A Comparative Histopathological and Immunohistochemically Study of Capillary Hemangioma, Pyogenic Granuloma and Cavernous Hemangioma in the Oral Region: with Special Reference to Vascular Proliferation Factors

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Abstract
Capillary hemangioma, pyogenic granuloma and cavernous hemangioma, benign vascular lesions affect in the oral regions. There have been some studies of them, but their pathological status is still controversial. The aim of the present study was to reveal the characteristic histopathological and immunohistochemical features such as cell proliferation activity, vascular proliferation factors and mesenchymal marker of these lesions in order to understand the pathological status of them. In histopathological findings of the present study, capillary hemangioma consisted of proliferating capillaries having endothelial cells and ovoid or spindle cells associated with mast cells and lobular structures circumscribed with PAS-positive matrices. Pyogenic granuloma exhibited proliferation of the capillaries having endothelial cells and a few perivascular mesenchymal ovoid or spindle cells beneath erosive and ulcerative lesions and inflammatory changes. Cavernous hemangioma was composed of remarkably dilated and hyperplastic blood vessels. Immunohistochemically, Ki-67 labeling index of capillary hemangioma and pyogenic granuloma was higher than that of cavernous hemangioma. Positive immunoreactivity for CD34, CD105 and Tie2 was observed in some perivascular ovoid cells supported to be endothelial precursor cells. Capillary hemangioma exhibited that positive immunoreactivity for VEGF was found in the perivascular cells, mast cells and/or macrophage, whereas pyogenic granuloma showed that the positive reactivity was also seen mainly in the endothelial cells. Positive immunoreactivity for α-smooth muscle actin was identical to perivascular spindle cells, and was supposed to be pericytes. Conclusively, the present study indicated that capillary hemangioma and pyogenic granuloma showed different histopathology and immune-profiles of vascular proliferation factors, suggesting they would have different pathological status.

Keywords: capillary hemangioma, pyogenic granuloma, cavernous hemangioma, vascular proliferation factors, VEGF

Introduction
Hemangioma, a relatively frequent benign soft tissue tumor, is also found in the oral region (1-8). It is subdivided into capillary hemangioma, pyogenic granuloma and cavernous hemangioma. Although World Health Organization (WHO) classification of the soft tissue tumors (2) has been widely used, pyogenic granuloma is not listed in the classification. There have been some reports concerning about comparative study of these tumors but the detail of the lesions have been unclear (3-5). Thus the subclassification of hemangioma is a little controversial. Recently, various vascular proliferation factors, cell proliferation factors and mesenchymal markers have been found and used for the diseases (3-8), however, the histopathological and immunohistochemical study using these antibodies of these tumors has not been performed comprehensively and conclusively, as yet.

The aim of the present study was to reveal the
characteristic histopathological feature and to examine the pathological status of capillary hemangioma, pyogenic granuloma and cavernous hemangioma by histopathological and immunohistochemical techniques in order to understand the pathological status of the disease.

**Materials and Methods**

*Materials*

The author retrieved the files on ten cases each of capillary hemangioma, pyogenic granuloma and cavernous hemangioma kept at the Department of Oral Pathology, Nihon University School of Dentistry at Matsudo. Control specimen comprised ten cases of normal oral mucosa including non-neoplastic vessels. Consideration was given to patient privacy, diagnosis, and the management and prognosis of the lesions (Ethics committee recognition number: EC 08-021).

*Methods*

For light microscopical observation, following fixation with 10% neutral formalin solution, specimens were cut into several pieces and routine paraffin sections (4 μm) were prepared. They were stained with hematoxylin and eosin (HE), toluidine blue pH 4.1 (TB) and periodic acid schiff reaction (PAS).

For immunohistochemical observation, deparaffinized sections were pretreated, and performed endogenous peroxidase blocking with 3% hydrogen peroxidase for 5 min. Next they were pretreated with microwave irradiation in citrate buffer (10 mM, pH 6.0, 5 min × 3) to unmask primary antibodies (exclude KP–1). Details of primary antibodies used in the present study (CD31, CD34, KP–1, SMA, Ki–67, VEGF, Tie2), dilution, clone, and antigen retrieval methods, manufacture are shown in Table 1. And then sections were incubated with Histofine Simple Stain MAXPO (MULTI) (Nichirei Co, Ltd, Tokyo, Japan), (MAXPO (G) for Tie2) for 1 hr at room temperature. The immunoreaction was visualized using simple stain DAB solution (Nichirei Co, Ltd, Tokyo, Japan) for 5 min at room temperature.

Double immunohistochemical stain method for colocalization of CD105 and Ki–67 was applied. Deparaffinized sections were pretreated, and endogenous peroxidase blocking with 3% hydrogen peroxidase for 5 minutes was performed, followed by pretreatment with proteinase K (DakoCytomation Glostrup, Denmark) for 15 min at room temperature. Incubation with primary CD105 antibody for 1 hr was done, and then sections were incubated with Histofine Simple Stain MAXAP (MULTI) (Nichirei Co, Ltd, Tokyo, Japan) for 1 hr at room temperature. The immunoreaction was visualized using BCIP/NBT Substrate System (DakoCytomation Glostrup, Denmark) for 5 min at room temperature. Secondary staining was pretreated with microwave irradiation in citrate buffer (10 mM, pH 6.0, 5 min × 3) to unmask the Ki–67 epitope. After 30 min incubation with Ki–67 antibody, they were incubate with Histofine Simple Stain MAXAP (MULTI) for 30 min at room temperature the immunoreaction was visualized using New Fuchsin Substrate Kit (Nichirei Co, Ltd, Tokyo, Japan) for 5 min at room temperature. It was also applied double stain method for co-localization of

<table>
<thead>
<tr>
<th>Primary antibodies</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Manufacturer</th>
<th>Clonality</th>
<th>Clone</th>
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<td>Ki–67</td>
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<td>Monoclonal</td>
<td>MIB–1</td>
</tr>
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<td>Monoclonal</td>
<td>JC70A</td>
</tr>
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<tr>
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<td>Proteinase K</td>
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<td>α–Smoth actin (SMA)</td>
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<td>Polyclonal</td>
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<td>Tie–2</td>
<td>1 : 100</td>
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<td>DakoCytomation</td>
<td>Monoclonal</td>
<td>KP–1</td>
</tr>
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</table>
SMA and PAS stains on the same section.
On analysis of proliferative activity, areas showing high positivity hot spots were chosen, and the average rates of positive cells (Labeling Index, LI) was calculated after analyzing about 500 cells in five fields at ×400 magnification. The average number of mast cells revealing metachromasia to TB stain was also calculated in five fields at ×400. Statistical significance was determined by Welch T test. Differences at p<0.05 were considered statistically significant.

**Results**

*Histopathological findings*

1) Capillary hemangioma

Capillary hemangioma was composed of nodular proliferation of capillary-sized vessels lined by endothelial cells having plump nucleus (Fig. 1a). Erythrocytes were frequently recognized in the lumen spaces. In many cases, the lobular formation surrounded by dense fibrous tissues was observed mainly at the lower area of the tumor tissues. Many perivascular mesenchymal cells which were ovoid- or spindled ones with plump nucleus, and the mast cells showing metachromasia with TB (Fig. 1c) were found in the matrix surrounding the lobular area revealing positive reaction to PAS (Fig. 1b). The average number of the mast cells was 10.8 (Fig. 1c). These findings are summarized in Table 2.

2) Pyogenic granuloma

Ulcer formation was recognized at the surface of the lesion. In the ulcer area, a layer of fibrin and entrapped neutrophils were found. Proliferation of plump endothelial cells forming small canalized capillaries and various-sized blood vessels lined with flatten endothelial cells were observed beneath the ulcer (Fig. 1d). The proliferating capillaries were also circumscribed with flatten pericyte-like cells.

<table>
<thead>
<tr>
<th>Mast cell (Mean±SD)</th>
<th>Capillary hemangioma</th>
<th>10.8±1.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyogenic granuloma</td>
<td>1.6±1.0</td>
<td>*</td>
</tr>
<tr>
<td>Cavernous hemangioma</td>
<td>1.8±1.5</td>
<td>*</td>
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<tr>
<td>Control</td>
<td>0.5±0.6</td>
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</table>

All p values are performed by Welch’s t test
* p<0.01

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Fig. 1a. Capillary hemangioma is composed of nodular proliferation of capillary-sized vessels and ovoid, spindle and mast cell-like cells. (HE, original magnification ×400)

Fig. 1b. In capillary hemangioma the matrix surrounding the lobules shows positive reaction to PAS. (PAS, original magnification ×100)

Fig. 1c. In capillary hemangioma of lobular formation, many numbers of the mast cells are found. (TB, original magnification ×400)

Fig. 1d. Pyogenic granuloma consists of plump endothelial cells forming poorly canalized capillaries, small and large blood vessels and lymphocyte infiltration. (HE, original magnification ×400)

Fig. 1e. In pyogenic granuloma, basal lamina of the endothelial cells reveals positive reaction to PAS. (PAS, original magnification ×100)

Fig. 1f. Cavernous hemangioma shows large, dilated or sinusoidal vessels having irregular lumen. (HE, original magnification ×200)
Some ovoid- or spindle perivascular cells were observed between the proliferating capillaries. Moderate inflammatory infiltration with neutrophils and lymphocytes was present around the proliferating blood vessels and formed the granulation tissue. The blood vessels showed a clustered or medullar pattern separated by less vascular fibrotic matrix. Basal lamina of the endothelial cells revealed positive reaction to PAS (Fig. 1e). A few mast cells showing metachromasia with TB were recognized and the average number was 1.6. These findings are shown in Table 2.

3) Cavernous hemangioma

Cavernous hemangioma was composed of large, dilated or sinusoidal vessels having irregular lumen with thin wall lined with flattened endothelial cells (Fig. 1f). Flattened, pericytes–like cells around the endothelial cells were frequently observed. The luminal spaces were filled with blood, and thrombus or phlebolith was also seen in some cases. Basal lamina around the endothelial cells showed positive reaction to PAS. A few mast cells showing metachromasia with TB were found around vessels and the number was 1.8 (Table 2–1).

4) Control

Some blood vessels were recognized in the connective tissue under the mucosa. Few mast cells were recognized and the average number was 0.5 (Table 2–1). The surface of the mucosa was covered with parakeratinized stratified squamous epithelium.

**Histochemical and immunohistochemical findings**

The results are summarized in Table 3

<table>
<thead>
<tr>
<th></th>
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<th>Pyogenic granuloma</th>
<th>cavernous hemangioma</th>
<th>Control</th>
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<tr>
<td><strong>Ki-67 (Mean±SD)</strong></td>
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<td>Capillary hemangioma</td>
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<tr>
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<td>Perivascular cell</td>
<td>23.5±1.8</td>
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<tr>
<td>Pyogenic granuloma</td>
<td>Endothelial cell</td>
<td>14.9±2.7</td>
<td>Endothelial cell</td>
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<td>Perivascular cell</td>
<td>19.0±2.3</td>
<td>Perivascular cell</td>
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</table>

3) Cavernous hemangioma

Ki-67-positive endothelial cells and perivascular mesenchymal spindled cells a were not observed in the tumor (LI, 0).

4) Control

The immunoreactivity to Ki-67 was similar to that of cavernous hemangioma. The LI was 0.

**Endothelial cells and mesenchymal cell associated antibodies**

The results are summarized in Table 4

1) Capillary hemangioma

Most of endothelial cells were positive to CD31, CD34 (Fig. 3a) and CD105. A few of endothelial cells were positive to VEGF (Fig. 3b), but many of them were positive to Tie2 (Fig. 3c). Some perivascular mesenchymal ovoid cells were positive to CD31, CD34, CD105 and Tie2 and many of them were positive to VEGF. Some perivascular mesenchymal spindle cells were positive to VEGF. Mast cells revealed are positive reactivity to VEGF. Perivascular mesenchymal spindle cells were positive to SMA (Fig. 3d).
Fig. 3a. Endothelial cells and ovoid cells are positive to CD34 in capillary hemangioma of lobular formation. (Original magnification ×600)

Fig. 3b. Some endothelial cells and many perivascular mesenchymal ovoid cells are positive to VEGF in capillary hemangioma of lobular formation. (Original magnification ×600)

Fig. 3c. Most of endothelial and ovoid cells are positive to Tie2 in capillary hemangioma of lobular formation. (Original magnification ×600)

Fig. 3d. Perivascular mesenchymal cells are positive to SMA in capillary hemangioma of lobular formation. (Original magnification ×600)

Fig. 3e. In pyogenic granuloma PAS-positive lines (pink) are irregularly recognized between and around these SMA-positive (brown) cells in double staining of SMA and PAS. (Original magnification ×400)

Fig. 3f. Some perivascular mesenchymal ovoid cells show positive reaction to KP-1 in capillary hemangioma of lobular formation. (Original magnification ×600)

Fig. 3g. Some endothelial cells and many perivascular mesenchymal ovoid cells are positive to VEGF in pyogenic granuloma. (Original magnification ×600)

Fig. 3h. Most endothelial cells are positive to CD34 in pyogenic granuloma. (Original magnification ×400)

Fig. 3i. Vascular surrounding cells reveal immunopositivity to SMA, but other perivascular mesenchymal cells are negative in pyogenic granuloma. (Original magnification ×400)

Fig. 3j. PAS-positive lines (pink) are regularly observed around the SMA positive cells (brown) in pyogenic granuloma in double staining of SMA and PAS. (Original magnification ×200)

Fig. 3k. Many perivascular mesenchymal ovoid cells show positive reaction to KP1 in pyogenic granuloma. (Original magnification ×400)

Fig. 3l. In pyogenic granuloma PAS-positivity (pink) is observed both at the inside and outside of the SMA positive cells (brown) in double staining of SMA and PAS. (Original magnification ×200)
Tabel 4. The result of immunohistochemistry

<table>
<thead>
<tr>
<th></th>
<th>CD31</th>
<th>CD34</th>
<th>CD105</th>
<th>VEGF</th>
<th>Tie2</th>
<th>SMA</th>
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<tr>
<td>Capillary hemangioma</td>
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<td>Pyogenic granuloma</td>
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- : negative, + : moderate positive, ++ : strong positive

PAS-positive lines were irregularly recognized between and around these SMA-positive cells in the double staining of SMA and PAS (Fig. 3e). KP-1-positive macrophages were seen around the capillaries (Fig. 3f).

2) Pyogenic granuloma

The immunoreactivity of endothelial cells was almost same as that of capillary hemangioma but many endothelial cells were positive to VEGF (Fig. 3g). The perivascular mesenchymal ovoid were almost negative for CD31, CD34 (Fig. 3h), CD105 and Tie2. The perivascular mesenchymal spindle cells revealed immunopositivity to SMA (Fig. 3i). PAS-positive lines were regularly observed around the SMA-positive cells in the double staining of SMA and PAS (Fig. 3j). KP-1-positive macrophages were seen around the capillaries (Fig. 3k).

3) Cavernous hemangioma

Some endothelial cells were positive to CD31, and many of them were positive to CD34, but negative to CD105. Some endothelial cells were positive to VEGF and many of them were positive to Tie2. Most of perivascular mesenchymal spindle cells were positive to SMA in the double staining of SMA and PAS. PAS-positive reactivity was observed both at the inside and outside of the SMA-positive cells (Fig. 3l). There was no positive reaction to KP1.

4) Control

Immunoreactivity of the endothelial cells and perivascular cells were similar to cavernous hemangioma.

Discussion

Hemangioma is the representative benign lesion among the vascular-related tumors in the head and neck region (1-13). Some authors consider that cavernous hemangioma is vascular malformation (9-11) and capillary hemangioma is a neoplastic lesion (3, 6, 12, 13). On the other hand, pyogenic granuloma is not listed in WHO of the soft tissue tumors (2). The term pyogenic granuloma was introduced by Hartzell in 1904, and firstly reported by Hullihen in 1844 (3-5). Thus, the pathological status of capillary hemangioma, pyogenic granuloma and cavernous hemangioma are still controversial.

There have been some histopathological and immunohistochemical studies about capillary hemangioma or pyogenic granuloma (1-6). They reported that capillary hemangioma was an underlying lesion of pyogenic granuloma which was frequently associated with marked ulcerative change, although the term capillary hemangioma is recognized today to be synonymous with pyogenic granuloma. On the other report, pyogenic granuloma is considered to be non-neoplastic in nature.

In the present study, there were some differences in the morphological features of the vascular element between capillary hemangioma and pyogenic granuloma. At first, thick PAS-positive membranous matrices and lobular appearance were observed in
capillary hemangioma but in pyogenic granuloma blood vessels showed a clustered or medullar pattern separated by less vascular fibrotic septa and PAS-positive membranous matrices.

Ki-67, a marker of cell proliferation activity, is useful to define the proliferation status of both endothelial cells and perivascular mesenchymal cells (5, 14). In the present study, the LI of endothelial cells in the capillary hemangioma was 17.4 and perivascular mesenchymal was 23.5. That of endothelial cells in the pyogenic granuloma was 14.9 and perivascular mesenchymal was 19. In each lesion, LI tended to be higher in the perivascular mesenchymal ovoid cells than the endothelial cells. No significant difference was recognized between capillary hemangioma and pyogenic granuloma. But no Ki-67 immunoreactivity was identified in endothelial cells of cavernous hemangioma and control blood vessels. These results suggested that capillary hemangioma and pyogenic granuloma had a possibility of true neoplastic character, whereas cavernous hemangioma was hamartomatous lesion. Further, the results suggested that the tumors consisted of both endothelial and perivascular mesenchymal cells proliferation.

It is well known that CD31 and CD34 are so representative specific markers for endothelial cells that many studies use them to detect endothelial cells (15–19). The target of CD31 is platelet endothelial cell adhesion molecule-1 (PECAM-1) and the expression of CD31 is recognized in endothelial cells and inflammatory cells (17). CD34 is known to express in the maturation of the endothelial cells and is also indicated in immature mesenchymal cells (18, 19). CD105, a kind of glycoprotein, is predominantly in the endothelial cells of the neoformed blood vessels (20, 21). In the present study, CD31, CD34 and CD105 were recognized in the almost endothelial cells in the tumors and some perivascular mesenchymal ovoid cells in capillary hemangioma suggesting to be endothelial precursor cells. Negative reaction of CD105 was found in the perivascular cells of cavernous hemangioma and control. These results suggested that CD105 had advantage as a marker of angiogenesis during the tumor growth of capillary hemangioma and pyogenic granuloma, because cavernous hemangioma was non neoplastic.

Several properties of VEGF make it universally accepted an important regulator of angiogenesis and tumor genesis of the vascular tumors; induction and promotion of endothelial cells, and inhibition of apoptosis. (22–26). Yuan et al. (12) reported that the positive immunoreactivity for VEGF was mostly localized in macrophages and fibroblasts in pyogenic granuloma. In the present study, the positive immunoreactivities for VEGF were recognized in the many endothelial cells of pyogenic granuloma, but the positivity was unremarkable in capillary hemangioma. Meanwhile, the degree of VEGF was apparently more abundant in the perivascular cells of capillary hemangioma, and many mast cells and some KP-1-positive macrophages regarding as macrophages were positive to VEGF. In pyogenic granuloma, some inflammatory cells and macrophage were also positive to VEGF.

Mast cells originate from hemopoietic stem cell around the blood vessels complete their maturation in vascularized peripheral tissues. On activation, mast cells can rapidly release VEGF, apparently from a preformed pool in the cytoplasm (27–30). The histopathological behavior of mast cells in hemangioma is indefinite (31–34). In the present study, mast cells showing metachromasia with TB were recognized in all lesions, but they were abundant in lobules with PAS-positive matrices of capillary hemangioma (Table 2). These results suggested that the mast cells might be associated with proliferation of the blood vessels of these tumors especially in capillary hemangioma.

Tie2 is an endothelium-specific receptor tyrosine kinase consisting of extracellular domain, a transmembrane domain and a split intracellular kinase domain (35–38). The tyrosine kinase receptor Tie2 accelerates the development of the embryonic vasculature and persists in adult endothelial cells during wound healing. Unlike the VEGF receptor system, Tie2 receptors are not required for angiogenesis but appear essential to support functions of the more mature endothelium. Tie2 is unregulated in capil-
laries during neovascularization processes and collaborates with VEGF in regulating angiogenesis and vascular maturation (35). Sato et al. (13) reported that in capillary hemangioma, positive immunoreactivity for Tie2 was recognized only in the perivascular ovoid cells and suggested that the Tie2-positive ovoid cells played an important role in the development and progression of tumor.

In the present study, positive immunoreactivity for Tie2 in pyogenic granuloma was observed only in endothelial cells, whereas in capillary hemangioma Tie2 positivity was recognized both in endothelial cells and perivascular mesenchymal ovoid cells around the capillaries. Since the perivascular mesenchymal ovoid cells which did not form the lumen revealed positive reactivity for CD34 or CD105, in the present study, Tie2 would be endothelial precursor cells.

Generally, fibroblastic mesenchymal cells are present in the outside of the endothelial cells. The basement membrane and the fibroblastic mesenchymal spindled cells are supposed to be pericytes, and capillary is restricted to vessels consisting of one layer only of endothelial cells and basal lamina, in which a few pericytes may be embedded (39).

In the present study, the perivascular mesenchymal spindled cells were found in the outside of the endothelial cells and basement membrane, exhibited positive immunoreactivity for growth factors and also SMA. In these perivascular mesenchymal cells, SMA-positive cells suggested pericytes.

Post-natal neovascularization in the physiological or pathological events is consistent with neovessel formation contributed by angiogenesis and vasculogenesis at various rates between their two mechanisms (40–42).

One mechanism: By angiogenetic stimulation (hypoxia, inflammation), pericytes leave endothelial cells and a basal membrane is digested by vascular endothelial cells protease and a vascular cavity is expanded. The extended blood vessel increases neovascularity by sprouting, bridging, intussusceptions. Finally pericytes build a mature blood vessel around neovascular circumference. Other mechanism: Endothelial progenitor cells, primary delivered from bone marrow, mobilize to the blood by stimulation such as VEGF; they migrate to a neovascularization site and differentiate into endothelial cells, and assist angiogenesis.

Angiogenesis and vasculogenesis are due to the activations of in situ endothelial progenitor cells and bone marrow–derived or in situ endothelial progenitor cells, respectively. In the present study, the growth of many endothelial precursor cells, pericytes and few inflammatory cells were found in capillary hemangioma, but whereas in pyogenic granuloma there are many inflammatory cells but a few endothelial precursor cells. As the result, in pyogenic granuloma an angiogenetic process suggests to similar to former processes and in capillary hemangioma the process similar to the latter.

Conclusively, the present study indicated that capillary hemangioma and pyogenic granuloma showed different histopathology and immune–profiles of vascular proliferation factors, suggesting they would have identical pathological status.

**Conclusion**

To examine and compare the histopathological status of the present study performed capillary hemangioma, pyogenic granuloma and cavernous hemangioma, histopathological and immunohistochemical analyzed and review of the literature, and the following results were obtained.

1. Histopathologically, capillary hemangioma consisted of proliferating capillaries having many endothelial cells and perivascular ovoid cells and associated with mast cells and lobular structures circumscribed with PAS-positive matrices, especially in lower area of the tumor tissues. Pyogenic granuloma exhibited proliferation of the capillaries having some endothelial cells and some perivascular ovoid cells with ulcerative lesions and inflammatory changes. Cavernous hemangioma was composed of remarkable dilated and hyperplasic blood vessels.

2. Mast cells showing metachromasia with TB were more frequent in capillary hemangioma than in
the other lesions.
3. Immunohistochemically, Ki-67 LI of capillary hemangioma and pyogenic granuloma was higher than that of cavernous hemangioma.
4. Positive immunoreactivity for CD34, CD105 and Tie2 was observed in some perivascular ovoid cells supported to be endothelial precursor cells.
5. Capillary hemangioma exhibited that positive immunoreactivity for VEGF was found in some perivascular mesenchymal ovoid cells, mast cells and/or macrophage; whereas in pyogenic granuloma, many endothelial cells and perivascular mesenchymal ovoid cells and macrophages were positive to VEGF.
6. Positive immunoreactivity for SMA was identical to perivascular spindle cells, supposed to be pericytes, with irregular avaragements of PAS-positive matrices.

These results indicated that capillary hemangioma showed proliferating activity of the unit of capillaries such as endothelial cells and pericytes-like perivascular cells with appearance of the mast cells and lobules of PAS-positive matrices, whereas pyogenic granuloma exhibited remarkable proliferative activity mainly of the endothelial cells and also identified with inflammatory changes, suggestively different pathological status in these tumors.

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References
38. Scholten T: Transforming growth factor–beta: Vasculogenesis, angiogenesis, and vessel wall integrity.