Immunohistochemical Expression of Endocan in Ameloblastoma

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ABSTRACT

Background: Ameloblastoma is the most common clinically significant odontogenic tumor, known for its locally invasive potential and frequent recurrences unless treated radically. Endocan is a soluble proteoglycan which is reported to have prognostic implications in multiple human diseases and tumors. This study aims to describe the expression of endocan in ameloblastoma.

Materials and methods: With immunoperoxidase method; tissue sections of formalin fixed- paraffin embedded blocks for ameloblastomas were stained with monoclonal antibodies to endocan, the localization of the endocan expression was examined and the resulting scores of the tissue sections were analyzed according to age, sex, site and tumor subtype.

Results: endocan was found to be expressed in peripheral and central epithelial cells of ameloblastoma tumor islands and stroma to different extents; a selectively increased expression was noted in epithelial cells with acanthomatous differentiation. Tumor epithelial cells of plexiform subtype tend to have higher expression levels of endocan. However, the associations did not reach statistically significant levels.

Conclusions: Endocan is expressed specifically in various populations of tumor epithelial cells and stromal elements of ameloblastoma. The prognostic significance of the expression needs to be clarified in further studies.

Keywords: Ameloblastoma, Endocan. **(J Bagh Coll Dentistry 2016; 28(4):68-71)**

INTRODUCTION

Amid odontogenic tumors; ameloblastoma (AB) is characterized to be the most common [(1)]. Except for the cystic subtype; it grows in an invasive fashion that often extends beyond radiographic borders [(2)], its local behavior prompt the clinicians for a radical surgical treatment [(3)].

ESM-1 (endothelial cell specific molecule-1) or as called later (endocan) is a dermatan sulphate proteoglycan that was first described in 1996 , it is a peculiar molecule that circulate freely in the blood stream in addition to its expression in endothelial cells. Experimental evidence showed that it plays a definite role in inflammation and tumor progression [(4, 5)].

Immunohistochemical expression of endocan was examined in multiple human normal tissues including lung [(4)], liver, brain, kidneys, skin and myocardium [(6)] and was expressed and positively related with the unfavorable outcome of several neoplastic processes such as pituitary adenoma [(7)], hepatocelular [(8)], ovarian [(9)] and colon carcinomas [(10)].

This study aims to evaluate the immunohistochemical expression and localization of endocan in ameloblastoma in relation to age, sex, site and histological subtypes.

MATERIALS AND METHODS

The study involved thirty seven archival formalin fixed-paraffin embedded tissue blocks of AB that were retrieved from the laboratory of the college of dentistry/Baghdad University and the medical city laboratories. Five *um* thick tissue sections of the blocks were mounted on positively charged slides, dewaxed and rehydrated in xylene and serial dilutions of ethanol.

Endogenous peroxidase activity and non-specific antibody binding were blocked with H_2O_2 and protein block respectively then, a monoclonal antibody to Endocan (ab56914; Abcam, Cambridge, UK) with a concentration of 1:2000 was added to tissue section and incubated for 2 hours at 37˚C then, "complement" and "conjugate" solutions of the (EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit , Ab80436; AbCam Inc., Cambridge, UK) were added to tissue sections and incubated for 10 and 15 minutes respectively. The antibody binding was finally visualized with DAB chromogen and counterstained with Mayer's hematoxylin. A positive control of a normal lung was included into each immunohistochemical run and a negative control section was selected in each slide that was stained with the omission of the primary antibody.

Two pathologists examined at least 5 high power fields of each stained tissue section independently, tumor epithelial and stromal expression was classified semiquantitatively to a 4 tiered scores where tissue sections with 0-24% positivity classified as negative, 25-49% as (+), 50-74% as (++) and 75% and above as (+++) [(10)].

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Statistical analysis was performed with SPSS.22 statistical software employing one way ANOVA, Mann-Whitney and Kruskal-Wallis tests. Test results with P values less than 0.05 were considered significant.

RESULTS

The mean age of the study sample was slightly more than 35 years with a predominance of females. The study included four maxillary and 33 mandibular cases. About eighty percent of the study sample was represented by solid AB that was further subdivided into follicular (56.8%), plexiform (16.2%) and acanthomatous (5.4%). The remaining 21.6% were of the cystic type (Table 1).

An intense cytoplasmic and nuclear epithelial expression was found within peripheral ameloblast like cells and stellate reticulum like cells.

Table 1: Study sample characteristics

Variable			Value
Total (%)			37 (100%)
Age (mean±SD)			35.16(±16.26)
Gender	Males		12 (32.4%)
	Females		25 (67.6%)
	M: F ratio		0.48:1
Site	Mandible		33 (89.2%)
	Maxilla		4 (10.8%)
Histologic subtype	SOL	FOL	21 (56.8%)
		PLEX	6 (16.2%)
		ACAN	2 (5.4%)
	CYS		8 (21.6%)

SOL, solid; FOL, follicular; PLEX, plexiform; ACAN, acanthomatous; CYS, cystic

Areas with acanthomatous differentiation showed selective antibody positivity as well. Stromal expression was noticed in a diffuse manner, accentuated at vascular endothelial cells, fibroblasts and focal inflammatory cells (figures1: A, B, C and D).

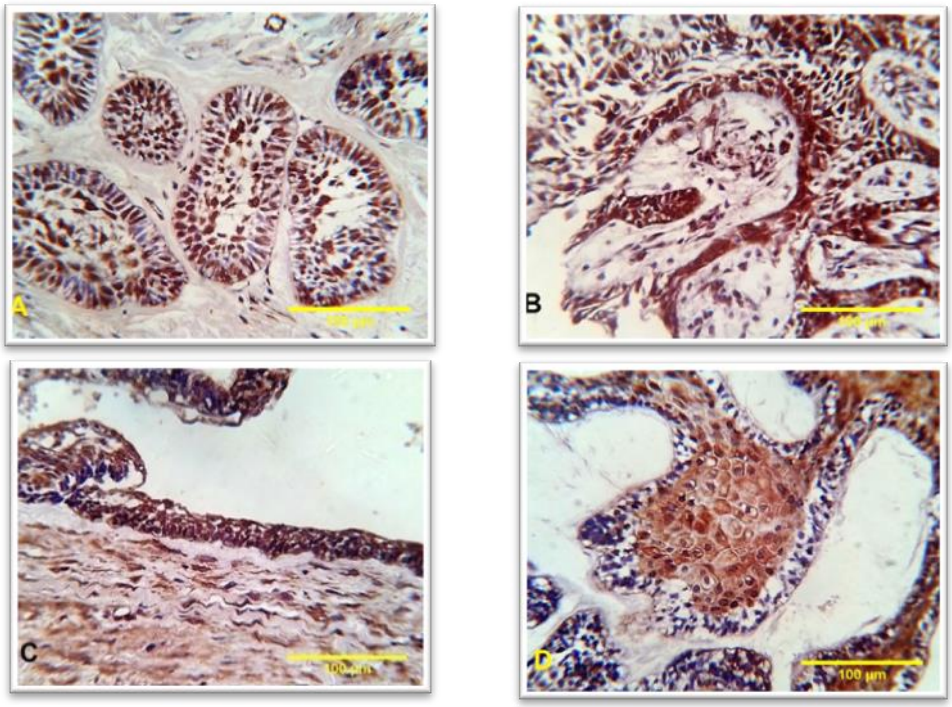

Figure 1: High power photomicrograph depicting immonohistochemical expression of endocan in follicular (A), plexiform (B), cystic (C) and acanthomatous (D) ameloblastomas (X40; scale bar = 100 *um*).

As it is detailed in tables 2 and 3; scored endocan tumor epithelial and stromal expression showed no significant correlation with age. Maxillary cases had a notably higher stromal expression than mandibular cases that did not reach statistically significant level. Mean tumor epithelial expression in males was higher than females and barely missed significance whereas stromal expression showed an opposite non-significantly higher expression in females. Though statistically non-significant; Cystic AB showed a higher expression in mural (n=1), luminal (n=5) and intraluminal (n=2) areas than solid subtypes in general with respect to tumor epithelial and stromal parts (p=0.77; p=0.09 respectively). Within solid AB, the highest mean expression values were found in plexiform

subtype for tumor epithelial part and in the follicular subtype for the stromal part.

Table 2: Mean scores of endocan expression in epithelial parts of ameloblastoma against age, sex, site and subtype

			Tumor Epithelium (N=37)						
			-	+	++	+++	*Mean score (±SD)*		*p*
Age (Mean±SD)			0	24.0 (±10.39)	34.71 (±11.31)	36.52 (±17.67)	35.16(±16.26)		0.461€
Sex	**M**		0	0	1	11	2.92(±0.289)		0.073¥
	F		0	3	6	16	2.52(0.714)		
Site	**Max.**		0	0	2	2	2.5(±0.577)		0.378¥
	Mand.		0	3	5	25	2.67(±0.645)		
SUB	**SOL**	**FOL**	0	3	3	15	2.57(±0.746)	2.62(±0.677)	(Among all)=0.824Ɨ (SOL Vs CYS)=0.776¥
		PLEX	0	0	1	5	2.83(±0.408)		
		ACAN	0	0	1	1	2.5(±0.707)		
	CYS		0	0	2	6	2.75(±0.463)		
Total			0	3	7	27	2.65(±0.63)		

Table 3: Mean scores of endocan expression in stromal parts of ameloblastoma against age, sex, site and subtype

			Stroma (N=37)						
			-	+	++	+++	*Mean score(±SD)*		*p*
Age(Mean±SD)			25	21.33 (±3.51)	33.4 (±13.03)	38.17 (±17.91)	35.16(±16.26)		0.336€
Sex	**M**		1	2	1	8	2.33(±1.07)		0.896¥
	F		0	1	9	15	2.56(±0.58)		
Site	**Max.**		0	0	0	4	3.0(±0.0)		0.111¥
	Mand.		1	3	10	19	2.42(±0.792)		
SUB	**SOL**	**FOL**	0	2	6	13	2.52(±0.680)	2.38(±0.82)	(Among all)=0.157Ɨ (SOL Vs CYS)=0.09¥
		PLEX	1	1	2	2	1.83(±1.169)		
		ACAN	0	0	1	1	2.5(±0.707)		
	CYS		0	0	1	7	2.88(±0.354)		
Total			1	3	10	23	2.49(±0.77)		

SUB, subtype; FOL, follicular; PLEX, plexiform; ACAN, acanthomatous; CYS, cystic; €, ANOVA test; ¥, Mann-Whitney test; Ɨ, Kruskal-Wallis test.

DISCUSSION

Despite it has been characterized to tag vascular endothelial cells [4,5]; endocan is known to be expressed in multiple tissue components including epithelial cells of gastrointestinal tract, renal tubules, respiratory alveoli and epithelia of normal skin and adnexal structures [6]. In this study, it was shown to be expressed in a variable extent within tumor epithelial islands at both peripheral ameloblast like cells and inner stellate reticulum like cells denoting a harmonious endocan immunoprofile within the tumor islands' various locations, however; a slightly increased selective expression was noted at epithelial cells with acanthomatous differentiation, although it did not substantially affect the overall expression values of acanthomatous subtype; such pattern of expression marks an intricate presence of the antigen among subsets of tumor epithelial cells with variable differentiation.

An earlier study by Zhang et al.[6] suggested that endocan expression is associated with neogenesis or in tissue parts that are in a non-quiescent state, an observation that is substantiated by several studies that demonstrated endocan as a soluble circulating marker for aggressiveness in disease processes and outcome of neoplastic conditions [5,7-9]. Tumor epithelial expression of endocan in AB found in this study does not depart from this general notion since that AB is a relentless tumor with a capacity for growth, invasion and a remarkable recurrence potential [3].

Although it is reported to harbor a vascular stroma [11]; plexiform subtype of AB had a lower stromal endocan score than other subtypes in this study, however; it had the highest tumor epithelial scores which could denote a trend toward aggressive behavior aside from its stromal components. Nevertheless; the small number of

each subtype within this study sample precludes a conclusive result in this context. Another notable finding is that cystic AB had a relatively high mean score when compared to the collective mean of solid subtypes; it actually approached significant levels of difference in stromal expression, keeping in mind that cystic ameloblastomas are much less aggressive than solid ones [11,12], this expression pattern may point to either an inverse relation to the outcome or that stromal expression of endocan is of no value in AB, nevertheless; these presumptions that to be accentuated in a more detailed studies.

Finally, to the best of the authors' knowledge; no previous studies were found that addresses the expression of endocan in ameloblastomas. This study showed the presence of endocan antigen in AB epithelial and stromal elements in addition to its potential for behavioral discrimination which would need further clarification.

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