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MVD CAN BE USED AS AN INDICATOR OF ANGIOGENESIS

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ABSTRACT
Angiogenesis plays a central role in the embryogenesis, growth and development of normal tissues and in a variety of pathological disorders such as diabetic retinopathy and rheumatoid arthritis. The study sample consisted of 30 cases of Normal buccal mucosa (NBM). The 30 archival samples were stained by Hemotoxylin and Eosin (H & E) and Masson Trichrome (MT). The stained sections were analysed using Image analysis software. Statistical analysis used: SPSS 12.0 statistical software was used. The combined Mean Vessel Density (MVD) of all the cases in H & E was 0.1112 and for MT 0.2150. The difference of MVD between H&E and MT was statistically significant. MVD can be used as an indicator of turn-on & turn-off phenomenon of angiogenesis. MVD can also be used as an adjunct with other diagnostic modalities. The baseline level of MVD for buccal mucosa was 13%. Further studies are needed to standardize baseline levels for different sites and age groups.

INTRODUCTION

Angiogenesis is a complex, multistep process involving endothelial cell growth, migration and capillary morphogenesis [1]. Tumors require a blood supply for growth and dissemination. Considerable attention has been focused on the mechanisms by which tumors acquire their blood supply. It is well-accepted paradigm that tumors recruit new blood vessels from the existing circulation by secreting growth factors like VEGF, FGF etc from the tumor cells. This has been shown in many human malignancies, especially lung, prostate and breast cancers. Nonetheless, in some cancers the relationship between tumor angiogenesis and cancer clinico-pathologic parameters are still controversial [2]. In clinical practice, lesions can only be termed potentially malignant only after malignant change has developed, since there are no means of predicting with certainty the risk of cancerous transformation. Microscopy has been the best available guide. The blood supply of the oral mucosa is extremely rich and the vascularity of the various parts of oral mucosa differs. Hence, this study used mean vascular density (MVD) as diagnostic tool for diagnosing lesions undergoing angiogenesis.

AIMS AND OBJECTIVES

The study attempts to

1) Evaluate vascularity in normal buccal mucosa
2) Evaluate the correlation between morphometric aspects of vascularity in normal buccal mucosa by H&E and Masson's Trichrome.

MATERIALS AND METHODS

The study involved the use of formalin fixed, paraffin-embedded tissue of Department of Oral Pathology, PDUDC, Solapur, Maharastra, India.

The study sample consisted of 30 cases of normal buccal mucosa.

The relevant information of age, sex, site, histopathological grading, lymph node status, metastasis, and recurrence was obtained. Mean vessel density was assessed of H&E and MT stained 30 normal buccal mucosa. Most intense vascular area was found by light microscopy in 40X (Figure 1 & 2) and counting performed in 400X field (Figure 3). The assessment was made by single trained observer. Images of histological samples were captured using Lawrence and Mayo (75X)

microscope and Nikon digitized camera. The images were saved to the computer and examined using high resolution RGB display monitor and the morphometric analysis was performed manually by using "Image-J" image analysis software.

The assessment included

a) The vascular tissue was identified by the presence of red blood cells and endothelial cells.

b) For MT method, blood vessels were encircled with red line; lymphatics were devoid of encircling line. The presence of red blood cells was also considered.

c) Most intense vascular area was found by light microscope in 40X.

d) Three such fields were selected under 40X for each slide.

e) The area representing vascular tissue in the said digital image.

f) The area representing total tumor tissue in the said digital image.

g) MVD – the ratio of vascular tissue to total area in the said digital image.

STATISTICAL ANALYSIS

The statistical analysis included mean, standard deviation; minimum and maximum values were calculated for each group and for NBM. The Student's t-test was used to determine significant differences present in the area while moving the slide clockwise and MVD between different groups. A p-value of less than 0.05 was considered for statistical significance.

One way ANOVA was used for the variance between groups and Student's t-test for variance within group using SPSS 12.0 statistical package.

A total number of 30 cases were taken for the study and the mean MVD of all the cases in H&E was 0.1112 and for MT 0.2150 with a p-value of 0.0017 which was statistically significant (Table 1). The percentage of distribution of study subjects by three groups and gender in normal revealed 33.33% and 66.67% for female and male respectively. The comparison of Normal buccal mucosa in H&E and MT by one-way ANOVA test resulted in a p-value of 0.0006 and 0.0000 which was statistically significant. (Table 2 &3) Pair wise comparison of three groups by student's t-test is studied for H&E and MT. For H&E the p-value for normal and leukoplakia is 0.1570 which is statistically not significant. The p-values for NBM was statistically significant. (Table 4 &5)

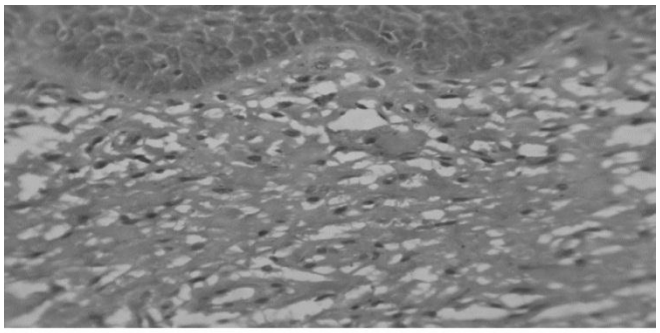

FIGURE 1. PHOTOMICROGRAPH OF NORMAL BUCCAL MUCOSA SHOWING H & E STAINED SECTION (40X)

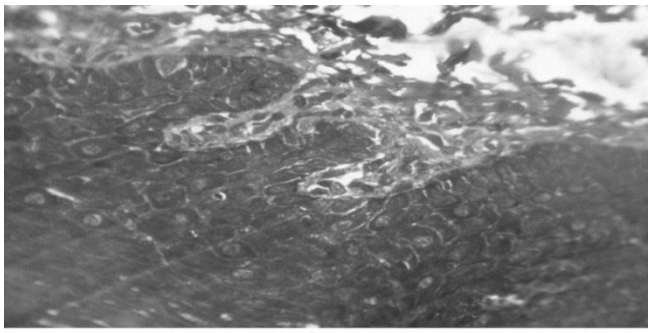

FIGURE 2. PHOTOMICROGRAPH OF NORMAL BUCCAL MUCOSA SHOWING MASSON'S TRICHROME STAINED SECTION (40X)

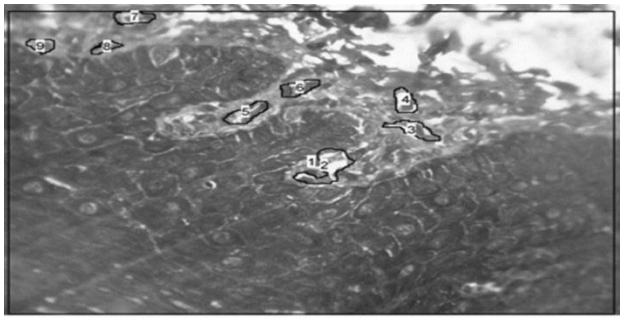

FIGURE 3. PHOTOMICROGRAPH OF NBM SHOWING MORPHOMETIRC ANALYSIS IN MASSON'S TRICHROME STAINED SECTION

Table 1.Comparison of H & E and MT groups in total of all the three groups.

Group	Mean± SD	t-value	p-value
H &E (n=30)	0.1112 ± 0.1937-3.1937	0.0017*	
MT (n=30)	0.2150 ± 0.2042		

Table 2.Comparison of two groups of in H & E by one-way ANOVA

Source ofvariation	Degreeof freedom	Sumof squares	Mean sum of squares	F-value	P-value
Between	2	0.52	0.2583	8.2300	0.0006*
Groups					

Table3. Pair wise comparison of three groups by Student's t-test for H&E

Group	Mean± SD	t-value	p-value
Normal (n=30)	0.0611 ± 0.0783-2.1085	0.0409 *	

Table 4. Pair wise comparison of three groups by Student's t-test for MT

Group	Mean± SD	t-value	p-value
Normal (n=30)	0.1314 ± 0.2358	-2.9862	0.0046*

Table 5. Comparison of two groups in MT by one-way ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F-value	P-value
Between 2	0.85	0.4259	13.7177	0.0000*	
Groups					

DISCUSSION

There are various studies on angiogenesis with different parameters [Mean Vascular Density (MVD), Mean Vascular Volume (MVV), Mast cell Density (McD)] [3-5] and immunohistochemical stains. However, none of the antibodies (CD34, CD31 and Factor VIII) could distinguish between blood vessels and newly forming blood vessels. α v β3 integrin and TGF-β receptor complex were till recently thought to differentiate neo-vasculature from pre-existing blood vessel, which were not found useful in the subsequent studies. Though the reasons for the observed variability are unclear, the possible explanations are anatomical variation of vascularity throughout different locations of oral cavity, methods of determining MVD, methods of staining (Antigens screened for CD 31, Factor VIII, CD 34 and different antigens), methods of observation, and reporting degree of angiogenesis [6,7]. Hence, digital MVD determination in MT stained sections is considered to be appropriate especially in developing countries like India, considering the cost and early detection of the tumor.

The present study included 30 cases of NBM. The objective of the study was the morphometric correlation of vascularity, its usefulness for the buccal mucosa. Hence, the results obtained were that the mean MVD of all the groups by MT staining was 0.2150 which was more than that of H&E, where it was 0.1112. The compared values of both the staining were statistically significant (p= 0.0017). Hence MVD using Masson's Trichrome can be used as cost effective tool to measure the rate of angiogenesis.

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