

## RESEARCH ARTICLE

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# Angiogenesis Intensity within Benign and Malignant Oral Mucosa Epithelial Tumor

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**Background:** Angiogenesis is an important and fundamental process for new blood vessels to provide nutrients and oxygen needed by tumor cells to grow, develop, and in case of cancer also to metastasize into other organs. This study aims to evaluate the intensity of angiogenesis within benign (papillomas) and malignant (squamous cell carcinoma) epithelial tumors.

**Materials and Methods:** This analytic observational study with cross-sectional design using histopathology slide sample that were clinically diagnosed as squamous cell carcinoma (n=3) and papilloma (n=3). Microscopically, the angiogenesis characterized with lumen lined by endothelial cells with or without red blood cells inside within sub epithelial connective tissue of papilloma and squamous cell carcinoma by Hematoxylin Eosin stain. Angiogenesis intensity was counted from four areas under magnification of (10x10), each area was scored under (10x40) magnification.

**Results:** Angiogenesis intensity of papilloma and squamous cell carcinoma are (45.17±14.573) and (55.18±6.26041) respectively. T-test analysis showed there was no significant difference ( $p=0.336>0.05$ ).

**Conclusion:** Angiogenesis intensity of papilloma is less than those of squamous cell carcinoma.

**Keywords:** angiogenesis, oral epithelial tumor, benign, malignant

## Introduction

Neoplasm can be in form of benign or malignancy.<sup>1</sup> Oral and oropharyngeal cancer occupied the most sixth malignancy in the world.<sup>2</sup> Other lesions such as oral lichen planus, oral submucous fibrosis, and leukoplakia are pre-cancerous lesions that have potency to become malignancy that is squamous cell carcinoma.<sup>3,4</sup> Epithelial dysplasia is the main previous pathognomonic features of malignant process in form of abnormal epithelial cells proliferation. Oral squamous cell carcinoma is the most frequently squamous

epithelium carcinoma as much as 90% of oral cancerous lesions.<sup>1,5</sup> The prevalence is higher on men with the highest among 41-50 years population.<sup>6</sup>

Benign epithelial tumor that commonly occur is papiloma as exophytic growth mass on skin and oral cavity with roughly surface such as verrucous surface with pedunculated or sessile and diameter of several millimeters. Histopathological features shows long thin papil come out of epithelial surface covered by stratified squamous epithelium with axis consisted of connective tissue stroma, blood vessels, chronic inflammatory cells such as lymphocyte

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and plasma cells<sup>7</sup> Papilloma in oral cavity related to Human Papilloma Virus (HPV) 6 and 11, proven that this does not show any progressive symptom toward malignancy since this is low virulent virus.<sup>8,9</sup>

Angiogenesis is the vessel new formation from the existing blood vessel.<sup>10</sup> This process is important and basic due to provide nutrient and oxygen needed by tumor cells to grow and metastasize into another organ. To induce angiogenesis, tumor cells release molecules that send signals to host organ with the impact of certain gene activation following by protein induce angiogenesis formation. Tumor could not grow and develop without angiogenesis.<sup>11</sup> Small blood vessels to supply nutrients needed for basic metabolic requirements of the neoplastic cells. Many retrospective studies show that angiogenesis is an important new prognostic indicator in breast cancers.<sup>12</sup>

The ability of a tumor to grow and eventually to infiltrate adjacent tissue requires a sufficient blood supply.<sup>13</sup> Tumor angiogenesis can be studied by various parameters like estimation of expression of VEGF (Vascular Endothelial Growth Factor), MVD (Mean Vascular Density), Chalky counting, MAGS (Microscopic Angiogenesis Grading System) scoring, Color Doppler study. The parameters of vascularity like MVD, MVA (Mean Vascular Area), and TVA (Total Vascular Area) can be used to histologically grade the tumors under light microscope by the use of H and E stained sections. Ultrasound imaging with color Doppler technology allows the visualization of blood flow in ovarian tumors, which may permit early diagnosis and treatment.<sup>12</sup> MAGS scoring is a quantitative technique of measuring degree of angiogenesis in a tumor. This is based on three parameters, vasoproliferation, endothelial cell hyperplasia and endothelial cytology. MAGS can be used to evaluate classes of tumors and the ability to elicit new endothelial growth.<sup>14,15</sup> The first used of MAGS scoring in a series of brain neoplasms revealed that higher degrees of scores were consistently displayed by more malignant neoplasms.<sup>14</sup>

Moriyama, *et al.*, examined vessel density immunohistochemically in oral SCC using JC-70A antibody to CD-31 which is the most specific vascular endothelial cell marker (VEGF) by counting the number of vessels under light microscopy at x200 magnification.<sup>16</sup> Jones and Haris evaluate the angiogenesis in different grades of oral squamous cell carcinomas (OSCCs) under light microscope using H and E stained sections to assess whether the parameters of vascularity like MVD, MVA, and TVA can be used to histologically grade the tumors.<sup>12</sup>

In examining turnout angiogenesis, not only vessel density but also expression of VEGF and its receptors may be important. It may be very risky to establish prognosis based only on vessel density in patients with OSCC. Therefore, in predicting metastasis and establishing prognosis, not only angiogenesis but also lymphatic vessel neogenesis should be further examined.<sup>16</sup>

Some studies have reported that there is a correlation between vessel density and prognosis in patients with OSCC.<sup>17,18,19</sup> Angiogenesis intensity within benign lesion is lower than those of malignant.<sup>13,21</sup> While others have reported that there is no correlation.<sup>22,23</sup> Moriyama, *et al.*, stated that supposedly, the reasons for this disagreement are methodological difference in immunostaining of endothelial cells, one using factor VIII antigen staining and the other using CD-31, and vessel density may be an inadequate measure of tumour angiogenesis for OSCC since such oral cavity consisted of so highly vascular region that distinguishing pre-existing microvessels from neovascularisation becomes difficult.<sup>16</sup> Therefore this preliminary study was done to evaluate the angiogenesis intensity by counting the blood vessels using Hematoxyllin Eosin staining under light microscope as a simple method within benign oral epithelial tumor (papilloma) and malignant oral epithelial tumor (well differentiated squamous cell carcinoma). The number of samples used in this study based on the previous study which related to the progressivity of lesions that was proliferation activity of cells within oral neoplastic and proliferative non neoplastic lesions using immunohistochemical proliferative activity stain Ki-67<sup>24</sup> and scored with the formula of Lameshow as follows:

$$n = 2 \left\{ \frac{(Z\alpha + Z\beta)\delta}{\mu_1 - \mu_2} \right\}^2$$

with  $\mu_1=20,11$ ;  $\mu_2=2,167$ ;  $Z\alpha=1.282$ ;  $Z\beta=0.842$  resulted in the number of  $n=2,17$ .

## Materials and methods

This was an observational analytic study with cross sectional design to evaluate angiogenesis intensity of oral papilloma and OSCC using Hematoxyllin eosin staining. Samples used in this study were slide glass of OSCC ( $n=3$ ) and oral papilloma ( $n=3$ ) from Indonesian Navy Oral Pathology Laboratory in Jakarta and Oral Pathology Department Faculty of Dentistry, Trisakti University.

The angiogenesis was characterized with lumen lined by endothelial cells with or without red blood cells inside stained with Hematoxylin Eosin within sub epithelial connective tissue of oral papilloma and OSCC microscopically. Angiogenesis intensity was counted from four areas under magnification of 10x10. Each area was evaluated under 10x40 magnification by three observers.

## Results

The angiogenesis of papilloma and SCC were characterized as blood vessels lined by endothelial cells with or without

erythrocyte inside shown at Figure 1, 2, 4 and 5. Angiogenesis intensity was counted from four areas under magnification of 10x10 (Figure 3). Each area was evaluated under 10x40 magnification by three observers (Figure 5).

Table 1 shows the distribution of angiogenesis of samples. The number of angiogenesis within OSCC was higher than those of papilloma. The distribution of data was normal with  $p>0.05$ . T-test showed no significant difference ( $p=0.336>0.05$ ) of angiogenesis intensity within papilloma and OSCC.

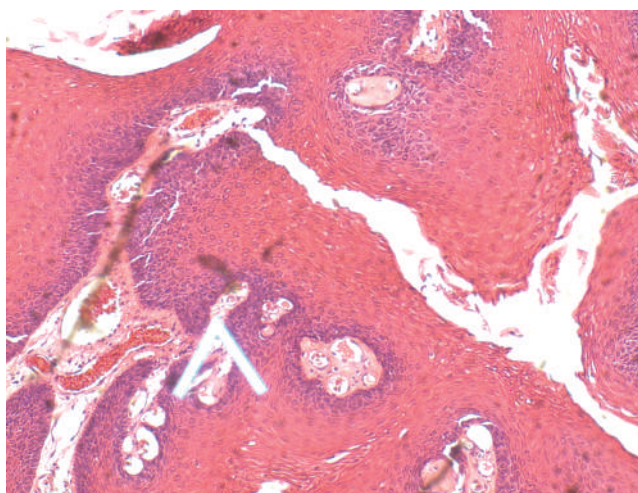


Figure 1. Angiogenesis of papilloma (HE, 10x10).

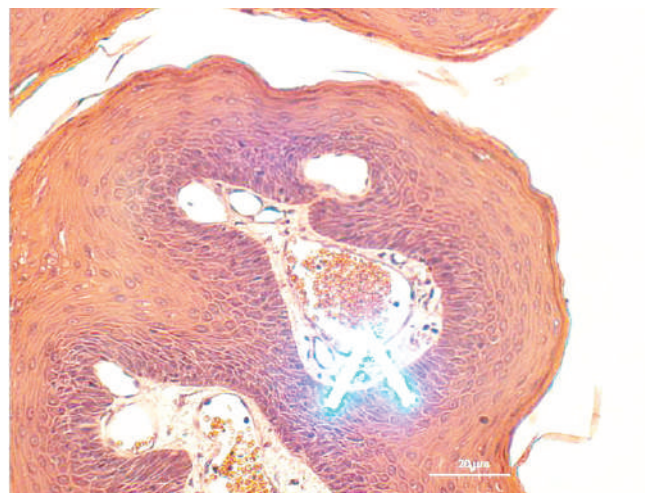


Figure 2. Angiogenesis of papilloma (HE, 20x10). The blood and lymphatic vessels within the stroma as normal subepithelial connective tissue.

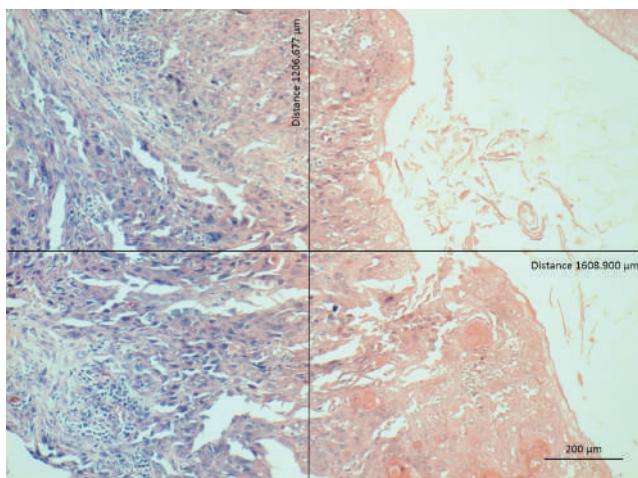


Figure 3. Four areas of evaluation (HE, 10x10).

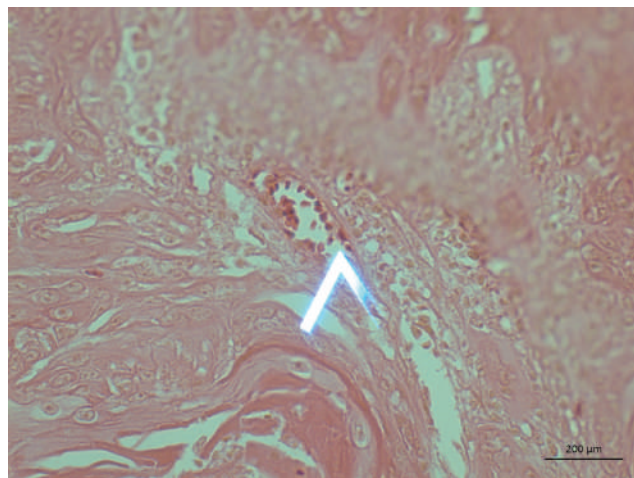
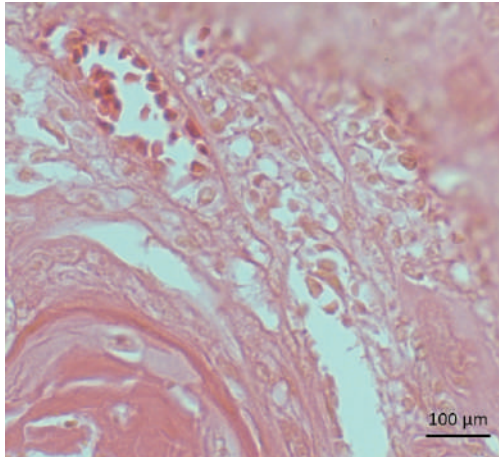


Figure 4. Angiogenesis of well differentiated OSCC (HE, 20x10).





**Figure 5. Angiogenesis of well differentiated OSCC (HE, 40x10).** The crowded blood vessels within the stroma invaded by cancerous cells.

## Discussion

Angiogenesis has important role for metastasis and progressivity of cancer.<sup>24</sup> In this study, angiogenesis intensity within OSCC ( $55.18 \pm 6.260$ ) was higher than those of papilloma ( $45.17 \pm 14.573$ ). The result of this study supported the statement that angiogenesis is an important factor for tumor to grow uncontrolled and develop continuously even though there is no more stimulant.<sup>6,25</sup> It is also in proper to the clinical features that the benign type like papilloma is less progressive than malignant type OSCC as used in this study. However, t-test showed no significant difference with  $p=0.336 > 0.05$ . This probably due to the OSCC used in this study is from well differentiated type. There is known that well differentiated tumor has less aggressive development than those of poorly differentiated type SCC. Tumor is called “well-differentiated if the cells of the tumor and the organization of the tumor’s tissue are close to those of normal cells and tissue. Well-differentiated tumors tend to grow and spread at a slower rate than tumors that are “undifferentiated” or “poorly differentiated,” which have abnormal-looking cells and may lack normal tissue structures.<sup>26</sup> The well differentiated has less vascular than those of poorly differentiated with its rich vascular supply as shown at the histopathological feature of poorly differentiated type as sarcomatous appearance with a lot of vascular supply among cancerous cells. Differentiation grade in SCC has been stated as an independent adjusted predictor for overall survival.<sup>27</sup> The vessel density of carcinomas with a well-defined tumour-stromal boundary was lower than that of diffusely invasive carcinomas.<sup>16</sup>

**Table 1. Distribution of angiogenesis of samples**

Lesions	Angiogenesis Intensity	Mean $\pm$ SD
Papilloma	43.44	45,17 $\pm$ 14.573
	31.55	
	60.54	
OSCC	48.55	55,18 $\pm$ 6.260
	60.99	
	56.00	

The result of this study suggested that certain gene activation of cells following by protein induce angiogenesis formation. The result of this study that showed lower angiogenesis in oral papilloma as benign tumor than those of well differentiated OSCC as malignant tumor is also in accordance with the previous study of Moriyama, *et al.*, that angiogenesis is essential for tumour proliferation and progression.<sup>16</sup> This result is also supported by study of Cassarino, *et al.*, who suggested that poorly differentiated behave a more aggressive manner and portend a worse prognosis than well and moderate differentiated SCC.<sup>28</sup> While other studies showed that poorly differentiated SCCs are deeper tumors<sup>29</sup> with greater recurrence rates.<sup>29,30</sup> Poorly differentiated SCC on the ear or mucosal lip has an increased risk of metastasis.<sup>30,31</sup>

## Conclusion

Angiogenesis intensity of oral papilloma as benign oral epithelial tumor is less than well differentiated oral squamous cell carcinoma as malignant type with no significant differences. It is assumed that the type of cells differentiation within malignant tumor influence the angiogenesis intensity.

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