

Macrophage and angiogenesis intensity within proliferative non-neoplastic and neoplastic oral lesions in-accordance with biological properties

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ABSTRACT

Introduction: The most common chronic inflammation involved dental and oral tissue are gingival polyp, pulp polyp, and fibroma, that are proliferative non-neoplastic and neoplastic condition. The previous study revealed that increasing macrophage followed by increasing angiogenesis intensity. Increasing angiogenesis or vascular proliferation indicates progressive growth in the form of proliferative non-neoplastic or neoplastic disease outside of easily bleeding clinical features. This study was aimed to evaluate the macrophage and angiogenesis intensity within lesions, and the conformity with their biological properties. **Methods:** Samples used were taken from oral mucosa excision with clinical diagnose of gingival polyp (n = 3); pulp polyp (n = 3); and fibroma (n = 3). The macrophage was detected using immunostaining with CD68 antibody, resulted in brown staining of cell membrane under light-microscope; while angiogenesis intensity was evaluated as numbers of blood vessels HE staining. **Result:** The CD68⁺ expression as macrophage marker showed no significant differences (p = 0.102 > 0.05) with the highest found in fibroma (51.32 ± 31.64%), followed by non-neoplastic pulp polyp (45.82 ± 15.94%), and the least in gingival polyp (29.98 ± 13.51%). One way-ANOVA test showed significant differences (p = 0.02 < 0.05) in angiogenesis intensity, with the highest found in pulp polyp (12.00), followed by fibroma (11.81), and gingival polyp (9.67). However, there was no significant difference in angiogenesis intensity between non-neoplastic lesion (pulp polyp) and neoplastic lesion (fibroma) with p = 0.102 > 0.05. The Pearson R-test showed a mild positive correlation of angiogenesis intensity and CD68⁺ as a macrophage marker (r = 0.31). **Conclusion:** Macrophage and angiogenesis intensity, and their correlation within oral proliferative non-neoplastic and proliferative neoplastic lesions were in accordance with their biological properties.

Keywords: Angiogenesis, macrophage, CD68⁺, oral proliferative non-neoplastic and neoplastic lesion

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INTRODUCTION

Gingival polyp, pulp polyp, and fibroma as a result of chronic inflammatory response conduct new matrix healing in the form of granulation tissue consisted of collagen and extracellular base substances. There is also new capillary formation or angiogenesis insisted by macrophage activity.¹

Macrophage is distributed widely within tissue including on inflammation process toward foreign bodies or microbes and also within the extracellular matrix of neoplastic growth that is tumour-associated macrophages (TAMs). However, the process and mechanism of TAMs as the main cellular components of human cancer is still not fully understood.¹ Macrophage has a role in angiogenesis induction by secretion of several mediator inflammation factors such as tumour necrosis factor- α (TNF- α), vascular endothelial growth factor (VEGF), angiogenin and urokinase.¹ Macrophage provides growth factor that stimulates fibroblast formation and angiogenesis. Fibroblast produces new extracellular matrix to support the growth of cells while vascular supply brings oxygen and nutrients needed for continuing cells metabolism.²

Pulp polyp or chronic hyperplastic pulpitis is an inflammation caused by caries which extend to the young pulp chamber with clinical feature shows the granulation tissue within large cavity come out from the pulp. This condition caused by pulp perforation as a result of slowly progressive caries which appear light red colour, easily bleeding, and sensitive.³

Most of the gingival polyp has the same colour as normal gingival, painless, occurred as a result of local irritation usually of caries in the proximal site without filling restoration. The histopathological feature shows hyperplastic tissue consisted of fibrous connective tissue, collagen and occasionally acute inflammatory cells beneath the stratified squamous epithelium.³

Most of the fibrous-tissue lesions are tumour-like or proliferative reaction of fibrous connective tissue such as fibroma. The histopathological feature is the proliferation of spindle cells fibroblast with the homogenous small length nuclei within the connective tissue matrix.⁴ Inflammation cascade monocyte as a chronic inflammatory cell is to migrate into the perivascular area and then

differentiate into macrophage and dendritic cell.⁵ Monocyte or macrophage has a crucial role within the fibrosis and angiogenesis process. Macrophage stimulates angiogenesis and facilitates remodelling in the period of regeneration or healing process by releasing protease and growth factors. Fibrosis is essential for tissue recovery and maintenance against environmental while angiogenesis is essential for tissue nutrition.² Moreover, monocyte or macrophage influence the growth of cancer cell through induction of fibrosis and angiogenesis; therefore, it is important to evaluate these factors and their correlation. Based on description above, this study was conducted to evaluate the macrophage and angiogenesis intensity within lesions, and the conformity with their biological properties.

METHODS

This study was observational analytic with cross-sectional design. The number of samples used in this study based on the previous research which related to the progressivity of lesion which was proliferation activity of cells within oral proliferative non-neoplastic and neoplastic lesions using immunohistochemical proliferative activity stain Ki-67⁶ and scored with the formula of Lameshow⁷, resulted in the number of $n = 2.17$. The sample used was non-neoplastic proliferative lesions diagnosed as gingival polyp ($n = 3$), pulp polyp ($n = 3$) and fibroma as the neoplastic lesion sample ($n = 3$). The gingival and pulp polyp were the surgical excision therapy specimen from a local health centre while the fibroma derived from paraffin blocks of Indonesian Navy' Oral Pathology Laboratory in Jakarta.

The conventional HE staining to evaluate angiogenesis intensity and CD68⁺ immunostaining as the marker of macrophage were carried out at Pathology Anatomy Laboratory of University of Indonesia and Dharmais Cancer Hospital respectively. The scoring was performed at Oral Pathology Laboratory of Trisakti University. Angiogenesis intensity and CD68⁺ was counted from four areas under magnification of 10×10 . Each area was evaluated under 10×40 magnification by three observers. Ethical clearance was given by Ethical Committee Biomedical Research on Human/Animal, Faculty of Dentistry Trisakti

University with the registration number of No.307/KE/FKG/03/2016.

RESULT

The immunostaining with CD68⁺ as macrophage marker was shown in Figure 1.A, 1.B, and 1.C. The positive expression of CD68⁺ showed as the brown cell membrane of macrophage.

The distribution of CD68⁺ among samples was presented in Table 1. The highest amount was found on the benign neoplastic lesion of fibroma (51.32 ± 31.64%), followed by proliferative non-neoplastic lesions pulp polyp (45.82 ± 15.94%) and gingival polyp (29.98 ± 13.51%) One-way ANOVA test showed no significant differences ($p = 0.102 > 0.05$) of CD68⁺ expression among lesions.

Figure 2.A, 2.B, and 2.C showed the expression of angiogenesis within lesions under HE staining. The angiogenesis was characterised with lumens lined by endothelial cells with or without red blood cells inside.

The distribution of angiogenesis intensity was shown in Table 2. The highest was found on pulp polyp (12.00 ± 1.23) followed by fibroma (11.81 ± 0.94) and gingival polyp (9.67 ± 0.52). One-way ANOVA test showed significant differences in angiogenesis intensity among lesions ($p = 0.02 < 0.05$). Pearson R-test showed a mild positive correlation ($r = 0.31$) between angiogenesis intensity and CD68⁺.

DISCUSSION

Oral proliferative lesions used as samples in this study clinically shown as tissue enlargement that have various biological natures following their biological properties. Each has an opportunity to grow progressively related to the type of stimulations either non-neoplastic stimulation such as chronic irritation or neoplastic stimulation such as disturbances in cells cycle. For example, in pulp and gingival polyp, the irritation will come from untreated caries or proximal caries that bacteria invade into the surrounding tissue. While there would be such a neoplastic transformation in the form of abnormal cells cycle that commonly occurred within lesions categorised as proliferative neoplastic type lesion such as fibroma as used in this study. All of these

stimulations may induce inflammation to respond within tissues that one of these is in forms of increase vascular supply (angiogenesis) and or accumulation of inflammatory cells.⁸

Angiogenesis is a complex process involving the extensive interaction between cells and extracellular matrix components⁸, such as increased permeability of capillary resulted in extravasation of acute inflammatory cells, exudation, and stasis as the respond of acute inflammation. Acute respond turns into chronic condition as the stimulation remains that commonly occurred in oral proliferative non-neoplastic lesions like pulp and gingival polyp and also in oral proliferative neoplastic of fibroma that used in this study. The monocyte as chronic inflammatory cells that migrate into the surrounding destroyed tissue turn into the macrophage.⁹

Previous in vitro and in vivo studies showed that there is angiogenesis activity in field congregation of macrophages within a neoplastic condition.¹⁰ There are parameters of vascularity can be used to histologically grade under a light microscope by the use of H and E stained sections as used in this study, like MVD (Mean Vascular Density, MVA (Mean Vascular Area), and TVA (Total Vascular Area).¹¹ This study used the MVD parameter as a simple method to evaluate vascularity or angiogenesis.

In this study, the highest expression of macrophage marker CD68⁺ was found on neoplastic lesion, in this case benign fibroma type fibroma (51.32 ± 31.64%) as shown in Figure 1.C, followed by non-neoplastic lesions of pulp polyp (45.82 ± 15.94%) and gingival polyp (29.98 ± 13.51%) as shown in Figure 1.A and Figure 1.B respectively. It was by the biological natures of lesions and assumed that monocyte or macrophage has an essential role in inducing fibroblast in the wound healing process of infection, toxic, traumatic as well as a mutagenic factor, as already stated by previous studies.^{1,11-18} The result of this study was also by the statement that fibroblast stimulated by macrophages to form fibrous and collagen fibres as found in the fibroma used in this study.^{17,19} Therefore, it revealed that there is monocyte or macrophage within fibroma which induce fibroblast activity.

Table 1 showed that the CD68⁺ as macrophage marker in this study was also found

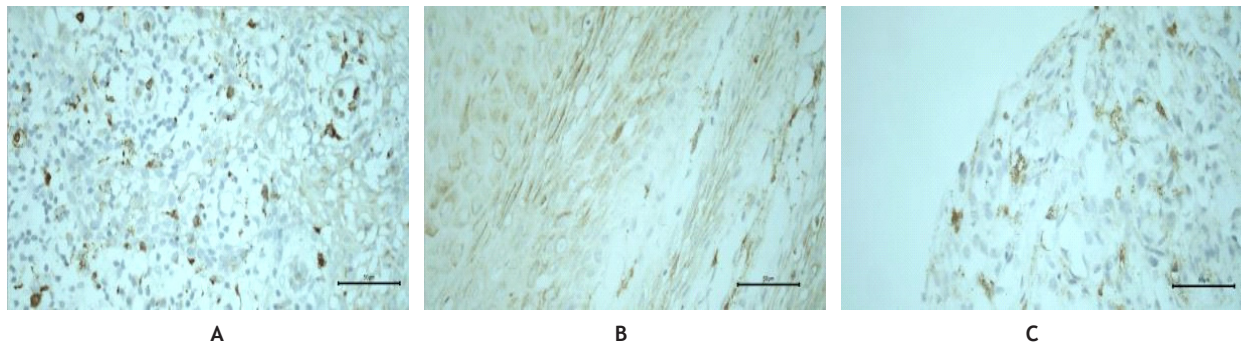


Figure 1. A. CD68⁺ as brown cell membrane of macrophage on pulp polyp (40x10); B. CD68⁺ as brown cell membrane of macrophage on gingiva polyp (40x10); C. CD68⁺ as brown cell membrane of macrophage on fibroma (40x10)

Table 1. Distribution of CD68⁺ among samples

% CD68 ⁺	Pulp polyp	Gingival polyp	Fibroma
Type of lesions			
Mean ± SD	45.82 ± 15.94	29.98 ± 13.5	51.31 ± 31.64
Median (%)	45.89	33.08	33.7
Minimum (%)	23.54	10.18	18.29
Maximum (%)	77.91	54.36	93.64

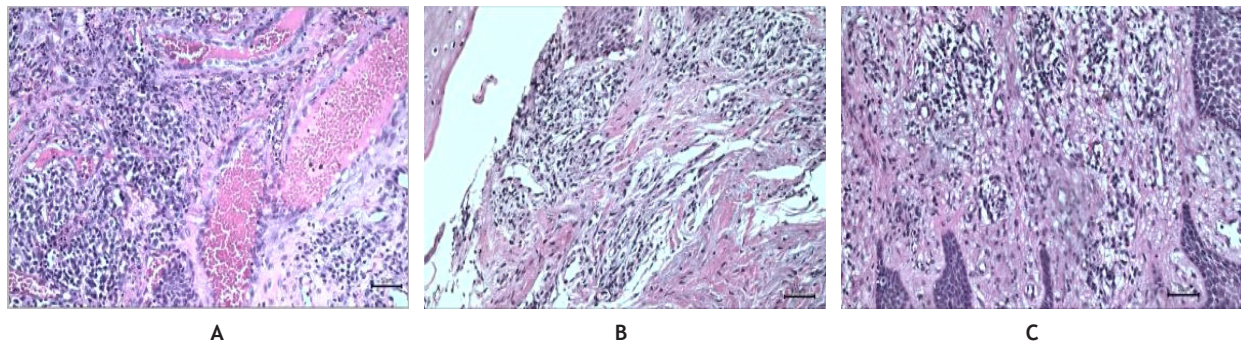


Figure 2. A. Angiogenesis in pulp polyp appear as rich vascular supply; B. Angiogenesis in gingiva polyp appear as capillary vessels lined by endothelial cells; C. Angiogenesis in fibroma appear as capillary vessels lined by endothelial cells (HE with magnification 20x10)

Table 2. Distribution of angiogenesis intensity among lesions

	N	Mean	SD	SE	95% Confidence interval for Mean		Minimum	Maximum
					Lower bound	Upper bound		
Gingival polyp	3	9.67	.52041	.3004	8.85	10.48	9.08	10.08
Pulp polyp	3	12.00	1.23041	.7103	10.89	13.11	10.58	12.75
Fibroma	3	11.81	0.93521	.5399	10.71	12.90	11	12.83
Total	8	11.16	2.68603	1.5507	10.55	11.76	9.08	12.83

higher on pulp polyp (45.82 ± 15.94%) compared to those of gingival polyp (29.98 ± 13.51%) suggested that pulp polyp derived from the granulation tissue growth excess of rich vascular supply within pulp tissue whereas gingival polyp came from gingival tissue with less vascular supply (Figure

2.B) than those within the pulp (Figure 2.A). This was already proved in this study that the highest angiogenesis intensity was on pulp polyp (12.00) followed by fibroma (11.81) and gingival polyp (9.67) as shown in Table 2. These results were also in proper to the biological properties of lesions in

the form of the easily bleeding and softer tissue found in pulp polyp compared to those of fibroma as well as gingival polyp.

Table 2 showed that the angiogenesis intensity in this study was found higher in pulp properties natures of lesions that increasing vascular supply will produce rich vascular granulation tissue and proliferation of cells as occurred in pulp polyp (Figure 2.A) as well as on neoplastic type lesion of fibroma (Figure 2.C). This result supported the statement that the existing of macrophages within the neoplasm process is a part of persistence inflamed reaction¹¹, in this study occurred in fibroma as the benign neoplastic lesion. This is also supported by the statement that the proper fibronectin with its receptor, fibrin or both of them on fibroblast act as the facilitator to form granulation tissue²⁰, as well as the study, suggested that fibrosis regulated definitely by macrophages population through the unique physiological process during initiation, maintenance and fibrosis phase.^{11,21}

In this study, the higher angiogenesis and CD68⁺ in fibroma that mainly consisted of fibroblast proliferation cells than those of gingival polyp may suggest the proliferation of fibroblast cells within fibroma might be not only due to angiogenesis intensity which is higher than those of gingival polyp but also to changes of cells cycle. It also supported previous studies that microenvironment of neoplasm which mostly regulated by inflamed cells quite influence the neoplastic process toward insisting the proliferation and migration of neoplastic cells.^{15,16,22} This reason was also in proper with studies that several molecular and biochemical cellular alterations and changes in the underlying fibrovascular stroma including neovascularisation influenced the occurrence of neoplastic lesion especially at those of malignant type such as oral squamous cell carcinoma whereas angiogenesis intensity within the benign lesion is lower than those of malignant.^{6,22} This fact was proven in this study that the angiogenesis intensity of fibroma is lower compared to those of pulp polyp probably caused by its benign nature of the fibroma.

There was a mild positive correlation ($r = 0.31$) of angiogenesis intensity and CD68⁺ as macrophage marker in this study, that means the increase of macrophage following by the slight

increase of angiogenesis. This was following the clinical, histopathological feature and biological natures of lesions used in this study especially for the pulp polyp in the form of hyperplastic granulation tissue come out of the pulp cavity as a result of the chronic inflammatory response that one of these chronic inflammatory cells are macrophages. This result also supported the study suggested that an increase of macrophages was followed by increase angiogenesis.¹

Macrophage as the chronic inflammatory cell was found higher in an oral proliferative neoplastic lesion of fibroma than those of oral proliferative non-neoplastic lesions of pulp and gingival polyp respectively. Angiogenesis intensity was found higher in pulpal polyp as oral proliferative pulp derived lesion compared to those of fibroma and gingival polyp. There was a slightly positive correlation between macrophage and angiogenesis intensity. Macrophage influence the angiogenesis intensity especially on the proliferative non-neoplastic lesion of pulp polyp and also induces the fibrous and collagen fibers formation as shown on neoplastic type lesion of fibroma. The results of this study suggested that macrophage and angiogenesis intensity could be used as a progres marker of oral proliferative lesions.

CONCLUSION

Macrophage and angiogenesis intensity, and their correlation within oral proliferative non-neoplastic and proliferative neoplastic lesions were in accordance with their biological properties.

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