Assessment Of Vascular And Lymphatic Vessels Density In Benign Vascular Lesions Using CD34 And D2-40 Immunohistochemical Markers

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ABSTRACT

Background: Vascular tumors and malformations, comprising a broad category of lesions often referred to as vascular anomalies. Hemangioma, represents a variety of vascular lesions (both malformations and tumor), while lobular capillary hemangioma is a common vascular lesion of the skin and mucous membranes that occurs mainly in children and young adults. Lymphangiomas are malformations of the lymphatic system. At the level of light microscopy the small lymphatics vessels may be similar to capillaries and sometimes are only tentatively identified by the nature of their contents or by immunohistochemical staining procedure. This study aimed to assess the vascular and lymphatic vessels density in benign vascular lesions using CD34 and D2-40 immunohistochemical markers.

Materials and Methods: Twenty two formalin-fixed paraffin-embedded tissue blocks of Hemangioma/vascular malformation, thirty of lobular capillary hemangioma and another twenty of lymphangioma.

Results: Lymphatic vessel density expressed by D2-40 immunomarker was found in all cases with mean (24.01±14.74) in lymphangioma ,for lobular capillary hemangioma it was (12.67±6.66) and for hemangioma was (9.77±6.82) where as the mean of microvessel density count measured by CD34 immunomarker was (49.87±31.97) for lobular capillary hemangioma , in hemangioma it was (37.42±23.40) and (25.90±12.23) for lymphangioma.

Conclusions: All vascular lesions are a mixture of blood and lymphatic vessels with different proportions, hemangioma shows high percentage of blood vessels and lymphangioma shows high percentage of lymphatic vessels.

Key words: Vascular tumor, immunohistochemistry, D2-40, CD34. **(J Bagh Coll Dentistry 2017; 29(2):61-64)**

INTRODUCTION

Vascular anomalies are heterogeneous group of congenital lesions of abnormal vascular development and may take place anywhere in the body. There is a main distinction between a vascular tumor, which grows by cellular proliferation and a vascular malformation, which represents a restricted defect in vascular morphogenesis. Some of the lesions are a source of esthetic problems, while some of them are malignant; thus, the therapeutic approach is variable [1]. The pathophysiology of vascular malformation, hemangioma and lymphangioma are interconnected [2].

Hemangioma is a term that encompasses a heterogeneous group of clinical benign vascular lesions, which is a proliferating mass of blood vessels that do not undergo malignant transformation [3].

Lobular capillary hemangiomas are rapidly growing, mostly exophytic lesions which may ulcerate. Most lesions develop at sites of superficial trauma; when seen early, it is a solitary, bright red mass, some authors use the term 'pyogenic granuloma' to describe this lesion.

Lymphangiomas are rare congenital tumors, with up to 70% reported in the head and neck.

They are alienated into three types: cystic (cystic hygroma), capillary, and cavernous. Lymphangiomas report for approximately 25% of all vascular neoplasms in children and adolescents. About 25% of cervical cysts are lymphangiomas. Differences between vascular and lymphatic capillary endothelium can be established by means of immunohistochemistry with antibodies targeted against lineage-specific substances, basal lamina, and pericytes.

D2–40 is a selective monoclonal immunohistochemical marker of lymphatic endothelium in adult human tissue; it does not stain vascular endothelium [4,5]. CD34, a sensitive marker for vascular epithelium was used to evaluate microvessel density in numerous tissues and intra-tumoral microvessel density [6].

MATERIALS AND METHODS

The sample is consisted of twenty two formalin-fixed paraffin-embedded tissue blocks of Hemangioma/vascular malformation, twenty of lymphangioma and another thirty of lobular capillary hemangioma.

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The samples were obtained from the archives of the department of Oral and Maxillofacial Pathology/College of Dentistry/ University of Baghdad and Al-Shaheed Ghazi Hospital/ Medical City / Baghdad dated from (1979 till 2015). After histopathological reassessment of haematoxylin and eosin stained sections for each block, an immunohisto-chemical staining was performed using anti D2-40 monoclonal antibody and anti CD34 monoclonal antibody, assessment of LVD and MVD Based on the criteria of Weidner [7].

RESULTS

D2-40 Expression

The immunostaning method of D2-40 was applied to lymphangioma, hemangioma and pyogenic granuloma, where the lymphatic vessels were stained with brown coloration as seen in **(Figures 1, 2 and 3).**

In **table 1**, the mean±SD of LVD evaluated by D2-40 immunomarker expression, according to ANOVA test imploded between samples groups. There was a high statistical significant difference in the mean of expression of D2- 40 in lymphangioma in comparison to pyogenic granuloma and hemangioma (p=0.000).

Table 1: Description of statistics obtained by immunohistochemistry of D2-40

D2-40	N	Mean (LVD)	SD	Sig.
Lymphangioma	20	24.01	17.74	0.000
Pyogenic granuloma	30	12.67	6.66	
Hemangioma	22	9.77	6.82	
Total	72	14.93	12.23	

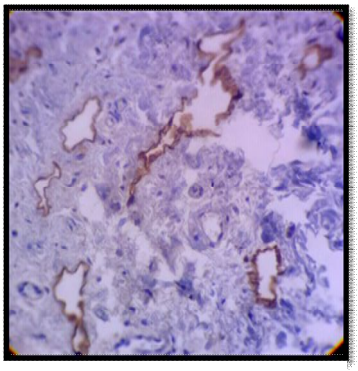

Figure 1: Positive lymphatic endothelium expression of D2-40 in hemangioma (400x)

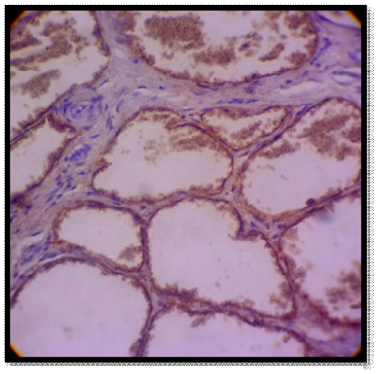

Figure 2: Positive lymphatic endothelium expression of D2-40 in lymphangioma (400x)

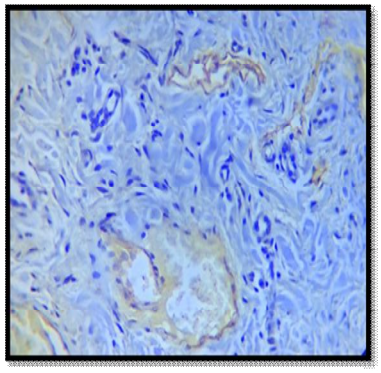

Figure 3: Positive lymphatic endothelium expression of D2-40 in lobular capillary

CD34 Expression

Table 2 shows the mean ±Sd of MVD evaluated by CD34 expression as brown stained blood vessels endothelial cells as seen in **(Figures 4, 5 and 6)**, according to ANOVA test imploded between groups found a statistical significant difference (P=0.006).

Table 2: Description of statistics obtained by immunohistochemistry of CD34

CD34	N	Mean (LVD)	SD	Sig.
Pyogenic granuloma	20	49.87	31.97	0.006
Hemangioma	30	37.42	23.40	
Lymphangioma	22	25.90	12.23	
Total	72	39.41	26.80	

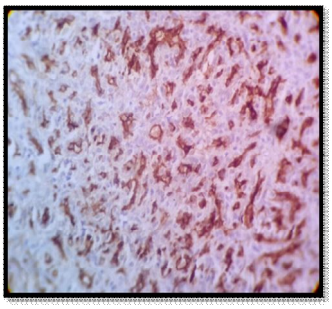

Figure 4: Positive blood vessels expression of CD34 in hemangioma (400x)

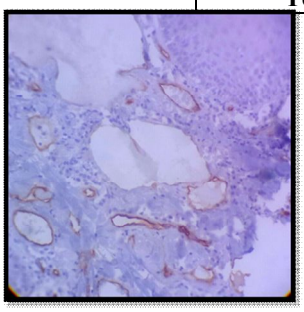

Figure 5: Positive blood vessels expression of CD34 in lymphangioma (200x)

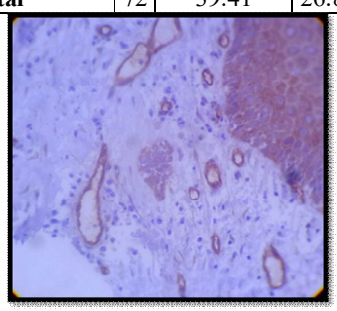

Figure 6: Positive blood vessels expression of CD34 in lobular capillary hemangioma (400x)

Table 3 shows the mean difference of lymphatic vessels density (LVD) measured by expression of D2-40 immunohistochemical marker subtracted from microvessels density (MVD) measured by expression of CD34 immunohistochemical marker. It also shows the percentage of lymphatic and blood vessels in hemangioma, pyogenic granuloma and lymphangioma. As lymphangioma is predominantly composed of lymphatic vessels (92.7%) while hemangioma predominantly composed of blood vessels (73.8%).

According to post hoc test, whereas multiple comparisons was made between hemangioma, lymphangioma and pyogenic granuloma with different markers, a highly significant difference was found between D2-40 expression in hemangioma and in lymphangioma (0.000), and the same result found between pyogenic granuloma and lymphangioma (0.001).

While a significant difference is found between CD34 marker expression in lymphangioma and hemangioma (0.002).Details explained in **(Table 4).**

Table 3: Mean difference of LVD from MVD and the percentage of lymphatic and blood vessels in hemangioma, pyogenic granuloma and lymphangioma

Groups	Mean difference LVD from MVD	Percentage of blood vessels	Percentage of lymphatic vessels
Pyogenic Granuloma	(49.87)-(12.67) **=37.2**	74.6%	25.4%
Hemangioma	(37.42)-(9.77) **=27.65**	73.8%	26.2%
Lymphangioma	(25.90)-(24.01) **=1.89**	7.3%	92.7%

Table 4: Multiple statistical comparisons by post hoc test

	Dependent variable		Mean difference	S.E.	Sig.
d240	**Hemangioma**	**Lymphangioma pyogenic**	-14.23	3.38	.000**
			-2.89	3.07	.349ND
	Lymphangioma	**Pyogenic**	11.34	3.15	.001**
cd34	**Hemangioma**	**Lymphangioma pyogenic**	11.52	7.80	.144ND
			-12.44	7.09	.084ND
	Lymphangioma	**Pyogenic**	-23.96	7.29	.002*

ND=non significant, **highly significant (P ≤ 0.001), *significant difference (p ≤ 0.05)

DISCUSSION

Vascular tumors are heterogeneous groups of disease with biological behavior which ranging from a hamartomatous growth to frank malignant. This study aims to define the type of vascular tissue in three benign tumors: hemangioma, lymphangioma, lobular capillary hemangioma and assessment of those tumors by using D2-40, CD34, immunohistochemical markers to identify the proportions of lymphatic and vascular vessels in those tumors.

In this study, two important parameters were considered concerning with behavior of vascular tumor namely (LVD, MVD) the assessment was done by using D2-40, CD34 immunomarkers respectively.

This study assessed the expression of D2-40 in benign vascular lesion, the results revealed positive D2-40 expression in all lymphangioma cases. Our result agree with Fukunaga [8] and Galambos and Nodit [9] whose found 100% positivity of D2-40 expression in lymphangioma, however it disagree with Fukunaga [8] finding of D2-40 in other vascular lesion. While our result disagrees with Bhawan et al. [10] whose observed a variable staining of lymphangiomas to D2-40. The source of this discrepancy according to them may be that some of the cases that were diagnosed as lymphangiomas were actually hemangiomas.

This may not be uncommon, as several studies have indicated that it is difficult to distinguish lymphatic channels from venules or capillaries histomorphologically.

Also, the results revealed strong positive CD34 expression in lobular capillary hemangioma and hemangioma that agree with North [11] who stated that the endothelial cells of hemangioma immunoreact positively for normal

endothelial markers of the blood vasculature, such as CD34, and disagree with Kang et al. [12] whom explained that in the pyogenic granuloma portion, CD34 was almost negatively detected, according to them this is due to heterogeneous characteristics of the lesion.

Also In this study we have observed CD34 positive expression in lymphangioma Similar results obtained in previous studies which were explaining Endothelial markers (factor VIII, CD31, CD34, and Ulex) expression by endothelial cells in both hemangiomas and lymphangiomas [13].

The obvious capillary growth (hyper plastic granulation tissue) in lobular capillary hemangioma suggests that there should be a strong activity of angiogenic potential [14]. This agreed with the finding of our study which found that CD34 expression being higher in lobular capillary hemangioma explaining the proliferative nature of that lesion.

D2-40 immunomarker expression was detected in lymphangioma, hemangioma and in lobular capillary hemangioma in different percentage and density. Although lymphangioma predominantly composed of lymphatic vessels detected by D2-40, however lymphangioma also containing vascular vessels. Similarly, hemangioma although predominantly showed blood vessels however it was also containing lymphatic vessels.

This proved that all vascular lesions are a mixture of blood and lymphatic vessels with different proportions hemangioma show high percentage of blood vessels while lymphangioma shows high percentage of lymphatic vessels.

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المستخلص

الخلفية : الأورام والتشوهات الوعائية تمثل مجموعات واسعة من اللآفات غالبا يشار اليها بالأوعية الدموية الشاذة .أن مصطلح الأورام الوعائية تستخدم عادة لشرح مجموعة متنوعة من اورام الاوعية الدموية والتشوهات الخلقية. الأورام الوعائية الشعرية المفصصة هي أورام الاوعية الدموية التي تصيب ألأغشية المخاطية والجلدية عند الاطفال والشباب.اما الأورام اللمفية فهي التشوهات التي تحدث في النظام اللمفي .عند مستوى الفحص بالمجهر الضوئي فأن الاوعية اللمفية الدقيقة تكون مشابهه للأوعية الدموية الدقيقة وفي بعض الاحيان يمكن تميزها فقط بواسطة طبيعة محتواها او بواسطة استخدام الفحص المناعي النسيجي الكيميائي.

الأهداف: تهدف هذه الدراسة لتقييم كثافة الأوعية الدموية و اللمفاوية في اورام الاوعية الحميدة وقد تم ذللك بإجراء الفحوصات المناعية النسيجية الكيميائية D2-40, CD34

المواد وطرائق العمل:في هذه الدراسة 22 عينة للورم الوعائي معالج بالفورمالين والمغمور بالبارافين و 30 عينة للورم الوعائي الشعرية المفصص و 20 عينة اخرى للورم اللمفي جمعت من ارشيف المختبرات تضمنت خلال هذه الدراسة .

النتائج: ان كثافة الاوعية اللمفاوية والموضحة من خلال الاجسام المناعية D2-40 قد وجدت في كافه الحالات وبمعدل (24.01±14.74) بالنسبة للأورام اللمفية بمعدل(12.67±6.66) للأورام الوعائية الشعرية المفصصة وبالنسبة للأورام الوعائية فكان المعدل(9.77±6.82) .وكان كثافة الاوعية الدموية الموضحة من خلال الاجسام المناعية CD34 قد وجدت في جميع الحالات وبمعدل(49.87±31.97) للأورام الوعائية الشعرية المفصصة(37.42±23.40) للأورام الوعائية(25.90±12.23) بالنسبة للأورام اللمفية .

الاستنتاجات:جميع الاورام الوعائية هي عبارة عن خليط من الاوعية الدموية واللمفية بنسب متفاوتة ,الاورام الدموية تظهر نسبة عالية من الاوعية الدموية بينما تظهرالاورام اللمفاوية نسبة عالية من الاوعية اللمفاوية.