Review Article

Influence of Ki-67 and Proliferating Cell Nuclear Antigen Expression on the Biological Behavior of Ameloblastomas

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Abstract

Background: Ameloblastomas, the second-most common odontogenic tumors, are locally aggressive benign lesions that derive from cellular components of the enamel organ. **Objective:** The objective of the study is to assess the possible influence of cell proliferation markers Ki-67 and proliferating cell nuclear antigen (PCNA) on the biological behavior of ameloblastomas. **Materials and Methods:** A PubMed database search through February 2018, using the following medical subject headings (MeSH) terms were performed: "ameloblastoma" and ("Ki 67 antigen" or "proliferating cell nuclear antigen"). **Selection Criteria:** Studies with findings on cell proliferation markers in ameloblastoma. There were no restrictions regarding language or date of publication. **Data Analysis:** The data were analyzed using statistical software RevMan 5.3 (The Cochrane Collaboration, Oxford, UK). For continuous outcomes, the estimates of effects of an intervention were expressed as mean differences using the inverse variance method together with 95% confidence intervals. **Results:** Fourteen studies on cell proliferation markers in ameloblastoma were included in this meta-analysis. A greater significant Ki-67 expression in recurrent ameloblastomas ($P < 0.01$), in follicular type of solid/multicystic ameloblastomas ($P < 0.05$) and maxilla located ameloblastomas ($P < 0.05$) was found. In contrast, no significant influence of Ki-67 expression was observed in the following parameters: clinical type of ameloblastoma, histological type of unicystic ameloblastoma, age, gender, or size of the lesion. Finally, PCNA expression had no significant influence in any case. **Conclusions:** Ki-67 but not PCNA antigens seem to have an influence on the biological behavior of ameloblastomas.

Keywords: Ameloblastoma, Ki 67 antigen, odontogenic tumors, proliferating cell nuclear antigen

Introduction

Ameloblastomas are benign locally aggressive tumors that originate from cellular components of the enamel organ. Ameloblastoma usually presents as a painless, hard, slow-growing lesion near the angle of the jaw. It is more frequently diagnosed in the third to fifth decades of life, and it can produce a large deformity if left untreated. Ameloblastomas are the second most common odontogenic tumor after odontomas.[1]

Ameloblastomas typically appear in the mandible, in the region of the lower third molar, although they may appear in any other location of the mandible (80% cases) or maxilla (20% cases). Although it is a benign tumor, it is locally aggressive with a high recurrence rate. Approximately 20% of cases are associated with dentigerous cysts and unerupted teeth.[2]

According to the World Health Organization classification,[3] the following four main clinical types of ameloblastoma are differentiated: conventional (formerly called solid/multicystic), unicystic, extraosseous/peripheral, and metastasizing (malignant). Solid/multicystic ameloblastomas have several histological types: follicular, plexiform, acanthomatous, granular cell, basaloid, and desmoplastic; whereas, unicystic ameloblastomas presents three histological types: luminal, intraluminal, and mural.[4]

The Ki-67 nuclear antigen is a marker of cell proliferation that is present in all active phases of the cell cycle (G1, S, G2, and mitosis), being absent in quiescent cells. The cell proliferating cell nuclear antigen (PCNA), also known as cyclin, is synthesized primarily in the G1/S phases of the cell cycle. The increased expression of both proteins is an indicator

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of tumoral growth.[5] The aim of this study was to assess the possible influence of cell proliferation markers (Ki-67 and PCNA) on the biological behavior of ameloblastomas.

Materials and Methods

A PubMed database search of studies on markers of cell proliferation in ameloblastomas to February 2018 was conducted. Search strategies included the combination of the following terms from the medical subjects headings (MeSH): "ameloblastoma" [MeSH Terms] AND ("Ki 67 antigen" [MeSH Terms] OR "proliferating cell nuclear antigen" [MeSH Terms]).

The inclusion criteria were as follows: (a) type of studies (clinical trials, clinical studies, comparative studies, and multicenter studies), (b) human studies, and (c) studies with full-text availability.

Exclusion criteria were clinical case studies and studies with irrelevant or no usable data, lack of an adequate ameloblastoma diagnosis and studies with important biases.

After applying the inclusion and exclusion criteria remained fourteen studies that were included in this meta-analysis [Figure 1].

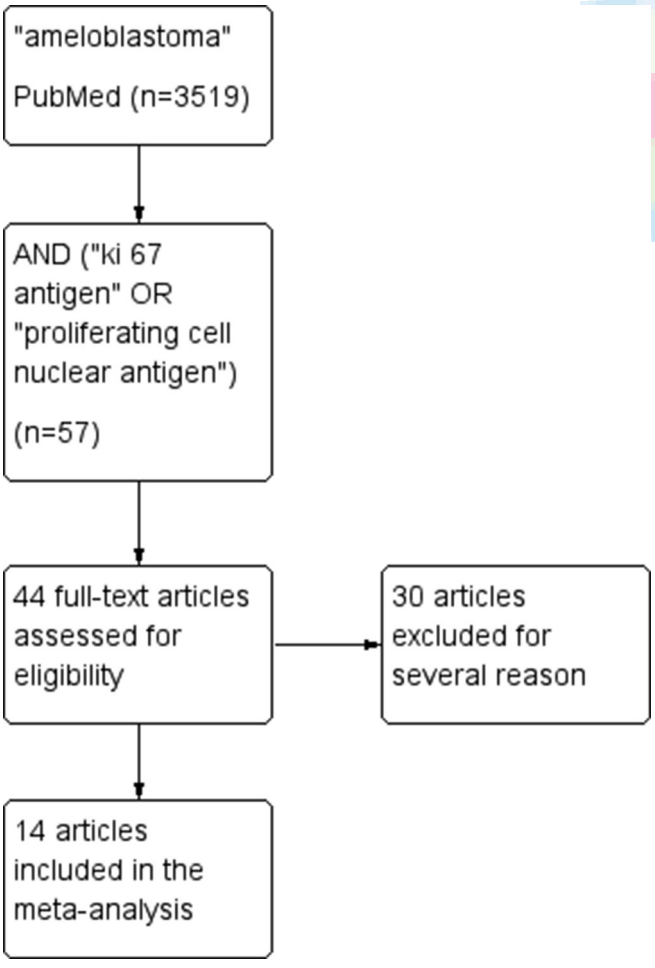

Figure 1: Study flow diagram

Statistical analysis

For the meta-analysis, the data were processed with the statistical software RevMan 5.3 (The Cochrane Collaboration, Oxford, UK). For the continuous variables, the inverse of the variance was used for the mean difference (MD) with 95% confidence intervals (95% CI). Heterogeneity was determined according to the *P* values and the Higgins statistic (I^2). In cases of high heterogeneity, the random effects model was applied. The statistical significance level was set at $P < 0.05$.

Results

Table 1 presents the descriptive characteristics of the 14 included studies in the meta-analysis.

The possible influence of parameters related to Ki-67 expression in ameloblastomas is shown in Table 2.

No statistically significant association was found between the Ki-67 expression and the clinical type of ameloblastoma (MD: 0.98, 95% CI: −1.37–3.33, $P = 0.41$), the histological type of unicystic ameloblastoma (MD: 0.72, 95% CI: −0.86–2.30, $P = 0.37$), younger patients (MD: 2.54, 95% CI: −0.41–5.48, $P = 0.09$), male gender (MD: 0.24, 95% CI: −3.03–3.50, $P = 0.89$), and larger lesions (MD: 1.60, 95% CI: −1.41–4.60, $P = 0.30$).

In contrast, the follicular histological type of solid/multicystic ameloblastomas showed a higher Ki-67 expression compared to other histological types with statistically significant differences (MD: 1.67, 95% CI: 0.29–3.05, $P = 0.02$). Similarly, recurrent ameloblastomas had also a higher Ki-67 expression with a strong statistically significant association (diabetes mellitus: 7.66, 95% CI: 2.01–13.32, $P < 0.01$). The ameloblastomas located in the maxilla presented a greater Ki-67 expression with statistically significant differences (MD: 1.35, 95% CI: 0.08–2.63, $P = 0.04$)

Table 3 represents the possible influence of parameters related to PCNA expression in ameloblastomas.

In the case of the PCNA expression, no statistically significant differences were found between this PCNA expression and the clinical type of ameloblastoma (MD: 2.30, 95% CI: −4.30–8.89, $P = 0.50$), the histological type of solid/multicystic ameloblastoma (MD: 0.04, 95% CI: −4.75–4.82, $P = 0.99$), or the histological type of unicystic ameloblastoma (MD: 0.47, 95% CI: −8.83–9.78, $P = 0.92$).

Discussion

In the present meta-analysis about the possible influence of the cell proliferation markers (Ki-67 and PCNA) on the biological behavior of ameloblastomas, data from 14 studies have been included.

In our study, unicystic ameloblastomas showed a higher Ki-67 expression although there were no statistically significant differences ($P = 0.41$). Of the six studies that considered the expression of Ki-67 according to the clinical type of

Table 1: Descriptive characteristics of included studies

First author	Year	Country	Study samples	Proliferating cell marker	Parameters analyzed
Kim[6]	1994	Korea	25 SMA, 13 UA	PCNA	Clinical type, histological type
Funaoka[7]	1996	Japan	23 SMA	PCNA	Clinical type, histological type
Piattelli[8]	1998	Italy	22 SMA	PCNA	Clinical type, histological type
Takahashi[9]	1998	Japan	46 SMA	PCNA	Clinical type, histological type
Sandra[10]	2001	India	27 SMA, 5 UA	PCNA, Ki-67	Clinical type, histological type.
Meer[11]	2003	South Africa	10 SMA, 10 UA	PCNA, Ki-67	Clinical type, histological type, recurrence status
Barboza[12]	2005	Brazil	16 SMA	PCNA	Clinical type, histological type, age, gender, recurrence status
Bologna-Molina[13]	2008	Mexico	45 SMA, 75 UA	Ki-67	Clinical type, histological type
Abdel-Aziz[14]	2012	Egypt	22 SMA	Ki-67	Clinical type, histological type, age, gender, size
Gadbail[15]	2012	India	14 SMA, 9 UA	Ki-67	Clinical type, histological type
Bologna-Molina[16]	2013	Mexico	66 SMA, 87 UA	PCNA, Ki-67	Clinical type, histological type
Sah[17]	2013	India	18 UA	Ki-67	Clinical type, histological type, age, gender, size, location
Olimid[18]	2014	Romania	18 SMA, 4 UA	Ki-67	Clinical type, histological type
Carreón-Burciaga[19]	2015	Mexico	38 SMA, 72 UA	Ki-67	Clinical type, histological type, age, gender, size, location, recurrence status

SMA=Solid/multicystic ameloblastomas; UA=Unicystic ameloblastomas; PCNA=Proliferating cell nuclear antigen; Ki-67=Ki-67 antigen

Table 2: Parameters related to Ki-67 antigen expression in ameloblastomas

Parameter	*n*	Reference value	MD	95% CI	I^2 (%)	*P*
Clinical type[10,11,13,15,16,18]	6	UA	0.98	−1.37-3.33	51	0.41
SMA histological type[10,13,14,16,18]	5	Follicular	1.67	0.29-3.05	0	0.02*
UA histological type[11,13,16-18]	5	Intraluminal	0.72	−0.86-2.30	0	0.37
Recurrence status[13,14,19]	3	Yes	7.66	2.01-13.32	55	<0.01*
Age[14,17,19]	3	Younger age	2.54	−0.41-5.48	52	0.09
Gender[14,17,19]	3	Male	0.24	−3.03-3.50	49	0.89
Size[14,17,19]	3	Larger size	1.60	−1.41-4.60	38	0.30
Location[17,19]	2	Maxilla	1.35	0.08-2.63	0	0.04*

*Statistically significant. Ki-67=Ki-67 antigen; *n*=Number of studies; SMA=Solid/multicystic ameloblastoma; UA=Unicystic ameloblastoma; MD=Mean difference; OR=Odds ratio; 95% CI=95% confidence interval; I^2=Higgins statistic for heterogeneity

Table 3: Parameters related to proliferating cell nuclear antigen expression in ameloblastomas

Parameter	*n*	Reference value	MD	95% CI	I^2 (%)	*P*
Clinical type[8,10,11,16]	4	SMA	2.30	−4.30-8.89	845	0.50
SMA histological type[6-10,12,16]	7	Plexiform	0.04	−4.75-4.82	55	0.99
UA histological type[11,16]	2	Intraluminal	0.47	−8.83-9.78	47	0.92

n=Number of studies; SMA=Solid/multicystic ameloblastoma; UA=Unicystic ameloblastoma; MD=Mean difference; OR=Odds ratio; 95% CI=95% confidence interval; I^2=Higgins statistic for heterogeneity

ameloblastoma (solid/multicystic vs. unicystic), four of them[11,13,16,18] coincided with our results, finding a greater Ki-67 expression in unicystic ameloblastomas. On the other hand, only two studies[10,15] found a higher Ki-67 expression in solid/multicystic ameloblastomas. However, in none of the cases, the results were statistically significant.

Although in principle, solid/multicystic ameloblastoma has a greater proliferative activity and an infiltrative nature that, together with the greater difficulty in surgical accessibility in its treatment, increases its tendency to recurrence,[15] Ki-67 expression seems to have no influence on its biological behavior.[10] Some types of unicystic ameloblastoma contain fewer cells similar to the stellate reticulum, making the cells located in the basal or suprabasal layer the ones that are marked primarily against Ki-67, giving apparently a greater Ki-67 expression in unicystic ameloblastomas.[16]

Regarding PCNA expression, it was higher in solid/multicystic ameloblastomas although without statistical significance ($P = 0.50$). In the four studies,[8,10,11,16] in which PCNA expression was analyzed according to the clinical type of ameloblastoma, two of them[11,16] observed more expression in unicystic ameloblastomas and another two[8,10] a greater expression in solid/multicystic ameloblastomas. These apparently discrepant results could be conditioned by the design of the studies. For example, one of them[11] considered a sample size too small ($n = 10$) to be able to extrapolate the

results. The characteristics of the PCNA antigen could also have influenced them. The PCNA expression depends on a series of factors: its limited half-life where it is only expressed when the cells enter the cell cycle, the type of fixative used in paraffin-embedded tissue samples or the dilution of the antibody.[16]

Expression of both cell proliferation antigens (Ki-67 and PCNA) according to the histological type of solid/multicystic ameloblastoma (follicular vs. other histological types) was also analyzed. Follicular ameloblastomas had a higher Ki-67 expression with statistically significant differences ($P = 0.02$). The five studies[10,13,14,16,18] considered Ki-67 expression coincided with our results.

In respect of PCNA expression on different histological types of solid/multicystic ameloblastomas, no statistically significant association was observed ($P = 0.99$) between follicular and other types of ameloblastomas. Four studies[7,10,12,16] found a higher PCNA expression in the follicular ones and three[6,8,9] in the plexiform ones. The histological type of solid/multicystic ameloblastoma seems to have little influence on the PCNA expression.[10]

When the expression of both Ki-67 and PCNA was compared with the histological type of unicystic ameloblastoma (intraluminal vs. other histological types), in this meta-analysis, no statistically significant differences were found between the histological type of unicystic ameloblastoma and Ki-67 expression ($P = 0.37$) or PCNA expression ($P = 0.92$). Of the 5 studies[11,13,16-18] that assessed Ki-67 expression, 4 of them[11,16-18] observed a greater expression in intraluminal unicystic ameloblastomas although without significant results. Studies with larger samples are required to determine the true role of Ki-67 expression on the different types of unicystic ameloblastoma.[11] Two studies[11,16] considered PCNA expression found conflicting results. The particularities of the PCNA molecule described above may have influenced these results.[16]

In our study, Ki-67 expression was quite higher in recurrent ameloblastomas with a strong statistically significant association ($P < 0.01$). This finding reveals the close relationship between this marker of cell proliferation and the recurrent nature of ameloblastomas. The 3 studies[13,14,19] that analyzed this fact confirmed our results. Lesions with a greater proliferative activity (higher Ki-67 expression) were those that also showed a greater predisposition to recurrence.

The possible influence of other parameters such as age,[14,17,19] gender,[14,17,19] size of lesion,[14,17,19] or its location[17,19] on Ki-67 expression was also studied. There was an increase in the Ki-67 expression as the age increased, in the male subjects, in the larger lesions and in those located in the maxilla. However, only a statistically significant association was found in the case of the location of ameloblastomas ($P = 0.04$). Most of these studies have biases in the sample selection with inequalities according to the different parameters, which may alter the results.

All findings of this meta-analysis must be interpreted with caution due to the high heterogeneity of some studies considered and the presence of various biases. The differences among studies could be conditioned by the study design; the methods used to collect data, the type of analysis used or the characteristics of the study populations and samples.

Conclusions

In this meta-analysis, a greater significant Ki-67 expression was found in recurrent ameloblastomas ($P < 0.01$), in follicular-type solid/multicystic ameloblastomas ($P < 0.05$), and in maxilla located ameloblastomas ($P < 0.05$). In contrast, no significant influence of Ki-67 expression was observed in the following parameters: clinical type of ameloblastoma, histological type of unicystic ameloblastoma, age, gender, or size of the lesion. Finally, the PCNA expression in ameloblastomas had no significant influence in any case.

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Conflicts of interest

There are no conflicts of interest.

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