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SCIENTIFIC ARTICLES

Ki-67 Expression in Dentigerous Cysts, Unicystic Ameloblastomas, and Ameloblastomas arising from Dental Cysts

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This study investigated whether or not an ameloblastoma developing in the wall of a dentigerous cyst is a distinct lesion from the unicystic ameloblastoma. An immunohistochemical evaluation of Ki-67 in dentigerous cysts, unicystic ameloblastomas, and ameloblastomas arising in dentigerous cysts was done. The values of Ki-67 positivity were 3.14 for the dentigerous cyst, between 5.32 and 16.56 for unicystic ameloblastoma, and 11.77 for ameloblastoma arising in a dentigerous cyst. Statistically significant differences were found between the dentigerous cyst and the unicystic ameloblastoma and between the dentigerous cyst and the ameloblastoma arising from a dentigerous cyst. No statistically significant difference was present between unicystic ameloblastoma and ameloblastoma arising from dentigerous cyst. These immunohistochemical data confirm the hypothesis that an ameloblastoma arising from a dentigerous cyst has a similar biological behavior to the unicystic ameloblastoma and should be considered as merely a histologic variant.

Robinson and Martinez (1) first described the unicystic ameloblastoma in 1977. It has also been termed mural, intracystic, cystic, and plexiform unicystic ameloblastoma (2). Some investigators believe that a unicystic ameloblastoma arises from a preexisting odontogenic cyst, whereas others think that it is a cystic neoplasm originating de novo (2). The epithelial lining of odontogenic cysts is of ectodermal origin and seems to originate from dental lamina remnants (3). The malignant transformation of jaw cysts seems to be extremely rare (3–5); however, the odontogenic epithelial lining can give rise to odontogenic or nonodontogenic tumors (5–7). Epithelial overgrowth may frequently occur in the wall of odontogenic cysts, resulting from chronic inflammation in these lesions (8). Some researchers believe that 5% to 30% of ameloblastomas originate from dental cysts (3), but this hypothesis is disputed by Shear (9), who did not find evidence to support it. In fact, dentigerous cysts are rarer in South African blacks than in Caucasians, whereas to the contrary, ameloblastomas are much more common in blacks (9). The walls of dentigerous cysts may present with a proliferation similar to an ameloblastoma, but lack the ameloblast-like appearance of the peripheral cells of the true ameloblastoma follicles (8). However, it has been emphasized that the two lesions are histologically distinct entities (8). Ackermann et al. (2) divided unicystic ameloblastoma into three groups:

1. Group 1: cysts lined by variable, often nondescript epithelium with no infiltration into the fibrous cyst wall.
2. Group 2: cysts showing intraluminal plexiform epithelial proliferation with no infiltration.
3. Group 3: cysts with invasion of the epithelium into the cyst wall in either (a) a follicular or (b) a plexiform pattern.

Ki-67 antigen expression has been observed in the nuclei of proliferating cells, and it can be a marker to estimate the state of tissue growth. It has been reported that Ki-67 antigen expression increases in preneoplastic and neoplastic lesions of the oral mucosa and in all states of high cell turnover (10–14). MIB-1 is the monoclonal antibody that reacts with the epitope of the Ki-67 nuclear antigen in formalin-fixed, paraffin-embedded sections (10–14). The aim of this study was to evaluate immunohistochemically the presence of Ki-67 in dentigerous cysts, unicystic ameloblastomas, and ameloblastomas arising from dentigerous cysts using the MIB-1 antibody.

MATERIALS AND METHODS

Eight dentigerous cysts, five unicystic ameloblastomas, and three ameloblastomas arising in dentigerous cysts were studied. The unicystic ameloblastomas were of type 3a (two cases) and 3b (three cases), according to classification used by Ackermann et al. (2). The age of the 16 patients ranged from 23 to 57 yr (mean, 35 yr). All specimens had been routinely fixed in 10% neutral buff-

TABLE 1. Percentage of Ki-67-positive nuclei in all groups

	No. of Cases	Measured Zone	Mean ± SD	Median	Range
Dentigerous cyst	8		3.14 ± 1.18	2.95	1.70–5.20
Unicystic ameloblastoma	5	Cystic lining	5.32 ± 0.86	5.20	4.30–6.50
		Intraluminal nodule	14.18 ± 1.06	14.50	12.60–15.30
		Infiltrating island	16.56 ± 0.71	16.50	15.60–17.30
Ameloblastoma in dentigerous cyst	3		11.77 ± 1.83	12.40	9.70–13.20

ered formalin (24 to 48 h), dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin. The hematoxylin-eosin-stained slides were all reviewed, the diagnoses confirmed, and slides for the quantitative evaluation were selected.

For MIB-1 immunostaining, the slides were pretreated with 3-aminopropyltriethoxysilane (APES; Sigma, Milan, Italy), which avoided the separation of the section from the slide during incubation in the microwave oven. For each case, a 5-micron section was cut and placed on a pretreated slide. The staining protocol of this slide consisted in the application of a series of reagents in the following manner:

- overnight drying at 37° C;
- dewaxing and rehydration;
- immersion in a plastic box containing 0.01 M citrate buffer at pH 6.0;
- incubation for 5 min in a microwave oven, initially at 750 watts until boiling began and then at 350 watts for the remaining time;
- incubation for 5 min in a microwave oven at 350 watts;
- cooling for 20 min at room temperature;
- washing in running water and then in distilled water for 5 min;
- washing in Tris-buffer saline (TBS) for 5 min;
- removal of any excess TBS;
- addition of primary monoclonal mouse anti-Ki-67 antibody (Immunotech, Marseilles, France) diluted 1:25 in TBS;
- overnight incubation at 4°C in a humidified room;
- washing in TBS for 5 min (3 times);
- addition of secondary prediluted biotinylated anti-mouse antibody (LSAB-Dako, Copenhagen, Denmark) and incubation for 10 min at room temperature;
- washing in TBS for 5 min (3 times);
- addition of prediluted streptavidin-peroxidase complex (LSAB-Dako) and incubation for 10 min at room temperature;
- washing in TBS for 5 min (3 times);
- immersion in 0.05% DAB and 0.01% H_2O_2 in TBS for 2 to 3 min at room temperature;
- washing in running water and then in distilled water for 5 min;
- counterstaining with ethyl green for 30 min;
- washing in distilled water for 30 s;
- washing in butanol I for 5 s;
- washing in butanol II for 30 s;
- dehydration and mounting in Permount.

The positivity to MIB-1 was evaluated by counting the number of positive cells in 1000 cells, and the values were expressed as a percentage. Descriptive statistical analysis was performed for each group of lesions, and the Mann-Whitney *U* and Kruskal-Wallis tests were used to test for statistically significant differences between the groups ($p < 0.05$).

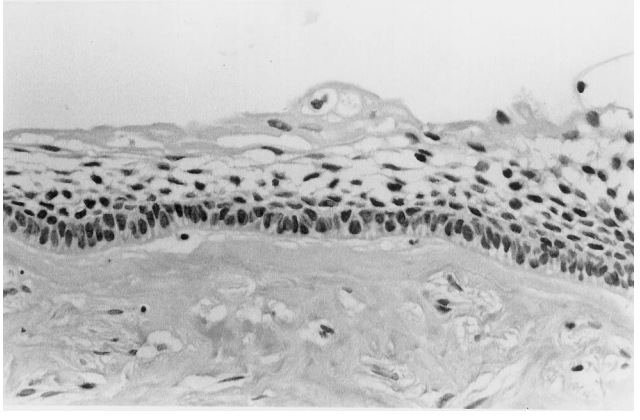

FIG 1. Unicystic ameloblastoma showing ameloblastoma-like epithelium lining the cystic component of the tumor (H & E × 200).

RESULTS

The percentage of Ki-67 positive nuclei was 3.14 ± 1.18 in dentigerous cysts, 5.32 ± 0.86 in the lining of unicystic ameloblastomas, 14.18 ± 1.06 in the neoplastic nodules containing ameloblastomatous epithelium and projecting into the lumen of the cyst, and 16.56 ± 0.71 in the infiltrating islands of unicystic ameloblastoma. The value for ameloblastoma arising in dentigerous cysts was 11.77 (±1.83) (Table 1). Statistically significant differences were found between the expression of Ki-67 in dentigerous cysts and unicystic ameloblastomas ($p = 0.0034$) and its expression in dentigerous cysts and ameloblastomas arising in a dentigerous cyst ($p = 0.0143$). In unicystic ameloblastomas, the expression of Ki-67 nuclear antigen was lowest in the cystic lining and progressively increased in the intraluminal nodules and in the infiltrating islands of the cyst wall; the difference in incidence at these locations was statistically significant ($p = 0.0019$). No statistically significant differences were found between the Ki-67 values of unicystic ameloblastoma and ameloblastoma arising in a dentigerous cyst. (Figs. 1 to 4.)

DISCUSSION

Ameloblastomas are tumors composed of odontogenic epithelium that, from a theoretical point of view, can arise from the epithelial lining of a dentigerous cyst (9). However, Shear (9) does not believe that ameloblastomas commonly arise in such situations, and therefore, dentigerous cysts need not be regarded as preamelo-

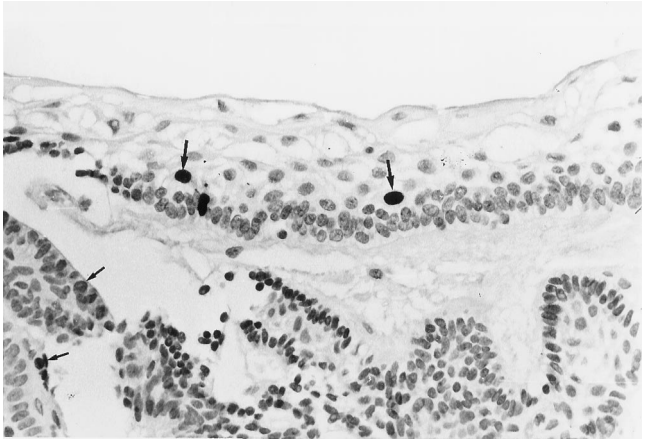

FIG 2. Unicystic ameloblastoma showing strong positivity of cells located in the cyst wall (*small arrows*) and in the epithelial lining to MIB-1 (*large arrows*) (H & E × 200).

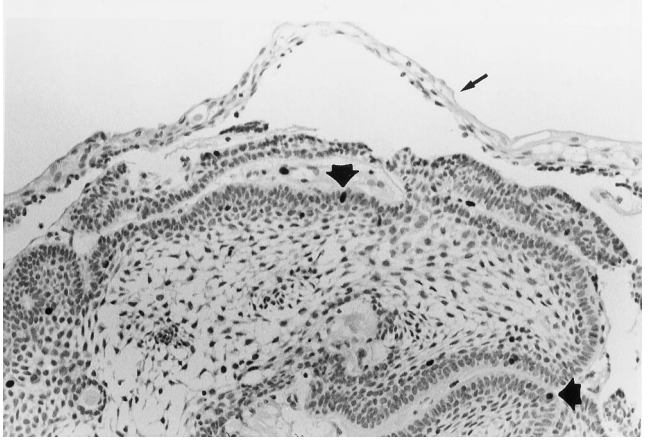

FIG 3. Dentigerous cyst showing the lining epithelium (*small arrow*) composed of 3 to 4 cell layers without ameloblastoma-like differentiation, typical of dentigerous cysts. There is strong positivity to MIB-1 of cells (*large arrows*) located in the ameloblastomatous proliferation of the cyst wall (H & E × 100).

blastomatous and treated with caution. On the other hand, in some cases, small mural thickenings of the cyst wall of dentigerous cysts may contain proliferating odontogenic epithelium, and these strands also may show follicular enlargements similar to an ameloblastoma (4). Such lesions have been called proliferations of hyperplastic epithelium in the wall of a cyst, pseudo-ameloblastomatous change in the wall of a cyst, ameloblastoma-like tissue (ameloblastoid cyst) (15), or plexiform unicystic ameloblastoma (16). The problem of differentiating between plexiform ameloblastoma and nonneoplastic proliferation in a cyst could be of considerable clinical importance because of treatment implications. Vickers and Gorlin (17) listed three features that, when observed together, justified the diagnosis of ameloblastoma in a cyst of the jaw:

1. Hyperchromatism of the basal cell nuclei of the epithelium lining the cystic cavity.
2. Palisading of the basal cells and polarization of the nuclei of the basal cells lining the cyst cavity.
3. Cytoplasmic vacuolization of these basal cells.

Our immunohistochemical data led to the conclusion that unicystic ameloblastoma and ameloblastoma arising from a dental

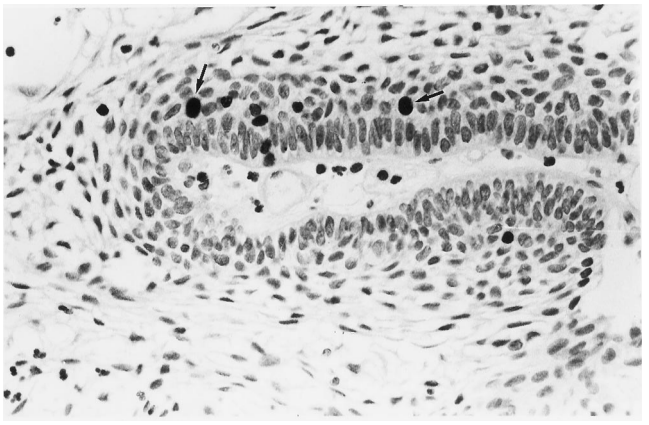

FIG 4. Dentigerous cyst showing strong positivity to MIB-1 of cells (*arrows*) located in the ameloblastomatous proliferation (H & E × 200).

cyst are both low-grade lesions with a low proliferation index. In all our cases, the ameloblastomas arising from dentigerous cysts were always very small lesions and were incidental findings during microscopic analysis. In a previous immunohistochemical study (18), comparing the expression of proliferating cell nuclear antigen (PCNA) in different types of cysts and ameloblastomas, we found that the value for unicystic ameloblastoma was 28.18 ± 3.84%, whereas for all types of ameloblastoma the value was 38.17 ± 9.70%, and for recurrent ameloblastomas it was 54.58 ± 3.32%. Moreover, the PCNA positivity of unicystic ameloblastoma was statistically significantly lower than the positivity of acanthomatous and plexiform ameloblastomas. Also, in contrast to what occurs in ameloblastomas arising in dentigerous cysts, a different percentage of positive cells were found in the different areas of the unicystic ameloblastoma. In conclusion, our data confirm the hypothesis of Gardner and Corio (16) that the biologic behavior of the plexiform unicystic ameloblastoma is the same as that of other types of unicystic ameloblastoma and should be considered simply as a histologic variant. The importance of this lesion lies in the fact that it should probably be recognized as an ameloblastoma and not as an hyperplastic epithelial proliferation, even if it lacks the criteria already described (17).

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