The expression of BMI-1 in ameloblastoma and dentigerous cyst

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Abstract:

Objective: To check for a possible relation between the expression of BMI-1 among two different odontogenic lesions; ameloblastoma and dentigerous cyst known for different biological behavior. **Materials and Methods:** The study was conducted on 14 formalin-fixed paraffin-embedded blocks (ameloblastoma (Am) group: seven blocks and dentigerous cyst (DC) group: seven blocks). These blocks were collected from archived files of the Department of Oral Pathology, Faculty of Dentistry, Mansoura University. Immunohistochemically (IHC), the BMI-1 polyclonal antibody expression in the retrieved sections was investigated. A computerized method was used to compare the IHC staining intensity between both groups. **Results:** The difference in the intensity of immunoreactivity to BMI-1 in two different studied groups was statistically non-significant to gender, age, and site ($P > 0.05$). The Am group showed significantly higher expression of BMI-1 than the DC group ($P < 0.0001$). **Conclusions:** BMI-1 is thought of as a useful marker probably delineating the variation in the biological behavior of Am and DC.

Introduction:

Ameloblastoma (Am) is the most common intra-osseous slowly growing odontogenic tumor.[1] Although Am is categorized as a benign lesion, yet, it is locally destructive, and aggressive with a high recurrence rate (50-90%) and sometimes causes distant metastasis. Ameloblastoma has an insidious growth pattern as it tends to infiltrate between intact cancellous bone at the periphery of the lesion before bone resorption occurs.2 Due to this insidious growth pattern of Am, it is difficult to predict its clinical course which in turn raised a couple of questions such as; whether cancer stem-like cells are present in Am and if its presence plays a role in aggressiveness and recurrence rate of tumor?[2,3]

A dentigerous cyst (DC) is the most common type of developmental odontogenic cyst with an indolent behavior and low recurrence rate.[4,5] These cysts are usually asymptomatic and are detected by routine radiographic examination except in large cases.[6] Larger cysts make great resorption of bone leading to thinning of cortex and eggshell cracking on palpation.[7] Pathological fractures may be seen in larger cysts.[7] Variation in biological behavior has been reported to depend upon certain factors among which is the cancer stem cells.[8] BMI-1 (B lymphoma-specific Moloney murine leukemia virus integration site-1) is an essential stem cell-related gene that plays a pivotal role in myriad cellular processes including cell cycle regulation, senescence, DNA damage response, angiogenesis, differentiation, and self-renewal.[9]

Materials and Methods:

Tissue samples:

This study was a retrospectively study done on 14 formalin-fixed and paraffin-embedded tissue blocks of Am (n=seven), and DC (n=seven). These blocks were collected from archived files of the Department of Oral Pathology, Faculty of Dentistry, Mansoura University. Hematoxylin and eosin staining for the tissue specimens was done to verify the diagnosis. Sample size calculation was performed using G*Power version 3.1.9.2, University Kiel, Germany. Copyright (c) 1992-2014. 10 The effect size d was 1.50 using an alpha (α) level of 0.05 and Beta (β) level of 0.05, i.e., power = 80%; the estimated sample size (n) should be at least 14 samples. A total sample size of 14 was required for both groups (Am and DC) that was represented by 7 samples in each group.

Immunohistochemical markers Universal Kit:

Polyclonal antibody BMI-1 (B lymphoma Mo-MLV insertion region 1 homolog): Rabbit IgG in phosphate-buffered saline, pH 7.4, 150 Mn NaCl, 0.02% sodium azide, and 50% glycerol. BMI-1 was obtained from Chongqing Biospes Co., Ltd. (Cat#: YPA2375. Lot# Y14/2021Y), China. The optimal dilution for immunohistochemical staining was determined by the investigator to be (1:25).

Sample preparation:

The available clinical data of studied cases were collected from the patients' medical reports with emphasis on age, sex, lesion site, and size. Four micrometers thick tissue sections were cut from the paraffin blocks for immunostaining. Sections were then mounted on Opti plus slides obtained from Bio GEMEX laboratory (4600 Canyon Road, San Ramon, USA, 2011). These slides are electrically charged to allow adhesion between the tissue sections and the slide surfaces. Immunostaining was performed using the Avidin-Biotin complex method according to the manufacturer's instructions (Biospes, Chongqing, China). The slides were deparaffinized by immersion in Xylene for 15 minutes then rehydration in descending grades of alcohol and then finally washing in water. Blocking the endogenous peroxidase activity by treatment of the sections with 0.5% H2O2 in methanol for 30 minutes then washing in phosphate buffer saline (PBS) for 5 minutes. Pretreatment of the tissue sections by immersing in 10 milli Molar boiling citrate buffer,

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pH 6.0 for 15 minutes, cooled at room temperature for 20 minutes, and then washed with distilled water. Followed by incubation of the slides in a solution of protease XIV 50 mg in 100 ml of 0.1 M, PH 7.4 PBS (pre-warmed to 37˚c) for 15 minutes at 37 c˚ then washed 3 times in PBS for 5 minutes to digest the proteolytic enzyme activity. Blocking of non-specific binding of antibody was done by incubating the slides in 4.0 % mouse serum for 30 minutes at room temperature. The primary antibody was applied, and the slides were incubated with the primary antibody overnight at room temperature then the sections were washed three times in PBS for five minutes. Avidin-biotin complex peroxidase solution was applied according to the manufacturer's instructions. Sections were incubated for 30 minutes at room temperature and then washed three times in PBS for five minutes. Application of chromogen for development of colored reaction product was done by using 3.3' diaminobenzidine-4HCL 1mg/ml in PBS supplemented with H2O2 (Sigma chemical Co. St. Louis, Missouri, USA) (10 µl of 50% H2O2 in 5 ml PBS). The chromogen yielded a brown reaction end product at the site of the target antigen.

Immunostaining evaluation

The immunoreactivity of BMI-1 was evaluated by digital morphometrics using computer-assisted digital image analysis. The resulting images were analyzed on an Intel® core I7® based computer using Fiji ImageJ (version 1.51r; NIH, Maryland, USA) software. To evaluate the immunoreactivity of BMI-1, the staining intensity quantification using the IHC profiler plugin for color deconvolution was performed by ImageJ.[11] The intensity of the immunostaining was measured in a deconvoluted DAB image. The regions of interest were selected manually to represent all examined samples, and staining intensity was measured as the "mean gray value". The average staining intensities for all measured regions from five fields of vision were calculated for each sample. The measured data were exported to an excel sheet. In ImageJ, the pixel intensity values for any color range from 0 to 255, wherein 0 represents the darkest shade and 255 represents the lightest shade of the color. Based on this, the staining intensities of the samples were divided into four groups; negative, weak, moderate, and strong according to thresholds established by creators of the IHC profiler plugin [11] as follows: strong for measured intensities ranging from 0 to 60, moderate for intensities ranging from 61 to 120, weak for intensities ranging from 121 to 180, and negative for intensities higher than 181.

Statistical analysis:

Data were presented as the means ± standard deviation (SD) of all the examined sections with comparable results. T-test was used to assess the differences between the two groups. The Chi-square test was used to evaluate the variation of qualitative data among the groups. The P-values < 0.05 were considered statistically significant and P < 0.01 were considered highly statistically significant. GraphPad Prism 8 software was used for the statistical analysis (GraphPad Software, San Diego, Canada)

Results:

The mean age of Am group was higher (42.0 ±21.7 years) with an age range from 15 to 76 years while the mean age in DC cases was (23.57 ±13.3 years) with an age ranging from 12 to 52 years. In both groups, male to female ratio was 5:2. The mandible was the most frequent site of involvement in Am (100%) and dentigerous cyst (71.4). Statistical analysis showed a non-significant difference between groups for age, gender, and sites at P <0.05, (Table 1).

In both the follicular and plexiform types of Am, the immunoreactivity for the BMI-1 was encountered predominantly in the nuclei and cytoplasm of the peripheral cells, (Figure 1). Some of the fibroblasts in the connective tissue were positively reactive to the BMI-1, (Figure 1).

The Immunohistochemical expression of BMI-1 in DC was detected mostly in the basal and suprabasal cells of the epithelial lining mainly as a cytoplasmic brown dot with occasional intranuclear expression, (Figure 2). Some fibroblasts in the connective tissue showed positive intranuclear reactions, (Figure 2).

Comparison of the immunoreactivity of BMI-1 in both groups by using the computerized analysis: The moderate staining intensity was measured in Am (88.87±10.74), while the lightest shade (weak) was measured in DC (126.26±12.41). There were significant differences in the IHC intensity between groups at a P-value< 0.0001, (Table 2).

Table 1: Demographic data of the studied group

	Ameloblastoma (N=7)	Dentigerous Cyst (N=7)	P-values
Gender N (%)			
Male	5 (71.4%)	5 (71.4%)	0.838
female	2 (28.6%)	2 (28.6%)	
Age (years)			
Mean± SD	42.0±21.7	23.57±13.3	0.075
Min-max	15-76	12-52	
Site N (%)			
Mandible	7 (100.0%)	5 (71.4%)	0.79
Maxilla	0 (0.0%)	2 (28.6%)	

Table 2: BMI-1 expression; Intensity quantification

Groups	Mean ± SD	t-test	P-value
Ameloblastoma	88.87±10.74	86.67	<0.0001**
Dentigerous Cyst	126.26±12.41		

**P: significant difference between groups at < 0.05

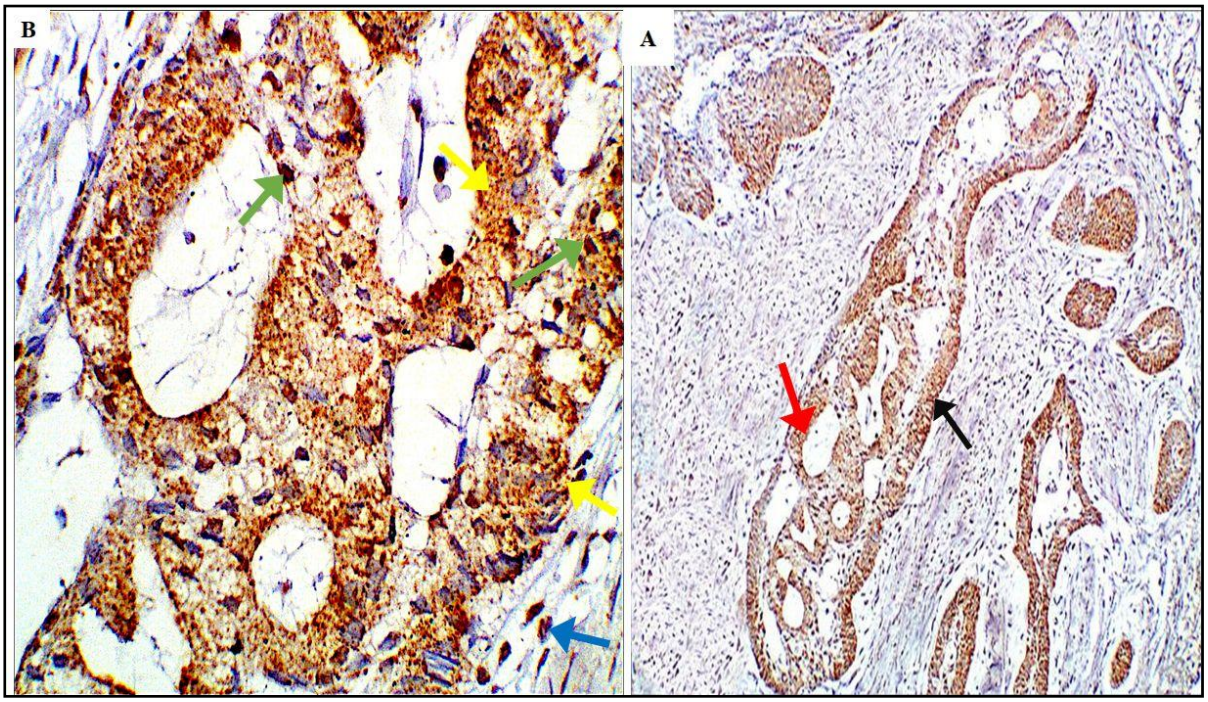

Figure 1: (A) Photomicrograph showing immunohistochemical expression of BMI-1 in follicular Am in both peripheral (black arrow) and central cells (red arrow) (BMI-1 ×200). (B) Higher magnification of (A) showing prominent cytoplasmic BMI-1 immunohistochemical expression (yellow arrow) together with nuclear staining (green arrow) in both peripheral and stellate reticulum-like cells in follicular Am. The connective tissue cells of the stroma show intranuclear reactivity (blue arrow) (BMI-1 ×400).

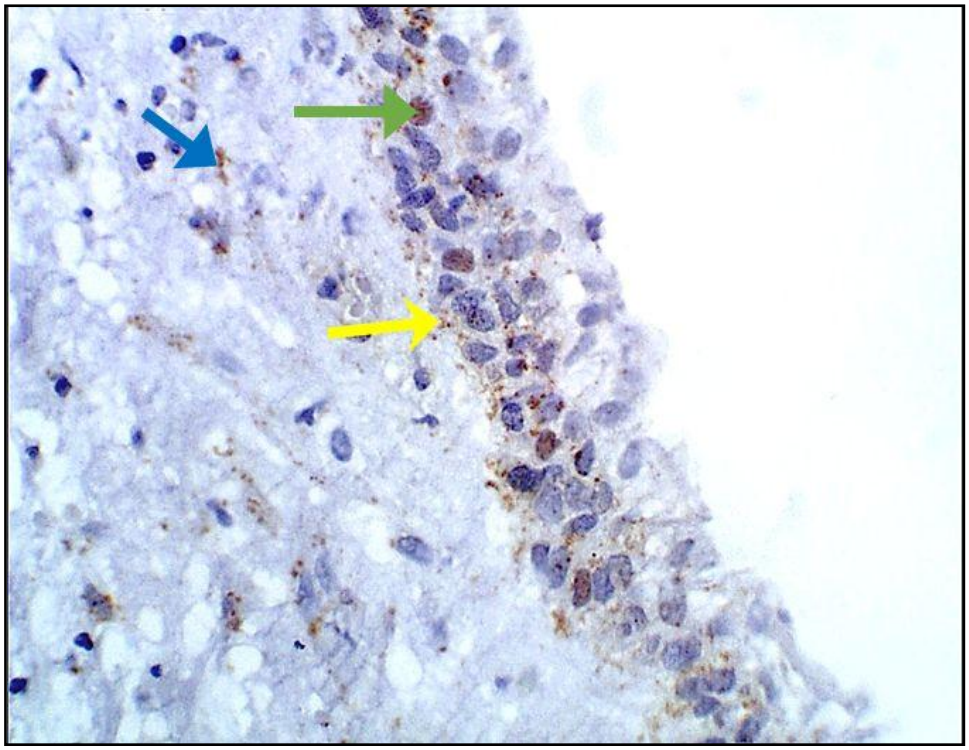

Figure 2: Photomicrograph of BMI-1 immunohistochemical expression showing cytoplasmic (yellow arrow) with occasional intranuclear expression staining (green arrow) in the basal and suprabasal cell layer of DC and some fibroblasts reaction (blue arrow) (BMI-1×400).

Discussion:

In the Am, BMI-1 was predominantly detected in the peripheral ameloblast-like cells of the tumor islands or strands while the central stellate reticulum-like cells showed less reaction. Our results are in agreement with the results of Kumamoto and Ohki[12] and Lapthanasupkul et al [13] who reported a similar pattern of BMI-1 expression in Am. The same distribution was described in previous studies employing ki-67 which is a known proliferation marker reported to be present during all active phases of the cell cycle and absent in the quiescent cells.[14–16] The currently observed localization stands in a line with previous studies suggesting that the peripheral cells might have an important role in the proliferative activity of Am neoplasm. Further support to this notion is provided by studies that reported that mutated p53; a tumor suppressor gene was mostly located in the peripheral columnar cells.[3,17,18] It was reported that BMI-1 inactivates p16INK4A and/or p14ARFp53 tumor suppressor pathways which result in promoting cell proliferation.[19]

In the present study, immunoreactivity for BMI-1 among the DC group was detected as scarce faintly stained cells in the basal and parabasal layers. The current result agrees with other studies employing the ki-67 which showed weak reactivity in the basal and suprabasal cells of the epithelial lining of DC.[16,20] This reflects the low activity in the proliferative component of the lining and consequently the known indolent course of DC. [3,5,16,21,22]

An original finding in our research is the dual staining pattern, nuclear and cytoplasmic, with a predominance of the former pattern in the Am group of neoplastic nature. The cytoplasmic immunoreaction in the current study is justified by the study which proved the presence of BMI-1 in the inner mitochondrial membrane. 23 The cytoplasmic localization partially contradicts the consensus about the sole nuclear localization of BMI-1 in the neoplastic and the potentially malignant lesions showing high proliferative activity reported in previous studies.[24–26] The stromal fibroblasts in both groups of the present study showed strong intra-nuclear immunoreactivity to the BMI-1. This is in agreement with what was previously reported that BMI-1 has been shown to immortalize normal fibroblasts via reactivation of the human telomerase reverse transcriptase gene in these cells.[27]

Conclusions:

The stronger expression in the peripheral cells in Am, basal and parabasal layers in DC compared to the stellate reticulum-like cells as well as the superficial cells points to BMI-1 as a major proliferation player in the former cellular component and a mild differentiation role in the latter ones. BMI-1 is thought of as a useful marker probably delineating the aggressiveness of odontogenic lesions. Studies on a higher number of cases are required to confirm the observed distribution of the BMI-1 expression in Am and DC.

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