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HEAD AND NECK

Expression of CD34 and CD105 as markers for angiogenesis in oral vascular malformations and pyogenic granulomas

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Abstract The objective of this study was to assess angiogenic activity by analyzing anti-CD105 and anti-CD34 immunostaining in 20 cases of vascular malformations (VMs) and 20 cases of oral pyogenic granulomas (OPG). In addition, the usefulness of these markers for the differential diagnosis of these two oral tumors was evaluated. The results showed no significant difference in mean microvessel count between the anti-CD105 ($P = 0.803$) and anti-CD34 ($P = 0.279$) antibody. The mean number of vessels was 18.75 and 59.72 for oral VMs immunostained with anti-CD105 and anti-CD34 antibody, respectively, whereas in OPG the mean number was 20.22 and 48.09, respectively. CD34 was found to be more effective than CD105 in identifying blood vessels. However, the anti-CD105 antibody seems to be more related to vascular neoformation. Overall, this study supports the role of angiogenic factors in the etiopathogenesis of oral VMs and PG, but the results showed that quantification of angiogenesis cannot be used as a marker for the differential diagnosis of these two types of lesions.

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Introduction

Angiogenesis is defined as the growth and development of new blood vessels from preexisting vasculature, and is fundamental for a series of physiological and pathological events such as inflammation, tissue repair, tumor growth, and invasion and metastasis. The process is dynamic and complex and involves the growth and migration of endothelial cells and capillary morphogenesis [1]. Studies have been conducted to better understand the main angiogenic mechanisms in an attempt to develop new therapeutic modalities that accelerate angiogenesis during tissue repair or inhibit this process in different tumor conditions [2].

Several biological markers with affinity for specific epitopes on endothelial cells, such as von Willebrand factor/factor VIII, CD31, CD44, CD105 (endoglin), and CD34, have been used to investigate the pathogenesis of head and neck tumors such as pyogenic granulomas and hemangiomas, which often exhibit similar clinical and histopathological characteristics that impair their diagnosis [1, 3].

CD105 (endoglin) is a 180-kDa homodimeric transmembrane glycoprotein, which is encoded by the CD105 gene located on chromosome 9q34. The endoglin is highly specific for endothelial cells of capillaries, veins, and arteries and is used as a marker for the assessment of vascularization in both normal and neoplastic tissues. Expression of CD105 is also observed in activated macrophages, immature pro-erythroblasts, syncytiotrophoblasts, and fibroblasts [2, 4].

CD34 is a transmembrane glycoprotein of 105–120 kDa, which shows high specificity for endothelial cells of blood vessels and for precursors of hematopoietic cells. This protein is expressed in various neoplasms including leukemia and vascular tumors, a fact suggesting that CD34 immunostaining can be used as a diagnostic and prognostic marker of these tumors [5].

Vascular anomalies are congenital errors in vascular development. They frequently involve the head, neck, and oral cavity. Subdivided into vascular tumors (hemangiomas) and vascular malformations (VMs), vascular anomalies remain poorly understood. However, growing interest and recent advances in the diagnosis, management, and molecular characterization of these lesions are improving treatment strategies [6]. Thus, the objective of this study was to quantify angiogenic activity by analyzing anti-CD105 and anti-CD34 immunostaining in oral VMs and pyogenic granulomas (PG) to better understand the pathogenesis of these tumors. In addition, the usefulness of these markers for the differential diagnosis of these two oral lesions was evaluated.

Methods

Twenty oral VMs and 20 PGs cases obtained from the files of the Pathological Anatomy Service, Discipline of Oral Pathology, Department of Dentistry, Federal University of Rio Grande do Norte, Brazil, were selected. For immunohistochemical analysis, 3-μm thick histological sections were obtained from tissue specimens fixed in 10% formalin and embedded in paraffin. The sections were mounted on previously cleaned, grease-free glass slides prepared with organosilane adhesive (3-aminopropyltriethoxysilane; Sigma Chemical Co., St. Louis, MO, USA), and analyzed immunohistochemically using the streptavidin–biotin complex. The sections were deparaffinized and antigen retrieval was performed by immersion of the slides in 0.4% trypsin, pH 7.9. Next, the slides were incubated with the anti-CD34 (clone QBEnd101, Dako Corporation, Glostrup, Denmark; 1:100, overnight) and anti-CD105 monoclonal antibodies (clone SN6H, Dako Corporation; 1:500, overnight). The reaction was developed with 0.03% diaminobenzidine chromogen (Sigma) and the sections were counterstained with Mayer's hematoxylin.

All slides were examined independently by two observers. The immunohistochemical staining pattern was analyzed by light microscopy considering the following parameters: angiogenic index determination based on the number of microvessels immunostained with the anti-CD34 and anti-CD105 antibodies, and qualitative analysis of the CD34 and CD105 immunostaining pattern. The angiogenic index of the tumors was determined based on microvessel count as described by Maeda et al. [7]. Five areas showing the highest vascularization were identified subjectively by evaluating histological sections at 40× magnification, and vessels were counted under an Olympus BX41 light microscope at 200× magnification. The method of Wakulich et al. [8] was used for the determination of the intensity of anti-CD34 and anti-CD105 staining, and a score ranging from 0 to 3 was attributed to subjectively assess vessel staining intensity (0, absence of staining; 1, focal or weak staining; 2, moderate or focally intense staining, and 3, generalized intense staining). Sections in which the step of incubation with the primary antibody was omitted were used as negative control.

For analysis, any cell or endothelial cell group stained separately from adjacent microvessels and other connective tissue components, as well as, lumen-containing vessels was counted as a single vessel. Branched and discontinuous structures were also counted as vessels.

Since the data were analyzed by pairwise comparison of the two groups, the Student's *t* test was chosen for analysis, with the level of significance set at 5% ($P < 0.05$).

Results

Of the 20 oral VMs studied, 52.63% were males and 47.36% were females and mean of patient was 35.84 years. All patients were older than 20 years. Most of patients were whites (70.58%). In relation to OPG, 65% were females with years mean of 34.73 and 66.6% were whites.

Qualitative analysis showed positive CD34 immunostaining in 100% of the oral VMs ($n = 20$) (Fig. 1) and OPG ($n = 20$) (Fig. 2) cases studied. In contrast, CD105 immunoexpression was observed in 50% of VMs cases ($n = 10$) (Fig. 3) and in 80% of PG cases ($n = 16$) (Fig. 4). A more homogenous immunostaining pattern was

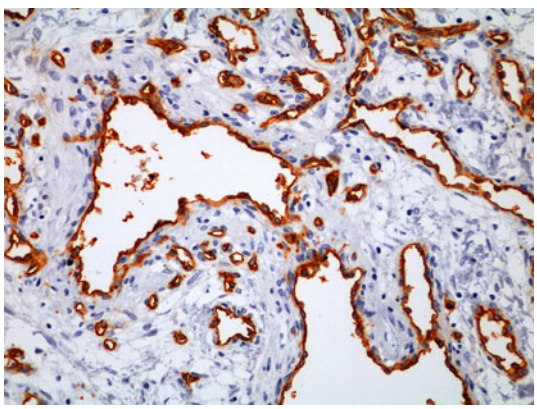

Fig. 1 Oral vascular malformation. Immunoexpression pattern of anti-CD34 (SABC, 400×)

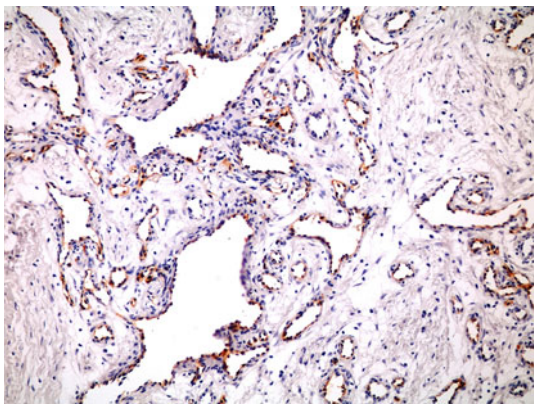

Fig. 2 Oral pyogenic granuloma. Immunohistochemical staining of CD34 (SABC, 400×)

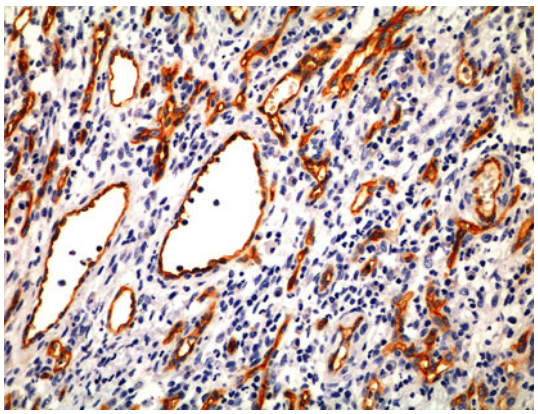

Fig. 3 Oral vascular malformation. Immunoexpression of anti-CD105 in blood vessels (SABC, 400×)

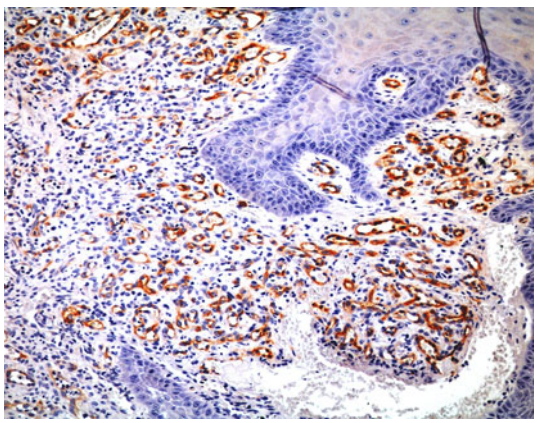

Fig. 4 Oral pyogenic granuloma. Immunohistochemical staining of CD105 in blood vessels (SABC, 200×)

obtained with the anti-CD34 antibody for both types of tumors.

With respect to the intensity of CD105 immunostaining, 4 (20%) of the 20 pyogenic granuloma specimens analyzed were negative, 9 (45%) presented focal or weak immunoexpression, 4 (20%) moderate and focally intense staining, and 3 (15%) generalized intense staining. For VMs, no staining was observed in 10 (50%) of the 20 specimens, 5 (25%) specimens presented focal or weak immunoreactivity, and 5 (25%) presented moderate and focally intense staining. No generalized intense immunostaining for this marker was observed in any of the VMs cases.

CD34 expression was generalized and strong in most (55%) PG cases, 4 (20%) cases presented focal or weak expression, and 5 (25%) presented moderate or focally intense staining. Analysis of CD34 expression in the hemangiomas showed strong or generalized staining in most specimens (60%) and moderate and focally intense staining in 8 (40%).

The mean number of blood vessels counted in VMs specimens stained with the anti-CD105 and anti-CD34 antibodies was 18.75 (range 0.0–64.2) and 59.72 (range 18.0–115.08), respectively. For PG, the mean number of blood vessels was 20.22 (range 0.0–38.6), and 48.09 (range 14.2–129.2) for specimens stained with the anti-CD105 and anti-CD34 antibodies, respectively. Analysis by the Student's *t* test showed no statistically significant difference in mean microvessel count between the two types of oral tumors immunostained with the anti-CD105 or anti-CD34 antibody (Table 1).

However, the Student's *t* test revealed a significant difference in mean microvessel count between the two antibodies tested for VMs and PGs (Table 2).

Discussion

In this study, angiogenic activity was quantified in oral VMs and PGs by analysis of the expression of CD105 and CD34 using anti-CD105 and anti-CD34 monoclonal antibodies. Hemangiomas and vascular malformations are congenital aberrancies of vascular development causing identifiable birthmarks of the skin and mucosa and a variable degree of underlying soft tissue abnormalities. Under

Table 1 Angiogenic index determined by microvessel count according to marker and type of lesion studied

Antibody	Lesion	*n*	Mean	SD	*t**	*P**
CD105	Vascular malformation	20	18.75	22.478	0.252	0.803
	Pyogenic granuloma	20	20.22	13.182		
CD34	Vascular malformation	20	59.72	36.533	−1.10	0.279
	Pyogenic granuloma	20	48.09	30.031		

SD standard deviation

* Student's *t* test

Table 2 Angiogenic index determined by microvessel count according to type of lesion and marker studied

Lesion	Antibody	*n*	Mean	SD	*t**	*P**
Vascular malformation	CD105	20	18.75	22.478	−4.271	<0.001
	CD34	20	59.72	36.533		
Pyogenic granuloma	CD105	20	20.22	13.182	−3.80	0.001
	CD34	20	48.09	30.031		

SD standard deviation

* Student's *t* test

the global heading of vascular anomalies, these lesions predominately occur within the head and neck and affect approximately one in 22 children. Depending upon the size and location, significant functional and esthetic impairment can result from the growth of 'problematic' hemangiomas or vascular malformations. Bleeding, pain, and disability are also common. Thus, an in-depth understanding of the natural history of vascular anomalies is critical for practitioners who diagnose and manage these lesions [6].

Little is understood regarding the pathogenesis, molecular make-up, and origin of vascular anomalies. The field, however, is rapidly progressing. The identification of molecular, genetic markers, and novel treatment protocols has emerged over the past decade that is changing the conceptual framework regarding the pathogenesis and management of vascular anomalies [6].

Angiogenesis plays an important role in metastatic mechanisms, vascularization of ischemic tissues, chronic inflammatory processes, oligodendrogliomas, vascular tumors such as hemangiomas, Kaposi's sarcoma, angiosarcoma, and some autoimmune diseases. Netto et al. [2] and Freitas et al. [3] agree about the clinical applications of data obtained in experiments regarding vascular neoformation for therapy aiming at the acceleration of angiogenesis in order to promote tissue repair and the inhibition of angiogenesis in various tumor conditions and regarding the quantitation of angiogenesis as a diagnostic and prognostic marker, thus representing an additional tool for monitoring the progression and efficacy of treatment in patients with these tumors.

In view of the different techniques recommended for the quantification of angiogenesis, in this study the authors first evaluated microvessel density, microvessel volume, and microvessel count. The last parameter was then used for the calculation of the angiogenic index since it was found to be the simplest, most effective, and easiest method for the quantification of angiogenesis in head and neck tumors.

Qualitative analysis showed positive immunostaining for CD34 in 100% of the hemangioma and pyogenic granuloma cases. In contrast, CD105 immunostaining was only observed in 50 and 80% of hemangioma and pyogenic granuloma specimens, respectively, demonstrating the high sensitivity of CD34 immunostaining as reported by Guttman et al. [9] for squamous cell carcinoma of the tongue.

Strong and generalized immunostaining of CD34 was observed in both PG (55%) and VMs (60%). In contrast, immunoexpression of CD105 was focal or weak in most PG specimens (45%) and absent in most hemangiomas (50%). These results agree with Guttman et al. [9] who emphasized the importance of CD34 immunostaining intensity in the study of angiogenesis.

Controversy exists regarding the evaluation of angiogenesis using immunohistochemical markers such as CD34 and CD105. Soares et al. [1] and Martone et al. [10] defend that CD105 represents an excellent indicator of angiogenesis, whereas Guttman et al. [9] and Ascani et al. [11] emphasize the importance of CD34 in the study of angiogenesis. This study showed a better efficacy of CD34, with the observation of more uniform results that were more easily interpretable, when compared to CD105. Nevertheless, no significant differences in the angiogenic indices obtained by microvessel count were observed between CD34 and CD105.

The CD34 and CD105 immunostaining results obtained for oral VMs and PGs reflect the clinical and histopathological similarity between the two types of tumors and, at the same time, indicate the inefficacy of quantification of angiogenesis as a differential diagnostic marker. These findings agree with the results of Freitas et al. [3], who used CD31 and von Willebrand factor antibodies for the quantification of angiogenesis in oral hemangiomas and PGs and concluded that these pan-endothelial markers should not be used for the diagnostic differentiation of angiogenic activity between these tumors by microvessel count.

In this study, the angiogenic indices obtained for oral VMs and PGs using the anti-CD105 antibody were much lower than those obtained with the anti-CD34 antibody. Furthermore, CD105 immunostaining was negative in most cases. Within this context, the small number of vessels and the lack of CD105 staining in some specimens might suggest that endoglin is an excellent marker of newly formed vessels, whereas CD34 is a pan-endothelial marker unable to differentiate newly formed vessels from preexisting ones [3, 12]. As a consequence, a larger number of positive vessels were observed when using the latter antibody. Some investigators emphasize that endoglin is preferentially expressed in proliferating vessels, with its expression being associated with a poor prognosis [2, 13]. This marker has therefore been used for the evaluation of the degree of tumor malignancy and for the estimation of survival rates in many patients.

These results regarding CD105 immunostaining also suggest that, based on the assumption that this antibody is only expressed on newly formed vessels, tumor specimens

that are negative for this marker might be in a more advanced or dormant stage in which a balance exists between angiogenic (VEGF, bFGF, TNF-α, PDGF, and IL-8) and antiangiogenic factors (TGF-β, TIMP-1, angiostatin, endostatin, and TPS-1) [14]. Thus, in this series 50% of oral VMs were in the non-proliferative phase and 80% of OPGs were in the phase of development or endothelial proliferation, a fact emphasizing the importance of angiogenic mechanisms for the etiopathogenesis of these tumors [15]. In this respect, it may be suggested that tumors in the proliferative stage mainly presented an elevated activity of vascular endothelial growth factor (VEGF). According to Freitas et al. [3], VEGF is a potent mitogen for endothelial cells, which is only expressed during the phase of tumor development and is absent in histological sections of involuted tumors or tumors in the phase of involution.

These results support the view that angiogenesis is an important process in the growth and development of different head and neck tumors. However, these findings do not emphasize only the similarity between oral VMs and PGs and the relevance of CD105 and CD34 immunostaining for the study of vascular neoformations, but also the tendency to consider that angiogenic factors play an important role in the pathogenesis of these tumors reported in several investigations [12, 14, 15]. However, the exact role of the angiogenesis and its relationship with the development of many tumors still needs to be clarified so that it can be used as a safe tool for the identification of the biological potential of the tumor and as a coadjuvant in available treatments.

Conflict of interest None.

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