340-1.
5. Pammar C, Nayak RS, Kotrashetti VS, Hosmani J. Comparison of microvessel density using CD34 and CD105 in oral submucous fibrosis and its correlation with clinicopathological features: An immunohistochemical study. J Cancer Res Ther 2018;14:983-8.
6. Folkman J. Tumor angiogenesis. Adv Cancer Res 1985;43:175-203.
7. Reynolds LP, Killilea SD, Redmer DA. Angiogenesis in the female reproductive system. FASEB J 1992;6:886-92.
8. Senger DR, Van De Water L, Brown LF, Nagy JA, Yeo KT, Yeo TK, *et al*. Vascular permeability factor (VPF, VEGF) in tumor biology. Cancer Metastasis Rev 1993;12:303-24.
9. Legan M. New marker of angiogenesis CD105 (endoglin): Diagnostic, prognostic and therapeutic role. Radiol Oncol 2005;39:253-9.
10. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis–A collagen metabolic disorder. J Oral Pathol Med 2005;34:321-8.
11. Tokunaga O, Minhajat R, Mori D. Tumor angiogenesis in cancers. Expression of CD105 marker. Method Cancer Diagn Ther Prognosis 2010;7:41-50.
12. Dallas NA, Samuel S, Xia L, Fan F, Gray MJ, Lim SJ, *et al*. Endoglin (CD105): A marker of tumor vasculature and potential target for therapy. Clin Cancer Res 2008;14:1931-7.
13. Marioni G, D'Alessandro E, Giacomelli L, Staffieri A. CD105 is a marker of tumour vasculature and a potential target for the treatment of head and neck squamous cell carcinoma. J Oral Pathol Med 2010;39:361-7.
14. Lai DR, Chen HR, Lin LM, Huang YL, Tsai CC, Lai DR. Clinical evaluation of different treatment methods for oral submucous fibrosis. A 10-year experience with 150 cases. J Oral Pathol Med 1995;24:402-6.
15. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966;22:764-79.
16. Lee AH, Dublin EA, Bobrow LG, Poulson R. Invasive lobular and invasive ductal carcinoma of the breast show distinct patterns of vascular endothelial growth factor expression and angiogenesis. J Pathol 1998;185:394-401.
17. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8.
18. Lu HH, Liu CJ, Liu TY, Kao SY, Lin SC, Chang KW. Areca-treated fibroblasts enhance tumorigenesis of oral epithelial cells. J Dent Res 2008;87:1069-74.
19. Mazure NM, Chen EY, Laderoute KR, Giaccia AJ. Induction of vascular endothelial growth factor by hypoxia is modulated by a phosphate dylinositol3-kinase/Akt signaling pathway in Ha-ras-transformed cells through a hypoxia inducible factor-1 transcriptional element. Blood 1997;90:3322-31.
20. Desai R, GS M, J Khatri M, J Shetty S. Immunohistochemical expression of vascular endothelial growth factor (VEGF) and its possible role in tumor progression during malignant transformation of atrophic epithelium in oral submucous fibrosis. Curr Angiogenesis 2012;1:347-53.
21. Murgod VV, Kale AD, Angadi PV, Hallikerimath S. Morphometric analysis of the mucosal vasculature in oral submucous fibrosis and its comparison with oral squamous cell carcinoma. J Oral Sci 2014;56:173-8.
22. Duff SE, Li C, Garland JM, Kumar S. CD105 is important for angiogenesis: Evidence and potential applications. FASEB J

2003;17:984-92.
23. Haider SM, Merchant AT, Fikree FF, Rahbar MH. Clinical and functional staging of oral submucous fibrosis. Br J Oral Maxillofac Surg 2000;38:12-5.
24. Raina C, Raizada RM, Chaturvedi VN, Harinath BC, Puttewar MP, Kennedy AK. Clinical profile and serum beta-carotene levels in oral submucous fibrosis. Indian J Otolaryngol Head Neck Surg 2005;57:191-5.
25. Hazarey VK, Erlewad DM, Mundhe KA, Ughade SN. Oral submucous fibrosis: Study of 1000 cases from central India. J Oral Pathol Med 2007;36:12-7.
26. Rajendran R, Paul S, Mathews PP, Raghul J, Mohanty M. Characterisation and quantification of mucosal vasculature in oral submucous fibrosis. Indian J Dent Res 2005;16:83-91.
27. Singh M, Chaudhary AK, Pandya S, Debnath S, Singh M, Singh PA, *et al*. Morphometric analysis in potentially malignant head and neck lesions: Oral submucous fibrosis. Asian Pac J Cancer Prev 2010;11:257-60.
28. Fang CY, Han WN, Fong DY. A morphometric study on the microvessel in oral submucous fibrosis. Hunan Yi Ke Da Xue XueBao 2000;25:55-7.
29. Sabarinath B, Sriram G, Saraswathi TR, Sivapathasundharam B. Immunohistochemical evaluation of mast cells and vascular endothelial proliferation in oral submucous fibrosis. Indian J Dent Res 2011;22:116-21.
30. Gadbail AR, Chaudhary MS, Sarode SC, Gondivkar SM, Belekar L, Mankar-Gadbail MP, *et al*. Ki 67, CD105 and α- smooth muscle actin expression in disease progression model of oral submucous fibrosis. J Invent Clin Dent 2019;00:e12443. doi: 10.1111/jicd.12443.
31. Pindborg JJ, Chawla TN, Srivastava AN, Gupta D, Mehrotra ML. Clinical aspects of oral submucous fibrosis. Acta Odontol Scand 1964;22:679-91.