IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 2 Ver. 12 February. (2018), PP 57-63
www.iosrjournals.org

Endoglin (Cd105) Expression in Oral Squamous Cell Carcinoma.

Dr. Srimahalakshmi. A[1], M.D, DR. ArunaLatha .P[2], M.D,
[1]*Assistant Professor, Government Mohan Kumaramangalam Medical College Hospital, Salem.*
[2]*Professor And HOD, Department Of Pathology, Stanley Medical College, Chennai.*
Corresponding Author: Arunalatha. P

Abstract
Background: *Increasing evidence suggests that angiogenesis is an essential factor in the tumour growth, invasion and metastasis. Research on related parameters such as micro vessel density(MVD) could be helpful in predicting the tumour behavior. Recently, Endoglin (CD105) has been introduced as a new powerful marker of neoangiogenesis that stains vessels in the proliferating stage. Many studies revealed that Endoglin (CD105) was intensively expressed in the tumour vessels and therefore could be both predictive and prognostic marker for the outcome in various malignancies.*
Aims And Objectives*: To determine and correlate the Endoglin (CD105) expression in normal oral mucosa, premalignant lesions of oral cavity and in oral squamous cell carcinoma.*
Materials And Methods: *Thirty (30) cases of normal oral mucosa, thirty (30) cases of oral pre-malignant lesions and thirty (30) histopathologically confirmed cases of oral squamous cell carcinoma, were immunohistochemically analyzed for Endoglin (CD105) expression and quantitated by evaluating micro vessel density through "Hot spot method".*
Results: *There was a significantly (P value <0.001) higher expression of Endoglin in oral squamous cell carcinoma than that of normal oral mucosa and premalignant lesions. Among carcinoma, Endoglin expression was significantly (P value 0.005) higher in poorly differentiated carcinoma when compared to well and moderately differentiated carcinoma. There was no association with this Endoglin expression and patients age, gender or tumour localization.*
Conclusion: *The finding of significantly higher MVD score in carcinoma than in normal oral mucosa and in premalignant lesions make us to emphasize that Endoglin could be a useful predictive and prognostic marker of malignancy.*
Keywords*: Endoglin, CD105, Microvessel density, oral squamous cell carcinoma*

Date of Submission: 09-02-2018 Date of acceptance: 24-02-2018

I. Introduction

Cancer is ubiquitous in human populations; the only way to avoid cancer is not to be born, but as to live is to incur the risk. Study of cancer patterns in the individuals has contributed the knowledge about the origin of cancers at specific sites.[5,18]

Head and neck is the most complex part of the body. Among head and neck cancers, oral cancers contribute around 85% of cases.[1,2,3] Oral cancers were diagnosed, when the cancer had reached or metastasized to the other location, most commonly to the lymph nodes of the neck. Hence, proper and better understanding regarding anatomy, histology and lymphatic drainage of the oral cavity will give us a better insight for the early diagnosis and appropriate management.[5,18]

Angiogenesis was proved to be one of the most essential component in the tumor growth and metastasis. Hence, the intensity of angiogenesis was considered to be a poor prognostic factor and it has become one of the major determinant for the development of oral cancers and also for other cancers.[3]

Endoglin is expressed in activated endothelial cells during the process of angiogenesis and therefore considered to be the most specific angiogenic marker for the detection of tumor angiogenesis. There is a strong association of Endoglin expression in tumor endothelium than in normal endothelium. Hence, used as a better immunohistochemical marker in assessing the microvascular density than any other endothelial marker.[3]In this retrospective and comparative study, Endoglin expression is assessed in normal oral mucosa, premalignant lesions and in oral SCC. The results were compared and analyzed.Endoglin was found to be a good predictive marker of malignancy as well as a good prognostic marker.[13]

II. Methods:

Totally, 5967 cases were reported in the Stanley Medical College during the period of January 2015 to December 2015. Out of which, 178 cases were from the oral cavity. In this, 91 cases were reported as malignancy, 47 cases being reported as premalignant lesions and 39 cases were found to be normal.

Detailed history regarding the patient's age, gender, site, side of lesion, personal history and the type of biopsy done were collected from the surgical pathology records.Sections of 4um thickness were taken from the corresponding paraffin blocks. The stained sections were then reviewed.30 normal reports, 30 cases among dysplasia and 30 cases from carcinoma were selected at random for staining with Endoglin immunohistochemical marker.Immunohistochemically stained slides were labelled, viewed and analyzed. The immuno positivity was confirmed by the presence of (brown) endothelial cells staining in the vessel wall. After confirming its positivity, micro vessel density was calculated by using hotspot method.

Micro vessel density was defined as the number of CD105(Endoglin) stained vessels per mm^2. The density (per mm^2 of newly formed blood vessels in the normal tissue, dysplastic and in the intra tumoral region were calculated based on the method described by Weidner et al. (Hot spot method).[17]At first, slides were screened using a low-magnification objective lens. The areas with the highest number of positively stained vessels (hot spots) were identified and selected. The same was viewed, under high power magnification lens. In this field, the number of positively stained vessels were counted. This should be done for three fields. The average of the three fields were taken. This will be the micro vessel density per high power field area($0.15mm^2$). The mean number of vessels were converted into number of vessels per mm^2 area and the resulting value was taken as micro vessel density.[6]Parameters such as age, gender and Endoglin expression (Micro vessel density) were evaluated.

III. Results:

Majority (34.4%) participants belonged to 61 to 70 years of age group. There were 21 (23.3%) subjects each in 41 to 50 years and 51 to 60-year age groups. The proportion of subjects below 40 years was 13.3% and 5.5% of the subjects were above 70 years. The proportion of males was 74.4% and females was 25.6% in study population. (Table 1)

Table 1: Frequency distribution for Age & Gender in study group (N=90)

Parameter	Frequency	Percent
Age Groups		
40 Years and less	12	13.3
41 to 50years	21	23.3
51 to 60 Years	21	23.3
61 to 70 years	31	34.4
71 -	5	5.5
Gender		
Male	23	25.6
Female	67	74.4

The most common sites of biopsy in the study were **tongue (42.2%)** and buccal mucosa in study population (34.4%). The above parameters were analysed with micro vessel density score. The mean MVD score was 34.04 in people below 40 years of age, 33.09 in people at 40 to 50 years & 54.11 & 44.86 are at 51 to 60 and 61 to 70 years of age respectively. And 53.75 at >70 years, which was statistically not significant. (P value 1.00). The mean MVD score was 46.83 in male and 33.09 in female, with a mean difference of 13.74 units (95% CI -7.44 to 34.93) which was statistically not significant. (P value 0.201).

Among the 30 subjects with premalignant lesions the number of subjects who had leukoplakia with mild, moderate and severe dysplasia were 19 (63.3%), 3 910%) and 6 (205) respectively. Remaining 2 (6.6%) subjects had sub mucosal fibrosis. Among 30 subjects with carcinoma, the number of subjects with grade 1, 2 and 3 cancers were 14 (46.6%), 13 (43.3%) and 3 (10%) respectively. These histopathological types were then compared with the MVD score.

Table 2: Association between HPE and MVD score in study group (N=90)

HPE New	Mean MVD score	Mean difference	P value	95% CI	
				Lower	Upper
Normal	**8.37±4.45**				
Pre malignant	**37.35±14.85**	28.97	0.002	9.10	48.85
carcinoma	**84.25±52.37**	75.88	<0.001	56.00	95.76

The mean MVD score was 8.37 in people with normal, 37.35 in people with Pre malignant and 84.25 with carcinoma. The MVD score was 28.97 units higher in premalignant group (95% CI 9.10 to 48.85, p value 0.002) and 75.88 units higher in malignant group (95% CI 56 to 95.76, p value < 0.001) when compared to normal subjects. Both these differences were statistically significant. (Table 2)

ROC ANALYSIS:

To conduct ROC analysis, the subjects in normal and premalignant categories were combined and labelled as non-carcinoma.(table 3)

Table 3: Frequency distribution of non-carcinoma and carcinoma groups (N=90)

HPE 2 Cat	Frequency	Percent
No carcinoma	60	66.66
Carcinoma	30	33.33

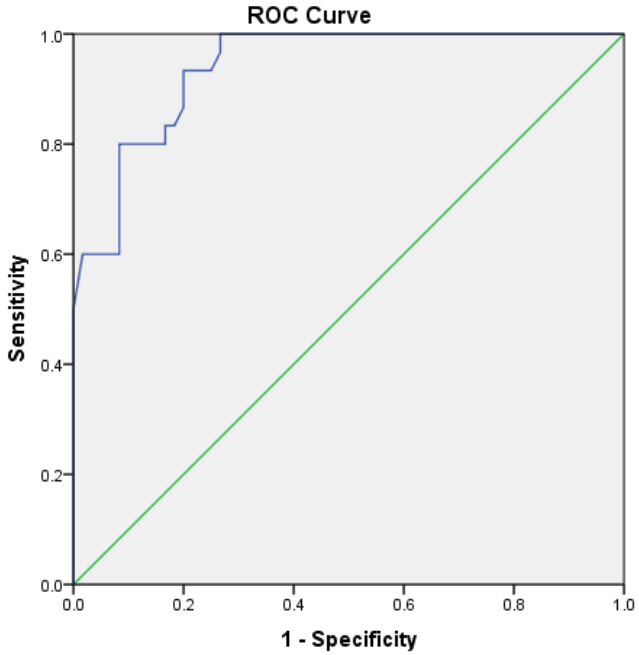

Chart 1: Receiver operating curve (ROC) analysis of MVD score and carcinoma (N=90)

Area Under curve	P value	95% Confidence Interval	
		Lower Bound	Upper Bound
.940	<0.001	.896	.984

Based on ROC analysis 34.43 was chosen as the best cut off value for MVD score. An MVD score of 34.43 and above was considered as high and a score up to 34.42 was considered as low. **The P value (<0.001) was statistically significant .The sensitivity, specificity and predictive values of MVD score is high in diagnosing carcinoma**

Against the gold standard, high MVD score had a sensitivity of 100% (95% CI = 20.53% to 39.47%) and specificity of 73.3% (95% CI was 33.74% to 54.26%), False positive rate was 26.67% (8.43% to 23.57%) with no false negative test results. The Positive predictive value & Negative predictive value was 65.2% (95%CI 20.53% to 39.47%) and 33.74% (95% CI 33.74% to 54.26%) respectively. The overall diagnostic accuracy was 74% (95%CI 64.94% to 83.06%).

IV. Discussion:

Oral cancer is an important global health concern accounting for 1, 28,000 deaths annually. Oral cancers are particularly dangerous, Because in more than 50% of the patients, the disease presents in an advanced stage and the expected survival rate for five years was found to be around 10-40% (Gold berg et al 1994).[19]This decreased survival rate is due to persistent uncontrollable disease and poor understanding of the pathogenesis at the molecular level and lack of knowledge regarding the significance of angiogenesis in the tumor progression.[4]

Many studies have used pan- endothelial markers such as CD31, CD34, VEGF-A in evaluation of micro vessel density which had low sensitivity and specificity therefore, the results were not convincing and at times contradictory too. But, Endoglin reacts specifically to the proliferating (angiogenic) endothelial cells and hence, proved its high specificity in Micro vessel density.Endoglin is a 180 Kda transmembrane protein and it shows higher expression in proliferating endothelial cells. It occurs in two isoforms (L and S) as a result of alternate splicing of the transcript. There is a strong association of Endoglin expression in tumor endothelium than in normal endothelium. Hence, used as a better immunohistochemical marker in assessing the microvascular density than any other endothelial marker.[7]

In the current study, histopathological and immune histochemical evaluation was done in 90 cases of oral cavity. They were categorized into three groups based on the histopathological report. Group 1 includes the normal oral mucosa; group 2 involves the biopsy specimens reported as dysplasia and group 3 highlights the specimens reported as neoplasia. An attempt was made to assess the strength of expression in three different groups, particularly in neoplasia and thereby, targeted therapy could be tried for better prognosis.[5]Among premalignant lesions (30 cases), mild dysplasia was observed in 19 (63.33%) cases, moderate dysplasia in 3 (10%) cases, severe dysplasia in 6 (20%) cases and submucosal fibrosis in 2 (6.66%) cases. while, in Marwah et al study 23% belongs to mild dysplasia, 59% belongs to moderate dysplasia and 18% in severe dysplasia.[7]Among malignant lesions (30 cases), 14 cases (46.66%) were diagnosed as well differentiated carcinoma, 13 cases (43.33%) were reported as moderately differentiated carcinoma and the remaining 3 (10%) cases were put under poorly differentiated carcinoma.

In this study, the correlation of MVD with age was not found to be statistically significant, since the P value was 1.000. This was in concurrence with other studies such as Schimming et al, Chien et al study, Miyahara et al and Martone et al study.[8,9,10.]In our study, the correlation of MVD expression with gender in carcinoma (predominantly males) group alone was proved to be statistically significant whose P value was 0.005.The mean MVD in normal mucosa was 8.37(figure1). The mean MVD score in the group 2 biopsy specimens was 37.35 and in evaluating MVD score in dysplasia group, was found to be statistically significant (P value 0.002). This was in concurrence with other study such as Maharudrappa n. Basnaker et al study.[3,6](figure 2,3&4)In this study, the expression of Endoglin in the tumor vasculature was found to be highly significant (P value <0.001). The mean MVD score in this group was 84.25 and the mean difference from that of the normal mucosa was 75.88. This was in concurrence with that of the other study (schimming et al, weidner et al, chien et al)[14,15,17]

In this study, we should also note that the intensity of Endoglin expression in various grades of carcinoma was statistically significant and the P value was 0. 005.This means that **MVD score was significantly higher in poorly differentiated carcinoma than that of well differentiated carcinoma**. **This was in concurrence with one study done by Weidner et al**, but all other studies showed negative correlation between MVD score and grade of tumor differentiation. CL Margaritetsu et al study did not observe any association and thereby did not report any correlation between the MVD score and the grades of tumor differentiation.[12,7,8,9,11,16]

In our study, the comparison of mean MVD score among all the three groups was also statistically significant (P value <0.001). This clearly explained that, Endoglin expression was definitely upregulated in the tumor vasculature when compared to that of the normal mucosa.(figure 5,6 &7).

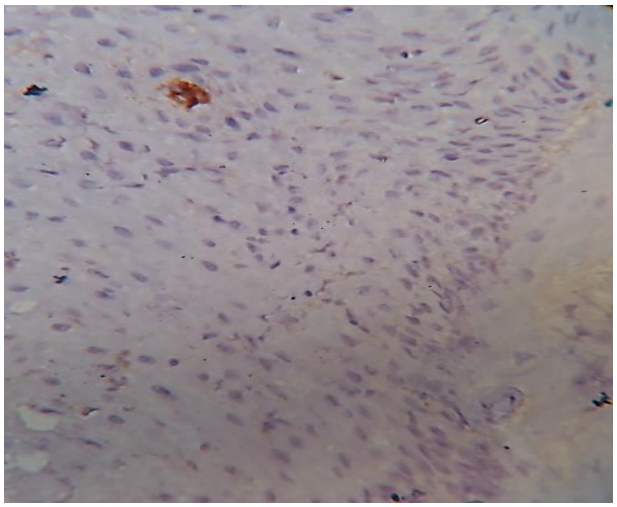

Figure 1: Showing Endoglin Expression in Normal Mucosa

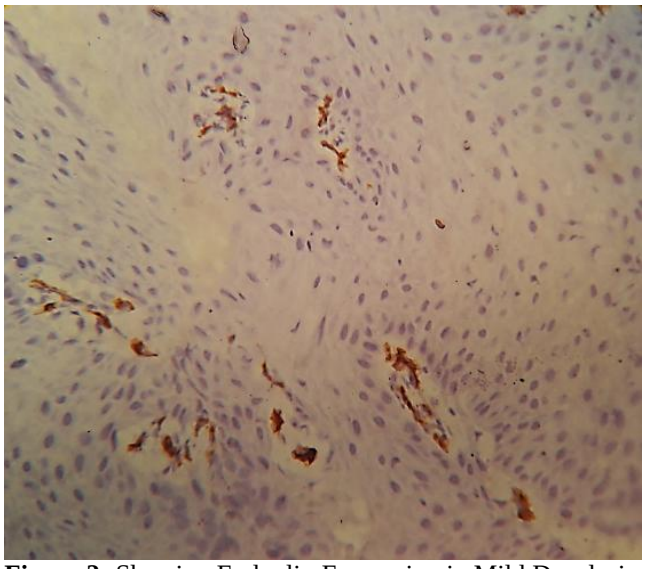

Figure 2: Showing Endoglin Expression in Mild Dysplasia

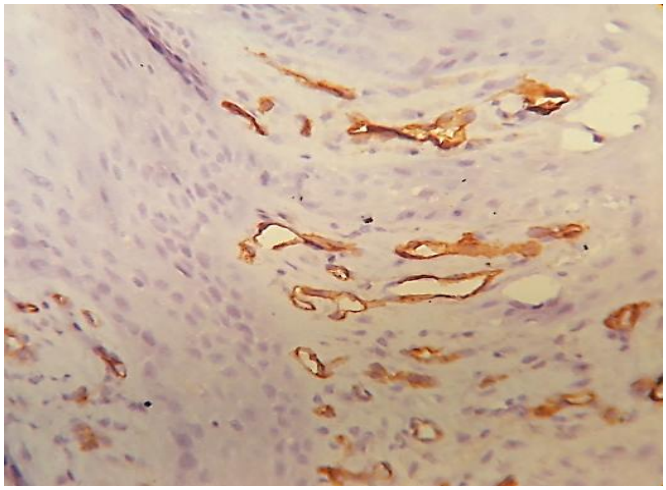

Figure 3: Showing Endoglin Expression in Moderate Dysplasia

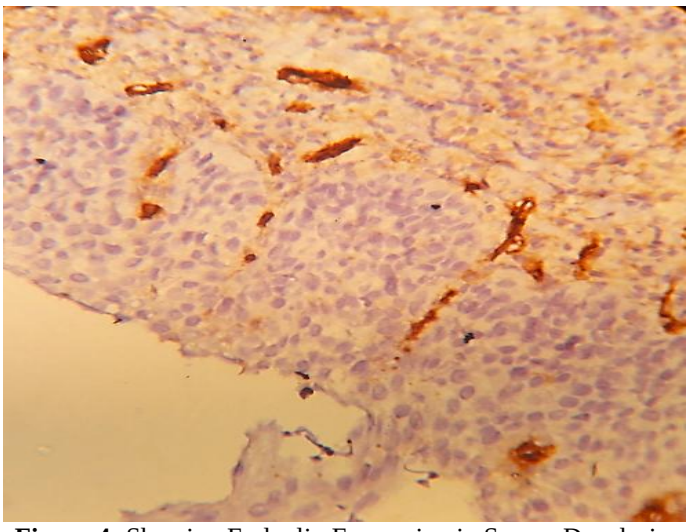

Figure 4: Showing Endoglin Expression in Severe Dysplasia

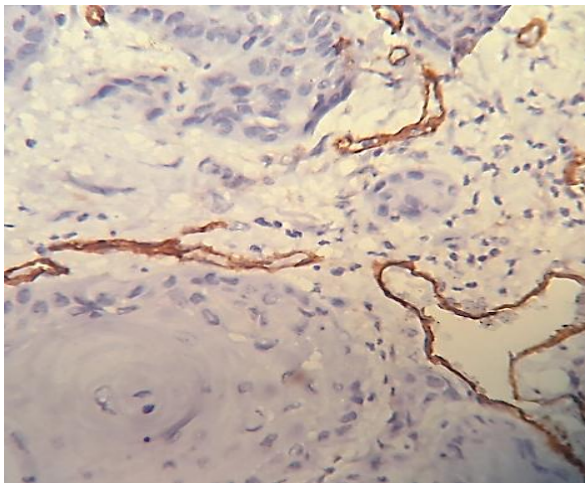

Figure 5: Showing Endoglin Expression in Well Differentiated Carcinoma

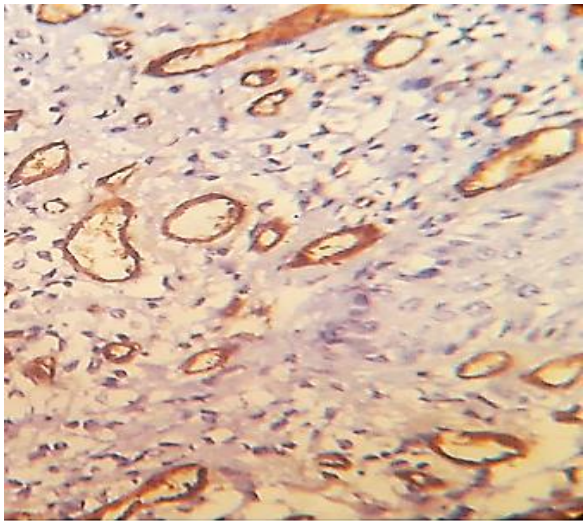

Figure 6: Showing Endoglin Expression in Moderately Differentiated Carcinoma

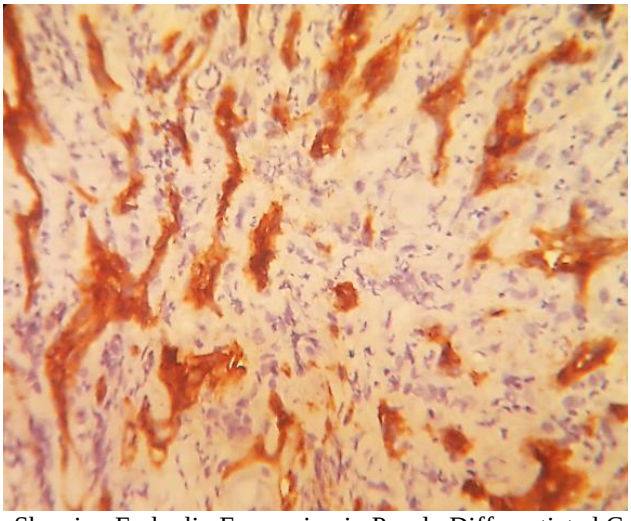

Figure 7: Showing Endoglin Expression in Poorly Differentiated Carcinoma

V. Conclusion:

This study showed the marked prevalence (51.12%) of malignancy (oral SCC) in the population. This study showed a good strength of Endoglin expression in carcinoma than that of the pre malignant lesion and normal mucosa. Hence, Endoglin proved to be a good predictor and a good prognostic marker as well. More

studies with good sample size would give us a better understanding regarding the tumor relation with neo angiogenesis and the significant role of Endoglin expression, so that in near future, **combined treatment modality like chemo radiation with the targeted therapy (anti-CD105 mono clonal antibody) could be considered. This approach would give us better therapeutic response and thereby, improving the survival of the patient**.

References:

[1]. Barnes L., Eveson J. W., Reichart P., Sidransky D. (Eds). Pathology & Genetics of Head and Neck Tumours, WHO Classification of Tumours Lyon: IARC Press; 2005.
[2]. DM, Parkin. Global cancer statistics in the year 2000. Lancet Oncol. 2001; 2(9): p. 533-543.
[3]. Cancer Incidence and mortality worldwide. ; GLOBOCAN 2008.
[4]. R A Cawson, P Speight, W H Binnie. Lucas's Pathology of Tumors of the Oral Tissues. 5th ed.: Churchill Livingstone; 1998.
[5]. Vinay Kumar, Abul K. Abbas, Jon C. Aster. Robbins and Cotran Pathologic Basis of Disease. 9th ed. Philadelphia: Elsevier Saunders; 2015.
[6]. Maharudrappa N, Basnaker et al. Expression of endoglin (CD105) and Micro vessel density in Oral dysplasia and Squamous cell carcinoma. Journal of clinical and diagnostic research. 2014; 8(9): p. ZC91–ZC94.
[7]. Barbara Young, Philip Woodford, Geraldine, O' Dowd. Wheaters Textbook of Functional Histology. 6th ed.: Elsevier; 2014.
[8]. Rosai, Juan. Rosai and Ackerman's Surgical Pathology. 10th ed.: Mosby; 2011.
[9]. Gnepp, Douglas R. Diagnostic Surgical Pathology of the Head and Neck. 2nd ed.: Saunders; 2009.
[10]. http://oralcancerfoundation.org. [Online]. Available from: http://oralcancerfoundation.org.
[11]. Bouquot JE, Farthing PM, Speight PM. Epithelial Dysplasia of the Oral Mucosa - Diagnostic Problems and Prognostic Features. 2006; 1(12): p. 11-21.
[12]. Gale N, Zidar N. Benign and Potentially Malignant Lesions of the Squamous Epithelium and Squamous Cell Carcinoma. In Cardesa A SP. Pathology of Head and Neck. 1st ed.: Springer Berlin Heidelberg; 2006.
[13]. Pindborg JJ, Reichert PA, Smith CJ, et al. Histological typing of cancer and precancer of the oral mucosa. 2nd ed. Berlin: Springer; 1997.
[14]. Schimming R, Marmé D. Endoglin (CD105) expression in squamous cell carcinoma of the oral cavity. Head Neck. 2002; 24(2): p. 151-6.
[15]. 15.Chien CY, Su CY, Hwang CF, Chuang HC, Hsiao YC, Wu SL., et al. Clinico pathologic significance of CD105 expression in squamous cell carcinoma of the hypopharynx. Head Neck. 2006; 28: p. 441-6. 48.
[16]. CL MARGARITESCU, CRISTIANA SIMIONESCU et al. Endoglin(CD105) and micro vessel density in oral squamous cell carcinoma. Romanian journal of Morphology and Embryology. 2008; 49(3): p. 321-326.
[17]. WEIDNER N., CARROLL P.R.,FLAX J., BLUMENFELD W.,FOLKMAN J.. Tumor angiogenesis correlates with metastasis in invasive prostrate carcinoma. Am J Pathol. 1993; 143(2): p. 401-409
[18]. CA, Waldron. Oral epithelial tumors Mosby. In Gorlin RJ GH, editor. Thomas' Oral pathology.: Mosby; 1970. p. 834-835.
[19]. Brad W. Neville, DDS, Douglas D. Damm, DDS, Carl M. Allen. Oral and Maxillofacial Pathology. 2nd ed. Philadelphia: WB Saunders; 2002..

Dr. Srimahalakshmi "Endoglin (Cd105) Expression in Oral Squamous Cell Carcinoma..". "IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), Volume 17, Issue 2 (2018), PP 57-63.