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Micro Pericytes Coverage in Relation to Different Grades of Oral Squamous Cell Carcinoma

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Abstract: ***Background: Angiogenesis is an important step in Oral Squamous Cell Carcinoma(OSCC)growth, progression, and metastasis. Micro Vessel Density(MVD) can reflect the angiogenic rate but not gives knowledge about the efficiency state of newly formed vessels. Measuring of micro vessel pericytes coverage index(MPI) for these blood vessels can determine the degree of their maturation. Aim of the study: this study established to determine the association of between MVD, MPI and clinicopathological parameter of OSCC. Material and Methods: Retrospective 36 formalin- fixed, paraffin-embedded tissue blocks histologically diagnosed as OSCC were examined for MVD and MPI were assessed by conventional immunohistochemistry staining using anti-CD105 and anti α-SMA antibodies respectively. Results: Highly significant association of MPI and histological grade of OSCC(P=0.000), while no significant difference between MVD and clinicopathological parameter of OSCC. Conclusion: Both of MVD (using CD105) and MPI can considered as signs of the angiogenic and functional status of a tumor vascularity.***

Keywords: Oral Squamous Cell Carcinoma, Pericytes, Micro Vessel Density

1. Introduction

Oral Squamous Cell Carcinoma (OSCC) characterizes about more than 90% of all oral malignancies worldwide[1]. Angiogenesis, newly formed vasculature from that already presented blood vessels, play an essential role in the development and progression of the tumor. [2,3]

Endoglin (CD105): is a powerful pleiotropic angiogenic factor expressed on activated endothelial cells (ECs) and it proposed to be a specific indicator to sense tumor angiogenesis.[4,5]. Alpha smooth muscle actin (α-SMA) is actin isoform that expressed within vascular smooth muscle cells (v SMCs), in macro vessels (arteries and veins), or within pericytes in micro vessels(capillaries and veinules). These mural cells are effective in providing stability and maturity to the newly formed vessels [6,7].

Micro vessel density (MVD): A measurement of tissue angiogenesis that made through measuring micro vessels number in a given area by one of endothelial markers [7]. Micro Pericytes Coverage Index(MPI) could be referred to the percentage the number of the micro vessels which positively stained with α-SMA antibody in the same vascular hot spot field in which MVD were numerated[8].
The aims of the current study were immunohistochemical evaluation of angiogenesis and vessel maturation, in addition to the evaluation of, MVD and MPI in relation to clinicopathological parameters of OSCC.

2. Materials and Methods

Tissue sample
Thirty six retrospective formalin- fixed, paraffin-embedded tissue blocks diagnosed histopathologically as OSCC which obtained from the archives of the Oral Maxillofacial Pathology Department / Dentistry Collage of Baghdad University were enrolled in this study. Demographic and clinical data: patients name, age, gender, clinical presentation, and tumor site,), were obtained from the archives. The positive tissue controls (formalin fixed, paraffin-embedded tissue blocks): normal lung, human breast adenocarcinoma for immunohistochemical evaluation of CD105, and α-smooth muscle actin antibodies respectively were obtained from the archives of Al-Shaheed Ghazi Hospital, Teaching Laboratory Department/ Baghdad Medical City.

Conventional immunohistochemistry (IHC)
Five micrometer (5µm) serial sections for all studied samples. Ordinary staining with Haematoxylin and Eosin (H&E) has done first to confirm diagnosis. CD105, and α-SMA expression were evaluated using abcam, expose mouse and rabbit specific HRP/DAB detection IHC kit (ab80436, Lot: GR288328-11). From the beginning deparaffinization and rehydration were done, then blocking of endogenous peroxidase by incubation the histological sections with 3 % hydrogen peroxide for 10 min. The blocking procedure for nonspecific antigens of the sample confirmed by binding with normal goat serum incubated for 1 hour. Antigen retrieval procedure was carried out (for those received anti α-SMA antibody) by incubating the samples in Citrate Buffer Saline (CBS) PH 6.0, and heating in water bath (95^{o}C) for 10 min. Sections were then incubated with one of primary antibody, an anti CD105 poly clonal antibody(1/200) dilution; (abcam company [ab49228]), and an anti α-SMA monoclonal antibody(1/200) dilution; (abcam company[ab125057]) overnight at 4^{o} C. Washing with phosphate-buffered saline (PBS, pH = 7.0). Then using the previous mentioned IHC detection kit, the slides were incubated with secondary antibody (rabbit anti mouse antibody unconjugated then Gout anti –rabbit HRP conjugated) 10min for each then incubation with 3,-3,-diaminobenzidine chromogen (DAB)for 1min at room temperature.

Haematoxylin was used as a counter stain, dehydration through alcohol solution in different concentrations, finally

mounting with cover slip. Positive and negative control tissues included in each IHC run.

Quantification of MVD and MPI

Any positively stained (endothelial lined lumen)micro vessels, ECs clusters or even single stained ECs which easily distinguish from neighboring micro vessels, malignant cells or other elements presented within connective tissue was considered as a single, measurable micro vessel [9].

In current study, Pro. Way, China light microscopy used for calculating the number of CD105- positively stained endothelial (MVD). The stained sections were screened at X40 magnification to recognize three regions which showed the maximum number of browned stained micro vessels, called vascular hot spots. Then the number of them was counted in each vascular hotspot at X200 magnification (X20 objective lens and X10 ocular lens). The mean of the number in those three hot spot fields was represented the MVD of that sections.

The MPI was defined as the percentage of α-SMA-positive vessels to CD105-positive vessels in the same vascular hot spot regions in which MVD was calculated. The MPI achieved as a quantity assessment of vessel maturation [8].

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-22. Data were presented in simple measures of mean, standard deviation, degree of freedom, for continuous variables. Anova test was used to compare the difference between means of several groups). Statistical significance was considered whenever the P value was less than 0.05.

3. Results

This study included thirtysix of histopathologically confirmed OSCC cases, males were 20 (55.6%) while female were 16 (44.4%). The mean age was 52.4 years. The most predominant age group was (70-79) years (27.78%).Ulcer was the most predominant clinical presentation in the study sample as 21 cases(58.33%). The most predominant effected site with tumor was tongue of 17 cases (47%), followed by floor of mouth of 7 cases (19%). Most predominant histological grade was moderately differentiated as 14 cases (38.89%).

Quantification of MVD

Quantitative analysis of angiogenesis was done by measuring MVD using anti CD105 antibody. MVD ranged between (8.8-29), the average was 18.9 . There is no statistical association between MVD and clinicopathological parameters (age, gender, clinical presentation, sites and histopathological grades of OSCC sample; P value>0.05).Table (1,2).
The highest mean value of MVD (18.04±4.72) was in(40-49) age group, in female(17.19±5.56), in the floor of the mouth (18.62±4.22), and in ulcer(16.93±3.91).

Quantification of MPI

Quantitative analysis of blood vessels maturation was obtained by calculating MPI as mentioned in material and method, using anti α-SMA and anti CD105 antibodies. MPI ranged (1-57) %, the average was 29%. There was no statistical association between MPI and the clinical parameters(age, gender, clinical presentation, and site) P value>0.05. (Table 3, 4, 5, 6).

There was highly significant difference between MPI and histological grade ofOSCC(P value<0.01).(Table 7).

Table1: The association of MVD and clinical sites developed OSCC in the study sample

	Sites	Mean± SD	ANOVA Test	P-value
MVD	Tongue	16.38±3.91	F=0.790	0.585
	Floor of the mouth	18.62±7.22		
	Buccal mucosa	16.44±4.51		
	Mandible	16.40±2.95		
	Soft palate	14.48±1.59		
	hard palate	14.83±.95		
	Alveolar ridge	8.80		

Table 2: The distribution of study sample according to MVD and grades of OSCC

	Grade	Mean± SD	ANOVA Test	P-value
MVD	W.D	15.18±2.29	F=0.728	0.490
	M.D	17.17±4.87		
	P.D	17.05±6.55		

(W.D=Well differentiated, M.D= Moderately differentiated, P.D=Poorly differentiated) Oral Squamous Cell Carcinoma.

Table 3: The association of MPI and age group developed OSCC in the study sample.

	MPI%			ANOVA Test	Sig
Age Group	N	Mean	SD±		
<0r=39	7	29.57	17.19	F=279	0.889
40-49	5	36.40	10.90		
50-59	5	35.20	9.88		
60-69	9	34.33	9,85		
>0r=70	10	31.40	9.29		

df=4

Table 4: The association of MPI and clinical presentation of OSCC in the study sample

Clinical Presentation	MPI			ANOVA	sig
	N	Mean	SD±		
Ulcer	21	33.71	10.37	F=0.148	0.702
Mass	15	32.00	9.86		

df=1

Table 5: The association of MPI and clinical sites developed OSCC in the study sample

SITE	MPI%			ANOVA Test	Sig
	N	Mean	SD±		
Tongue	17	35.76	10.76	F=1.244	0.313
Floor of the mouth	7	24.29	8.39		
Buccal mucosa	4	30	8.24		
Mandible	3	44	8.71		
Soft palate	2	34.5	6.36		
hard palate	2	34	8.78		
Alveolar ridge	1				

Table 6: The association of MPI and gender

Gender	MPI %			ANOVA Test	sig
	N	Mean	SD±		
Male	20	35.55	8.08	F=1.77	0.19
Female	16	29.81	10.23		

df=1

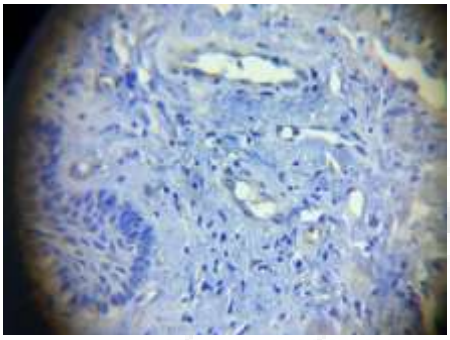
Table 7: The association of MPI and histological grade of OSCC in the study sample

Grade	MPI %			ANOVA Test	Sig
	N	Mean	SD±		
W.D	13	45.61	7.34	F=21.422	0.000*
M.D	14	27.57	6.54		
P.D	9	23.22	6.64		

df=2

(W.D=Well differentiated, M.D=Moderately differentiated, P.D=Poorly differentiated)Oral Squamous Cell Carcinoma.

* = highly Significant difference



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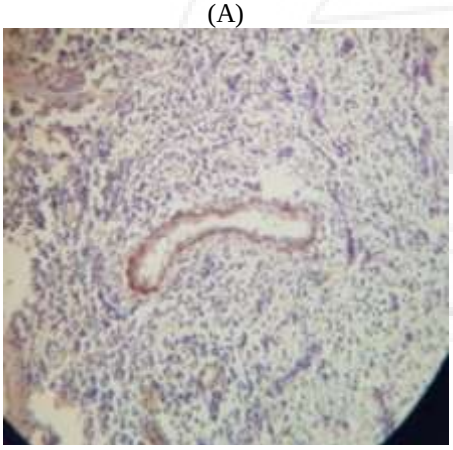

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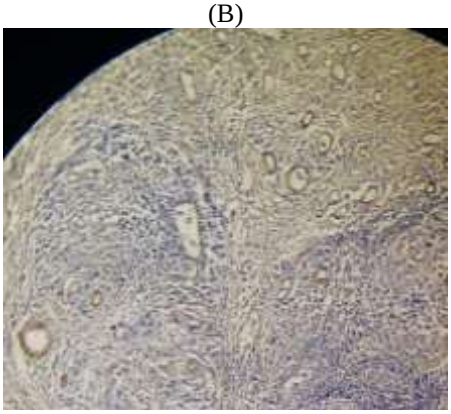
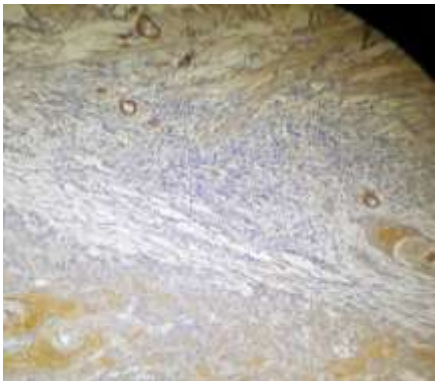
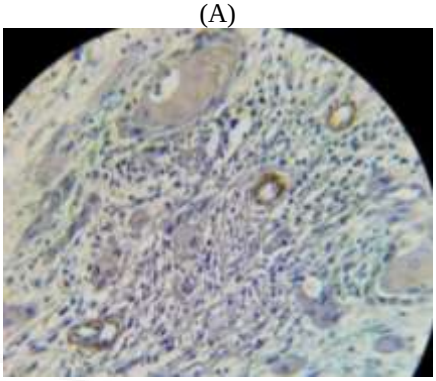


(C)

Figure1: Expression of CD105 in OSCC(a. well differentiated, b. moderately differentiated, c. poorly differentiated)



(A)



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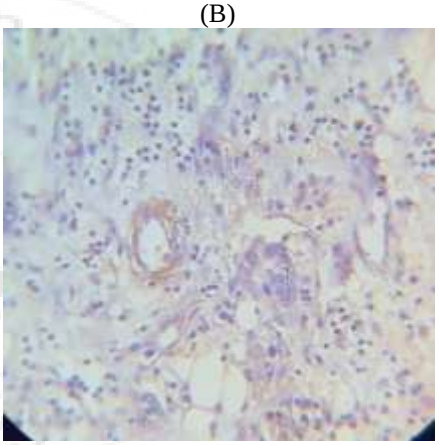

(C)

Figure 2: Expression of α-SMA antibody in OSCC samples(a. well differentiated, b. moderately differentiated, c. poorly differentiated).

4. Discussion

Tumor angiogenic blood vessels become most acceptable targets in searching for newly anticancer therapeutic approach because of the fact that these vessels have dissimilar expressed molecules than that surrounding normal vessels [10]. In addition to less possibility todevelop treatment resistance when using antiangiogenic antibodies [11].

CD 105 expressed on activated endothelial cells, has advantages upon the other pan endothelial cell markers, such as (CD31 or CD34, and factor VIII) which stained both angiogenic vessels and normal vessels confined within tumor tissues [12], so could be targeted to achieve cancer therapy [13].

Mural cells in normal blood vessels presented within different organs are completely covering them, making them stable and mature in addition, they control endothelial cells proliferation[14, 15], while tumorblood vessels are mostly with little envelopment with pericytes (immature and less stabilized), irregularly shaped, perforated, and more sensitivity to various angiogenic stimuli [16, 17].

In the present study, the association between MPI and the histological grade of OSCC was found to be statistically highly significance (P value=0.000).

Pericytes coverage is a correct functional reflection of the degree of microvessel maturation [18,19]. The present study has demonstrated that different grades of OSCCare characterized by varying degrees of pericytes coverage which found highly in cases of well differentiated OSCC (ranged 34-57%), while decreased to (11-40%) in poorly differentiated OSCC which in consent with previous studies that showed if the tumor vessels with less pericytes coverage (immature blood vessels) leads to increase the possibility ofpoor prognosis of the tumor [8, 20].

5. Conclusion

MVD using CD105 and MPI can considered as signs of the angiogenic and functional status of a tumor vascularity because they reflect the amount of pericytes recruitment to the newly formed tumor vessels which could be targeted by antiangiogenic therapy.

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