Immunohistochemical expression of endocan, as a marker of assessment of angiogenic potential in benign vascular lesions (hemangioma, lymphangioma and lobular capillary hemangioma) of head and neck region

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ABSTRACT

BACKGROUND: The biological behavior of vascular tumors ranging from a hamartomatous growth to frank malignant. The pathophysiology of vascular malformation, hemangioma and lymphangioma is interrelated, blood vessels known to be the site of origin of venous malformations, hamartomas and some neoplasms as benign, tumor-like growth of vessels (hemangiomas). Angiogenesis is the process of formation of new blood vessels from an existing structure.

Aims of study Assessment of angiogenic potential in benign vascular lesions (hemangioma, lymphangioma and lobular capillary hemangioma) of head and neck region.

Materials and Methods: Twenty formalin-fixed paraffin-embedded tissue blocks of lymphangioma, thirty of lobular capillary hemangioma and another twenty-two of Hemangioma/vascular malformation to be stained with ndothelial cell-Specific Molecule-1 (ESM-1) monoclonal antibody.

Results: Microvessel density expressed by Endothelial cell-Specific Molecule-1 (ESM-1) immunomarker was found in all cases with mean density of (25.02±13.89) for hemangioma and (37.44±23.16) for lobular capillary hemangioma and (6.34±3.52) for lymphangioma. Along with post hoc test ESM-1 marker expression showed a high significant difference between (lymphangioma and hemangioma =0.001), (lymphangioma, pyogenic granuloma=0.000), and it was significantly different between (hemangioma, pyogenic granuloma=0.011)

Conclusions: The obvious capillary growth in lobular capillary hemangioma revealed that lobular capillary hemangioma showed the highest activity of angiogenic potential in comparison to hemangioma and lymphangioma.

Keywords: Endocan,vascular tumor,angiogenic potentional. **(Received: 15/7/2018; Accepted: 28/8/2018)**

INTRODUCTION:

Vascular system is a highly heterogeneous and non-identical organ system**,** hence to make a judgment whether the entire lesion is composed of only venous component or whether there is a lymphatic channels cannot be easy**(1).**

To increase the flow of blood to ischemic tissue the human body grows new blood vessels from an existing structure, the process of formation of new blood vessels termed angiogenesis **(2)**.

Vascular development starts by the gathering of a vessel plexus from single cell precursors, adjustment then undergoes to this plexus by sprouting growth and remodeling (angiogenesis), followed by recruitment of vessels into target tissues, finally according to the specific needs of the tissue, new vessels differentiate**. (3)**

Endocan also named as Endothelial cell-Specific Molecule-1(ESM-1) has acted since its detection as a dermatan sulfate proteoglycan, with unique functional and structural properties. Endothelial cells naturally expresses ESM-1 , a highly controlled in existence of proinflammatory and proangiogenic molecules, binds to growth factors, matrix proteins, integrin and cells, and considered as an precise marker of endothelial activation **(4).**

MATERIALS AND METHODS

The sample is consisted of twenty formalin fixed paraffin embedded tissue blocks of lymphangioma, thirty of lobular capillary hemangioma and another twenty-two of hemangioma/vascular malformation. The samples gained from the archives of Al-Shaheed Ghazi Hospital/ Medical City / Baghdad and the department of Oral & Maxillofacial Pathology/College of Dentistry/ University of Baghdad & dated from (1979 till 2015).

After histopathological reexamination of haematoxylin and eosin stained sections for all blocks, an immunohisto-chemical staining was achieved using endocan, assessment MVD based on the criteria of weidner [(5)].

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RESULTS

ESM-1 Expression

The immunostaning procedure of **ESM-1** was applied to hemangioma, pyogenic granuloma and lymphangioma, where the endothelial cells of blood vessel were stained with brown coloration as seen in **(Figures 1,2 , 3,4and 5).**

In **(Table 1)**, the mean±Sd of MVD assessed by **ESM-1** immunomarker expression, in relation to ANOVA test imploded between samples groups. There was a high statistical significant difference in the mean of expression of ESM-1 in pyogenic granuloma in comparison to lymphangioma and hemangioma (p=0.000).

Table (1): Description of statistics gained by immunohistochemistry of ESM-1

ESM-1	N	SD	mean	Max.	Min.	F	SIG.
hemangioma	22	13.89151	25.0182	62.60	10.00	20.17	0.000
lymphangioma	20	3.51604	6.3400	15.60	2.30		
Pyogenic granuloma	30	23.16239	37.4400	95.30	8.60		
Total	72	21.04732	25.0056	95.30	2.30		

Numerous comparisons were made between lymphangioma, hemangioma and pyogenic granuloma with endocan marker as stated by post hoc test, a highly significant difference was establish between ESM-1 expression in(lymphangioma and hemangioma=0.001), (lymphangioma and pyogenic granuloma=0.000) and it was significantly different between pyogenic granuloma and hemangioma (0.011).**(Table .2)**.

Table (2): several statistical comparisons by post hoc test

ESM-1	Dependent variable		Std. Error	Mean difference	Sig.
	hemangioma	Lymphangioma	5.24	18.67	.001**
		Pyogenic	4.76	-12.42	.011*
	Lymphangioma	Pyogenic	4.89	-31.10	.000**

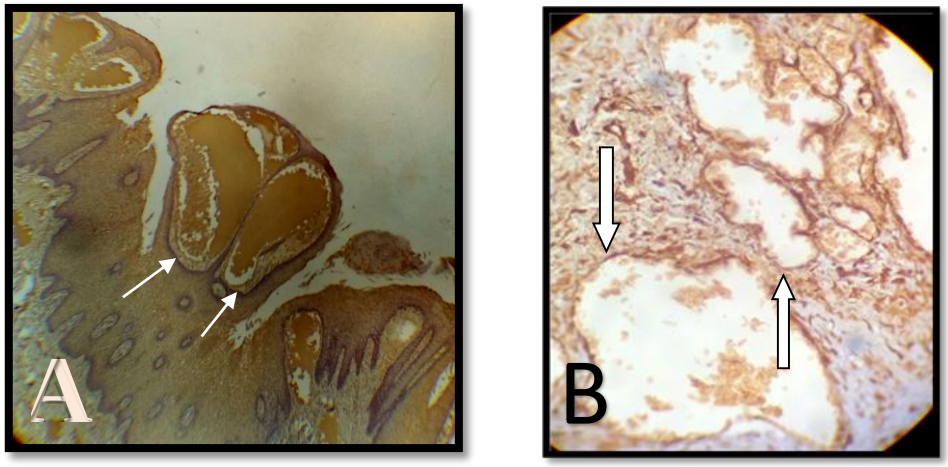

Figure (1) A: Photomicrograph in lymphangioma showing ESM-1 immunostaining - negative stain lymphatic vessels and positive blood vessels X100) B: Positive blood vessels X400) *(thin arrow for lymphatic while, thick arrow for blood vessels*

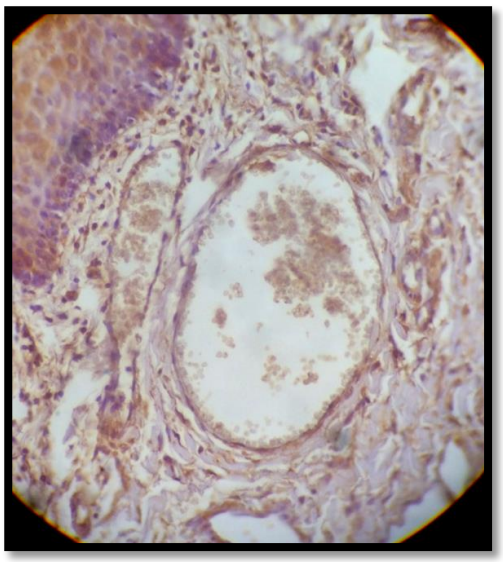

Figure (2): Photomicrograph in lymphangioma display ESM-1 immunostaining positive blood vessels (X400)

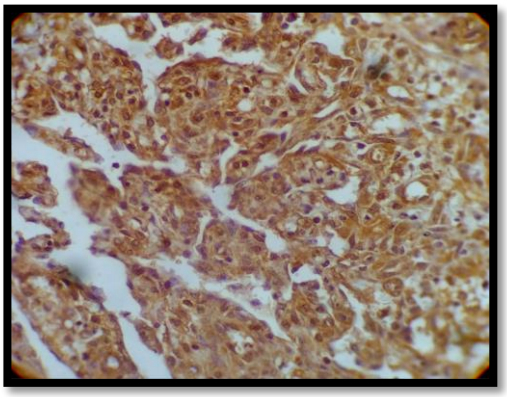

Figure (3): Photomicrograph in hemangioma viewing ESM-1 immunostaining - positive blood vessels(X400)

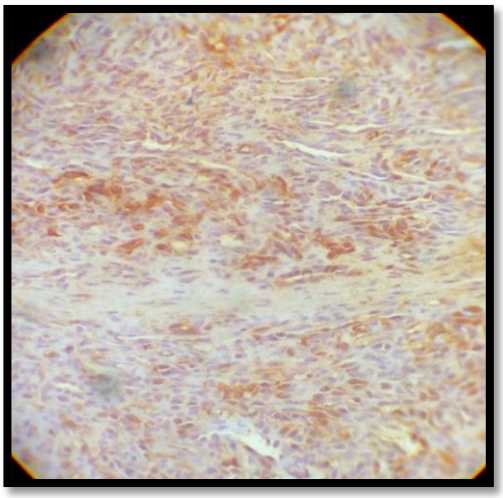

Figure (4): Photomicrograph in lobular capillary hemangioma viewing ESM-1 immunostaining- positive blood vessels (X200)

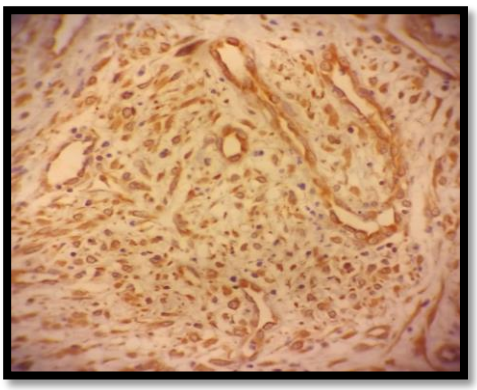

Figure (5): Photomicrograph in lobular capillary hemangioma viewing ESM-1 immunostaining- positive blood vessels (X400)

DISCUSSION

This study exhibited the highest positive expression of endocan in lobular capillary hemangioma that is in agreement with previous studies, which determined ESM-1 as critical proangiogenic molecule [7], also it agrees with Chen LY et al., [8] whom found *in vitro* that ESM-1 being over-expressed in the course of angiogenesis. This can be clarified by understanding the pathogenesis of lobular capillary hemangioma at the molecular level that considered as the imbalance between angiogenesis inhibitors and enhancers, that is overexpression of VEGF and bFGF and decreased quantity of angiostatin direct to the development of pyogenic granuloma **[9].**

In this study, endocan was positively expressed in lymphangioma and that agrees with Shin JW et al., [10] whom clarified that endocan was a potential target for the inhibition of VEGF-A– or VEGF-C– induced pathologic lymphatic vessel growth and activation and considered it as a novel mediator of lymphangiogenesis. In lobular capillary hemangioma the obvious capillary growth (hyperplastic granulation tissue) suggests that there should be a strong activity of angiogenic potential [9]. This agree with the findings of the present study, which found that ESM-1 expression being higher in lobular capillary hemangioma, which explains the proliferative nature of this lesion.

REFERENCES:

1. Jackson IT, Carreno R, Potparic Z, Hussain K. Hemangiomas, vascular malformations, and lymphovenous malformations: classification and methods of treatment. Plast Reconstr Surg 1993;91:1216–30

2. Igin RK, Camrmeliet PF. Vascular endothelial growth factor (VEGF). Scientific American 2001; 29-35.
3. Johnstone S, Logan RM. The role of vascular endothelial growth factor (VEGF) in oral dysplasia and oral squamous cell carcinoma. Oral Oncolo 2006; 42(4), 337-42
4. Sarrazin S, Maurage CA, Delmas D, Lassalle P, Delehedde M. Endocan as a biomarker of endothelial dysfunction in cancer. J Cancer Sci Ther. 2010;2(2):47-52
5. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. New England Journal of Medicine. 1991 Jan 3;324(1):1-8.
6. Huang GW, Tao YM, Ding X. Endocan expression correlated with poor survival in human hepatocellular carcinoma. Digestive diseases and sciences. 2009;54(2):389-94
7. Brütsch R, Liebler SS, Wüstehube J, Bartol A, Herberich SE, Adam MG, Telzerow A, Augustin HG, Fischer A. Integrin Cytoplasmic Domain–Associated Protein-1 Attenuates Sprouting Angiogenesis. Circulation research. 2010;107 (5):592-601.
8. Chen LY, Liu X, Wang SL, Qin CY. Over-expression of the Endocan gene in endothelial cells from hepatocellular carcinoma is associated with angiogenesis and tumour invasion. Journal of International Medical Research. 2010;38(2):498-510.
9. Yuan K, Jin YT, Lin MT. The detection and comparison of angiogenesis-associated factors in pyogenic granuloma by immunohistochemistry. Journal of periodontology. 2000;71(5):701-9.
10. Shin JW, Huggenberger R, Detmar M. Transcriptional profiling of VEGF-A and VEGF-C target genes in lymphatic endothelium reveals endothelial-specific molecule-1 as a novel mediator of lymphangiogenesis. Blood. 2008;112(6):2318-26.

المستخلص

الخلفية : ان مصطلح الأورام الوعائية تستخدم عادة لشرح مجموعة متنوعة من اورام الاوعية الدموية والتشوهات الخلقية. الأورام الوعائية والأورام اللمفية هي اورام مرتبطة فسلجيا.تعتبر الاوعية الدموية هي المصدر الرئسي المولد للاورام الوعائية ,

الأهداف: تهدف هذه الدراسة لتقييم القابلية لتكوين الاوعية الجديدة في الأورام الوعائية والأورام اللمفية في منطقة الراس والرقبة.

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: كثافة الاوعية الدموية الموضحة من خلال الاجسام المناعية ESM-1 قد وجدت في جميع الحالات وبمعدل(37.44±23.16) للأورام الوعائية الشعرية المفصصة(25.02±13.89) للأورام الوعائية (6.34±3.52)بالنسبة للأورام اللمفية .

الاستنتاجات: االتطور الوعائي الواضح للاورام الدموية الشعرية المفصصة تظهر نسبة عالية من الاوعية الدموية بالمقارنة مع الأورام الوعائية والأورام اللمفية.