Original Research Article

Study of Ki67 in odontogenic benign tumors

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ABSTRACT

The aim of the present study was to investigate the Ki67 expression level and to measure the cell proliferation index (Ki67) in odontogenic benign tumors.

Materials and Methods: This was an analytical cross-sectional study of odontogenic benign tumors. The sampling was non-probabilistic with an exhaustive recruitment including all biopsy or surgical excision specimen of odontogenic benign tumors. Ki67 immunohistochemistry was performed on histological sections of paraffin-fixed tissues of 3μ thickness with the Ki67 (MM1) monoclonal antibody. The studied variables were sociodemographic, clinical and histopathological. The data have been analysed with SPSS 20.0 software.

Results: Ameloblastoma represented 50.9% of cases and cemento-osseous dysplasia 36.4%. Among the 28 ameloblastoma cases, the 15 (53.6%) were plexiform type, the 7 (25%) follicular type and the 6 (21.4%) unicystic type. The percentage of Ki67 labelled cells was less than 1% in 74.6% (n=41) of the tumors and was equal to 5% in 12.7% (n=7) of cases. A Ki67 law labelling was found in 87.3% (n=48) of tumours and a negative labelling in 12.7% (n=7) of cases.

Conclusion: The odontogenic benign tumors had a weak Ki67 expression level and a low epithelial cell proliferation index. The Ki67 could not therefore be used as a predictive biomarker of tumor cell proliferation and the risk of recurrence in odontogenic benign tumors.

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1. Introduction

The odontogenic benign tumors are lesions that are developed from the odontogenesis cellular rests.[1] They represent around 9% of all the oral cavity tumors[2] and more than 75% of benign tumors affecting the maxillomandibular bone bases.[3,4] These are slowly evolving lesions with different histological types and variable clinical expressions.[1] Some of these lesions such as ameloblastomas and odontogenic keratocysts are locally aggressive with a recurrence potential after surgical removal.[1,2] This recurrence could be associated with biological phenomena like key genes alterations in odontogenic lesions.[4,5]

These key genes alterations will lead to the production of necessary molecules for the growth of tumor cells via their cellular receptors.[6]

Several cellular biological markers allow to better appreciate the clinical characteristics of odontogenic tumor lesions. Among these markers, the (Ki67) cell proliferation index is a biological marker for assessing the proliferative capacity of tumor cells.[7–10] In fact, the Ki67 is a nuclear protein expressed during the G1, S, G2 and M phases of the

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cell cycle and not during the G0 quiescence phase.

It is a prognostic biological marker whose study could help understand the behaviour of certain odontogenic benign tumors that have a high recurrence rate after surgical removal.[4,11–13] Its level of expression, evaluated by the percentage of tumor cells labelled with an anti-Ki67 antibody, would be directly correlated to the mitotic index and to the proliferation of tumor lesion cells, allowing then to measure the proliferative index.[14]

Furthermore, the Ki67 could also be used as indicator of the aggressiveness of odontogenic benign lesions.[15] It is in this context that we initiated this study with the objective of studying the expression level of Ki67 and measuring the proliferation index in odontogenic benign tumors.

2. Materials and Methods

It is a cross-sectional study with an analytical focus on odontogenic benign tumors. The study lasted two years and seven months from the beginning of January 2020 to the end of July 2022. The study has been approved by the Cheikh Anta Diop University ethics committee of Dakar, Senegal (Ref.:CER/UCAD/AD/MSN/046/2020).

The sampling was non-probabilistic with an exhaustive recruitment of all the surgical parts of odontogenic benign tumors from two dentistry departments.

The histological study has been realized at the anatomical pathology department of Fann's Univerity National Hospital Center (UNHC) in Dakar.

The Ki67 immunohistochemical study on histological sections of paraffin-fixed tissues of 3μ thickness has been realized at the pathology anatomy department of Cheikh Anta Diop University of Dakar.

The inclusion criteria were all the surgical specimen of odontogenic benign tumors whose biopsy or surgical excision specimen were available.

The non-inclusion criteria were all the surgical specimen of odontogenic benign tumors from smokers, alcoholics, alcohol-smokers, undergoing chemotherapy or radiotherapy patients. The studied variables were sociodemographic (sex and age), clinical (duration of evolution and location of lesions) and histopathological (histological type and Ki67 expression).

The Ki67 monoclonal antibody (MM1; Leica BIOSYSTEMS; Germany) had allowed to identify the fraction of proliferating cells in order to assess the effective activity of cell multiplication and to determine the tumor proliferation index.

To quantify the Ki67 labelling of epithelial cells, the following ranges had been proposed :

1. Negative if lack of labelling
2. Weak: $0 < Ki67 \leq 7\%$.
3. Moderate: $8 \geq Ki67 \leq 15\%$.
4. Intense: $Ki67 > 15\%$.[11,12]

The proliferation index is obtained by doing the ratio of the calculation of the tumor cells number expressing the Ki67 out of the total number of tumor cells in a tissue tumour area.

The data have been analysed with SPSS20.0 software with an univariate analysis performed for sociodemographic, clinical, paraclinical and therapeutic variables.

A bivariate analysis had been performed between Ki67 labelling and the other studied variables with a Chi2 test realized for a significance level of 5%.

3. Results

A total of 55 surgical specimen of odontogenic benign tumors that filled the inclusion criteria has been obtained.
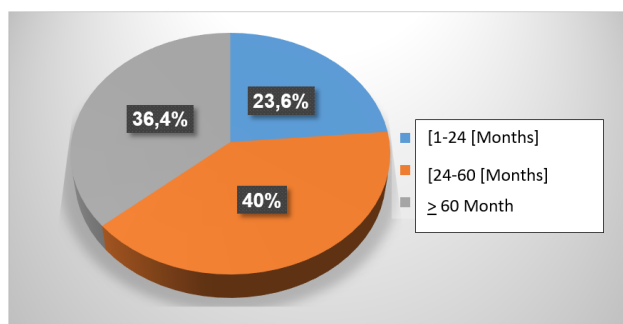
3.1. Demographic aspects

Women represented 63.6% (n=35) with a sex ratio of 0.57. The average age was 40 years with quartiles of 25 and 57 years.

3.2. Clinical aspects

The evolution average duration was 46.3 ± 34.2 months with extremes of 6 and 144 months.

An evolution duration range of 24 to 59 months was found in 40% (n=22) of patients and that of 60 months and more in 36.4% (n=20) (Figure 1).

Fig. 1: Distribution of the population according to evolution duration range

The lesions were located in the mandible in 89.1% (n=49) of patients and in the maxilla in 10.9% (n=6).

Radiographically, the images were radiodense in 49.1% (n=27) of cases and multilocular radiolucent in 29.1% (n=16) of the patients (Figure 2).

3.3. Histopathological aspects

Ameloblastoma represented 50.9% (n= 28) of odontogenic benign tumors and the cemento-osseous dysplasia 36.4% (n= 20) of lesions (Figure 3).
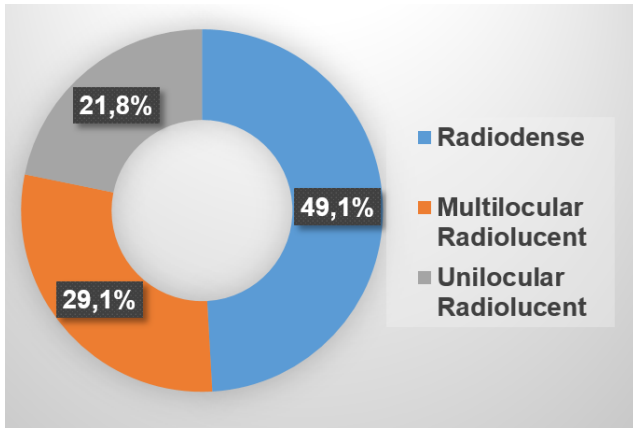
Fig. 2: Distribution of the population according to tumors' radiographic characteristics

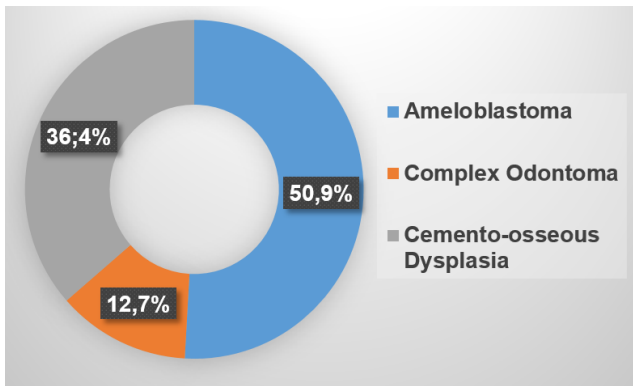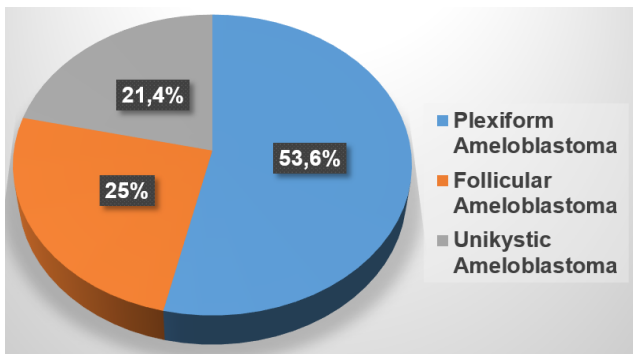**Fig. 3:** Distribution of the population according to histological types of odontogenic benign tumors.

Among the 28 cases of ameloblastoma, the 15 (53.6%) were of plexiform type, the 7 (25%) of follicular type and the 6 (21.4%) of the unikystic type (Figure 4).

Fig. 4: Distribution of the population according to histological types of ameloblastoma

3.4. Distribution of the population according to the Ki67 proliferation index of the tumor cells.

The percentage of Ki67 labelled cells was less than 1% in 74.6% (n=41) of tumors and was equal to 5% in 12.7% (n=7) of cases (Figure 5 and 6).
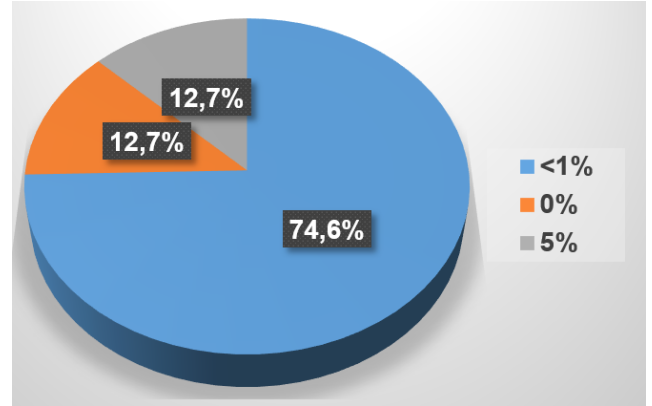
Fig. 5: Distribution of odontogenic benign tumors based on the proliferation index
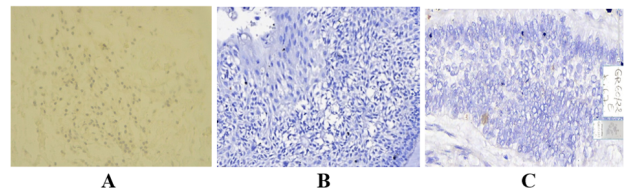
Fig. 6: Ki67 labelling at 5% in follicular ameloblastomas (**A**), at less than 1% in squamous ameloblastomas (**B**) and plexiform ameloblastomas (**C**)

3.5. Ki67 expression in odontogenic benign tumors

A weak labelling was found in 87.3% (n=48) of odontogenic benign tumors and negative labelling noted in 12.7% (n=7) of cases (Table 1).

Table 1: Distribution of odontogenic benign tumors based on Ki67 expression

Ki67 Marking	Numbers (n)	Percentage (%)
Weak	48	87,3
Negative	7	12,7
Total	55	100,0

3.6. Ki67 expression based on evolution duration intervals

Among the 22 patients whose evolution duration was between 24 and 59 months, all the 20 ones (90.9%) had

Table 2: Ki67 expression based on evolution duration intervals

Ki67 Marking	Evolution duration Intervals			Total n (%)	P-value
	(1-23) Months n (%)	(24-59) Months n (%)	≥60 Months n (%)		
Weak	8 (61,5)	20 (90,9)	20 (100)	48 (87,3)	
Negative	5 (38,5)	2 (9,1)	-	7 (12,7)	0,004
Total	13 (100)	22 (100)	20 (100)	55 (100)	

a weak Ki67 labelling. Similarly, all the patients with evolution duration superior or equal to 60 months (n=20), had low Ki67 labelling with a significant difference (p-value = 0.004) (Table 2).

3.7. Proliferation index based on evolution duration intervals

Among the 22 patients whose evolution duration was between 24 and 59 months, the 17 (77.3%) had a percentage of Ki67 labelled cells below 1% and among the 20 patients with an evolution duration superoir or equal to 60 months, the 17 (85%) had a percentage of Ki67 labelled cells below 1% with a significant difference (p-value = 0.027) (Table 3).

3.8. Ki67 expression based on radiographic image appearance

All the 16 lesions with a multilocular radiolucent appearance and all the 12 lesions with a unilocular radiolucent appearance had a weak labelling of Ki67 with a significant difference (p-value = 0.030) (Table 4).

3.9. Ki67 expression based on lesion histological types

A weak Ki67 marking was noted in all the follicular ameloblastoma cases, the squamous ameloblastoma, the plexiform ameloblastoma and cemento-osseous dysplasia with a significant difference (p-value = 0.030) (Table 5).

4. Discussion

Benign odontogenic tumors are diverse and can be encountered at any age affecting both men and women with variable clinical manifestations.[16,17] In the present study, a predominance of women (63.6%) was observed with a sex ratio of 0.57. These results are similar to those reported by Alsaegh and al, 2017 with 66.7% of females.[4]

However, a male predominance has been described by other authors.[18–20] This difference could be explained by a large number of cemento-osseous dysplasia cases, often seen and encountered in melanodermal adult women.[21] These tumors can be found at any age with a peak in young adults.[10,11] In this study, odontogenic benign tumors were more seen in adults with a median of 40 years old. These results are similar to those reported by some authors in literature.[13,19,20] Similarly, according to Sabea and al,

2017,[10] Mithra and al, 2021,[11] odontogenic benign tumors were predominantly (78%) found in adults. The evolution duration of these benign tumors is variable, ranging from a few months to several years.[18,20,22] The average of the evolution duration in the present study was 46.3 ± 34.2 months with extremes of 6 months and 144 months.

Similar results have been reported by several authors in literature.[17,18] However, Monteiro and al, 2021 in Portugal had noted a lower evolution duration average with 20.4 ± 7.5 months.[20] This difference could be explained by the fact that in our countries, in addition to the weak technical platform, the health infrastructures are insufficient and the recourse to consultation is the first reflex of the populations as soon as the oral cavity tumor lesion appears. Therefore, an evolution duration that is between 24 and 59 months was more frequent (40%) in the study population followed by that of 60 months and more with 36.4%. This could be explained by the delays in consultation often described in our developing countries.[21,22]

These odontogenic benign tumors can be located in both the mandible and the maxilla with a predominance in the mandible.[19,23] The lesions were at location more mandibular (89.1%) in this work. These results are similar to the data in literature.[4,20] The radiological aspects of these benign tumors are variable and may be radiolucent, unilocular or multilocular,[17,19,20] but also radiodense.[16] In the present study, the more frequent radiodense appearance (49.1%) could be explained by the high number of osteocondensing radiological lesions, such as cemento-osseous dysplasia, found in this population.

Multilocular radiolucent aspects came in the second position (29.1%). According to Aramanadka and al, 2018, Ajila and al, 2022 and Monteiro and al, 2021 ; the radiographic aspects of odontogenic benign tumors are dominated by multilocular radiolucent images with more than 60% of cases.[17,19,20] The histological aspects of odontogenic benign tumors are numerous and variable with different clinical manifestations.[16,20] In odontogenic benign tumors, ameloblastoma is the most common lesion according to literature.[7,20] These results are similar to those observed in this study with a predominance of ameloblastoma found in 50.9% of the collected surgical specimens. The follicular and plexiform histological types are the most frequently described in literature.[11,12,15,18]

This is in perfect agreement with the noted results in this work where a predominance of follicular and

Table 3: (Ki67) proliferation index based on evolution duration intervals

Ki67 index proliferation	Evolution duration interval			Total n (%)	P-value
	(1-23) Month n (%)	(24-59) Month n (%)	≥ 60 Month n (%)		
<1%	7 (53,8)	17 (77,3)	17 (85)	41 (74,6)	
0%	5 (38,5)	2 (9,1)	-	7 (12,7)	0,027
5%	1 (7,7)	3 (13,6)	3 (15)	7 (12,7)	
Total	13 (100)	22 (100)	20 (100)	55 (100)	

Table 4: Ki67 expression based on radiographic image characteristics

Ki67 Marking	Radiology Image			Total	P-value
	Multilocular Radiolucent	Unilocular Radiolucent	Radiodense		
Weak	16 (100)	12 (100)	20 (74,1)	48 (87,3)	
Negative	-	-	7 (25,9)	7 (12,7)	0,030
Total	16 (100)	12 (100)	27 (100)	55 (100)	

Table 5: Ki67 labelling based on lesions' histological types

Ki67 Marking	Histological type					Total	P-value
	Améloblastoma Follicular	Améloblastoma Unikystic	Améloblastoma Plexiform	Dysplasia Cémento-Osseous	Odontoma Complex		
Weak	7 (100)	6 (100)	15 (100)	20 (100)	-	48 (87,3)	
Negative	-	-	-	-	7 (100)	7 (12,7)	0,030
Total	7 (100)	6 (100)	15 (100)	20 (100)	7 (100)	55 (100)	

plexiform histological types of ameloblastoma has been noted. Furthermore, the cemento-osseous dysplasia comes in second place with 36.4%. This could be explained by its frequency in the melanodermal population in elderly subjects, particularly in women.[21] A Ki67 low labelling indicating a low proliferative index was found in 87.3% of odontogenic benign tumors. These results are similar to those reported in literature.[4,9,12,24]

However, according to some authors a Ki67 moderate labelling is noted on epithelial cells in a few cases of ameloblastoma.[10,12,13] In addition, an absence of labelling of epithelial cells in ameloblastoma has been described by Martín-Hernána and al., 2022[9] and Mithra and al., 2021.[11]

However, a strong Ki67 cell labelling giving a high proliferative index has been found in some cases of ameloblastoma.[10,12,13,25] These variable expressions noted could be explained by the difference of ranges retained to quantify Ki67 cell labelling. The association noted between the weak Ki67 epithelial cell marking and an evolution duration of at least two years and more could be explained by the fact that despite the aggressive locally character of certain odontogenic benign tumors such as ameloblastomas, these lesions are often of a progressive and slow evolution,[20] resulting in a low proliferation index.

In addition, a weak Ki67 marking has been noted in cemento-osseous dysplasia. These data are in full agreement with studies reported in literature.[26,27] The association between the weak marking of Ki67 epithelial cells and

lesions' unicolor and multilocular radiological aspects could be explained by the fact that, apart from cemento-osseous dysplasia, the majority of lesions are dominated by ameloblastomas which can be multilocular or unilocular on radiography.[19,23]

In addition, the association described between the weak Ki67 marking of ameloblastomas epithelial cells and cemento-osseous dysplasia could be explained by the fact that these lesions are slow-growing ones (low proliferative index) with perceptible clinical expression after several months of evolution.[17,26]

5. Conclusion

The majority of odontogenic benign tumors in the present study had a weak Ki67 expression level and a low proliferation index of epithelial cells. These lesions were predominantly composed of cemento-osseous dysplasia and ameloblastoma.

This corroborates the literature data which report that the Ki67 cannot be used as a predictive biomarker of tumor cell proliferation and the risk of recurrence of odontogenic benign tumors. Furthermore, thorough investigations are needed using other cellular markers to better understand the biological mechanism of these tumor lesions, some of which, like ameloblastoma, in addition to being locally aggressive, has a very high recurrence rate.

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6. Source of Funding

None.

7. Conflict of Interest

No funding from an organization or structure was received for realization of this study.

This is work that has involved only the contribution of authors of this study.

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