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Analysis of cell proliferation index using the Ki-67 Antigen in odontogenic keratocyst: a systematic review

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• Conflicts of interest: none declared.

Abstract

Objective: this review aims to analyze scientific articles about cell proliferation index using Ki-67 in odontogenic keratocyst and compare these papers to estimate the average index of this lesion. **Material and Methods:** two researchers performed a literature search independently in the MEDLINE/PubMed database and 28 articles containing relevant data were selected. **Results:** the immunohistochemical analysis methodology showed great variability among all the papers, with unclear and unified methodologies, making the comparison among different studies difficult. **Conclusion:** considering odontogenic keratocyst as a lesion with an uncommon clinical behavior, an adequate classification for it is necessary, so an appropriate treatment with a good prognosis for the patient can be established according to its nature. A standardization is needed so immunohistochemical analyses will find reliable data to classify properly this lesion.

Keywords: Odontogenic keratocyst; Ki-67 antigen; Proliferation index.

Introduction

The odontogenic keratocyst (OKC) was first classified as an odontogenic developmental cyst by Pindborg & Kramer (1971), on the first official classification of odontogenic cysts and tumors by the World Health Organization (WHO).[1] However, this lesion has three characteristics that raised discussions about its classification, which are locally destructive and aggressive behavior, high recurrence index and tendency toward multiplicity.[2] In 2005, the WHO changed the classification of OKC to an odontogenic tumor, based on its high cell proliferation activity when compared to other odontogenic cysts and the presence of mutations in p53 and *patched* (PTCH) genes that are also found in other neoplasms.[2]

Some authors did not accept this new classification, claiming that the existing evidence are insufficient to support the neoplastic origin of OKC and that further researches are needed.[3] The WHO published its new classification in 2017, and OKC was again categorized as an odontogenic cyst.[3]

OKC represents about 10 to 20% of all odontogenic cysts, usually occurring between the second and third decade of life, with a slight predilection for men.[3] The mandible bone is often affected[4] and the cyst can be solitary or multiple – the last former is usually one of the components of inherited nevoid basal cell carcinoma syndrome (NBCCS).[4,5]

The evaluation of cell proliferation activity is one of the most common methods to assess the biological behavior of a lesion, being reported as an important prognostic marker and indicator of biological aggressiveness.[5,6-8]

The Ki-67 antigen is a highly specific marker of proliferating cells[4,6-8] and has been widely used to evaluate the proliferative activity of preneoplastic and neoplastic lesions.[5-7,9] This protein is present in all phases of the cell cycle, except in the G0 phase[4,6-12], with a 60–90 minutes half-life.[11]

Over the years, many researches have evaluated the proliferative activity of OKC cells, comparing it to other cysts and tumors to better understand its pathogenesis and behavior.[4,5,7,8,11-21] However, each study presents a different cell proliferation index (PI) and the methodology used is not always clear, making the comparison between the OKC index with other odontogenic lesions difficult, impairing the improvement of our knowledge about its nature and biological behavior, as well as the possibility of using Ki-67 as a prognostic marker for OKC.

This review aims to analyze and compare scientific articles that used Ki-67 as reference to evaluate the cell proliferation activity in OKC, comparing their methodology and results to define an average PI for OKC to facilitate future studies and comparisons. To the best of our knowledge, this is the first systematic review about this issue.

Material and Methods

Two researchers, independently, conducted a literature search on the MEDLINE/PubMed database in June 2018. The search had no year restriction, using the abstract format and sorted by most recent. The Medical Subject Headings (MeSH) terms used were: Ki-67 antigen, odontogenic cyst, keratocysts and mitotic index. Those words were combined with other terms: odontogenic keratocyst, keratocystic odontogenic tumor, Ki-67 and cell proliferation index.

The search was performed in two stages: the first used the keywords chosen in different combinations, and the articles were selected based on their titles. Articles that were case reports and that were not in English language were excluded.

On the second stage, the researchers accessed the full texts of articles that were considered eligible for inclusion for further evaluation and selection. Some studies were excluded according to the established exclusion criteria: articles with an unclear methodology, articles without the PI, and articles that used NBCCS-associated OKC as samples. However, for articles that included syndromic and non-syndromic OKC in their samples with individualized results, only sporadic OKC was considered.[10,19,22] The same approach was taken with the article that included recurrent and non-recurrent OKC, only the last one was considered.[9,22]

From the various keywords combinations, 54 papers were pre-selected respecting the exclusion criteria for stage one. All articles appeared repeatedly in the different searches, these were only counted once, justifying the total of papers selected in this stage (Figure 1).

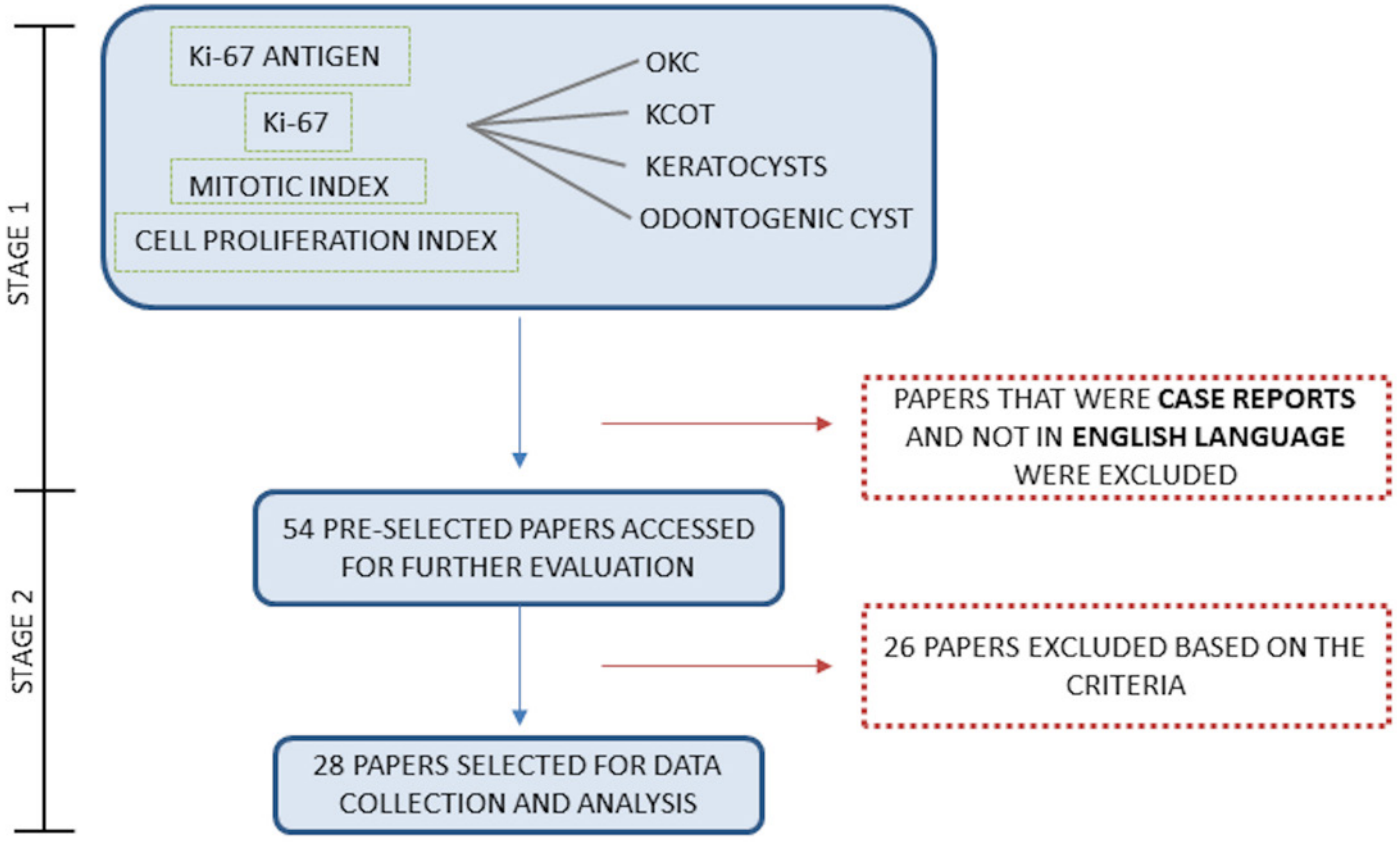

Figure 1. Flowchart of the articles selected for this literature review, from the initial identification to the final selection for review

After the analysis of all these papers on the second stage, 28 articles were selected according to the described criteria and all relevant data were obtained from these papers: Ki-67 PI, number of analyzed cases and the immunohistochemistry analysis method in each study (Figure 1). Table 1 describes the reasons for the exclusion of 26 articles.

Results

The selected papers were divided into two groups for comparison: articles that show the index as mean (Table 2) and articles that present it as median (Table 3), because it is not possible to compare these different values.

Table 1. Number and reasons of excluded articles

REASONS FOR THE EXCLUSION	N° OF ARTICLES
No cell proliferation index	6
Index expressed only as a graphic, making it impossible to precise the PI	3
Unclear methodology	2
Impossible to reach a single PI value with the information on the paper	11
PI calculated counting the positive cells per millimeters of basal membrane	2
Article with median and divided into two scores: more than 10% and less than 10% of immunopositivity.	1
The authors did not provide the PI separately for syndromic OKC and sporadic OKC	1

Table 2. Articles with PI as mean

ARTICLE	YEAR	n	MEAN (%)	SD (%)	M	FIELDS	CELLS	L
Ninomiya et al.31	2002	25	26.20%	4.10%	x100	3	1000	-
Kim et al.21	2003	32	18.41%	12.00%	x200	5 RAF	-	-
Kaplan et al.25	2004	45	10.00%	-	x400	10 CF	-	-
Kichi et al.20	2005	20	18.50%	1.50%	-	10 RAF	500	B/ SB/ S
Clark et al.24	2006	16	19.90%	-	-	-	500	-
Gonzalez-Moles et al.30	2006	29	22.50%	11.30%	x400	4 RAF	-	B/ SB
Gadbail et al.13	2009	36	12.20%	4.69%	x400	10 RAF	1000	B/ SB/S
De-Vicente et al.18	2010	11	40.00%	-	-	CF	200	-
De-Oliveira et al.17	2011	13	19.98%	11.66%	x400	5 REF	-	B/ SB
Gadbail et al.8	2011	38	14.64%	3.52%	x400	10 RAF	1000	B/ SB/ S
Gadbail et al.11	2012	32	12.92%	4.23%	x400	10 RAF	1000	-
Guler et al.16	2012	7	16.00%	13.46%	-	-	100	-
Kadlub et al.28	2013	5	51.43%	-	x400	10 RAF	-	-
Alur et al.22	2014	3	30.00%	-	x400	10 RAF	1000	B/ SB
Ramos et al.15	2014	11	25.30%	11.10%	x400	10 CF	1000	-
Johann et al.10	2015	8	13.00%	-	x400	-	-	B/ SB/ S
Cosarca et al.14	2016	20	22.40%	-	x400	-	100	B/ SB
Awni et al.23	2017	14	10.83%	5.00%	x400	5 RAF	-	B/ SB
Ledderhof et al.27	2017	14	18.64%	-	x200	5 RAF	500	-
Zivkovic et al.26	2017	30	19.00%	-	-	5 REF	-	-
Modi et al.5	2018	15	12.76%	4.78%	x400	5 RAF	1000	B/ SB/ S
Doll et al.4	2018	54*	21.80%	13.20%	x400	10	1000	-

M = magnification; RAF = randomly selected fields; REF = representative fields; CF = consecutive fields; L = evaluated layers; B = basal layer; SB = suprabasal layer; S = superficial layer; "-"= no data available.
*Authors did not say how many samples of sporadic OKC were evaluated, they consider that 54 lesions included sporadic and syndromic OKC. But the indexes were given separately.

Table 3. Articles with PI as median

ARTICLE	YEAR	N	MEDIAN (%)	VARIATION (%)	M	FIELDS	CELLS	L
Mateus et al.19	2008	11	9.83%	7.19-19.78%	x400	10	-	B/ SB/ S
Johann et al.12	2011	8	12.00%	-	x400	-	-	B/ SB
Amaral et al.6	2012	11	9.83%	7.19-19.78%	x400	10	-	B/ SB/ S
Selvi et al.29	2012	22	3.50%	3-4%	x400	5 CF	-	-
Metqud et al.7	2013	15	10.91%	8.29 - 20.49%	x400	10	-	B/ SB/ S
Naruse et al.9	2017	52	5.00%	0-17.3%	-	5 RAF	-	B/ SB

M = Magnification; RAF = randomly selected fields; CF = consecutive fields; L = evaluated layers; B = basal layer; SB = suprabasal layer; S = superficial layer; "- "= no data available.

Three papers divided the indexes into layers (basal and parabasal).[14,17,23] One provided only the number of positive cells and the total number of cells counted but did not provide a PI.[24] Two papers divided the OKC evaluation according to inflammatory density[25] and in follicular and extrafollicular OKC.[21] To include these papers in our study, an overall PI was calculated for each study. All data collected are shown in Tables 2 and 3.

Discussion

The classification of OKC has changed over the years and many studies are being developed to determine a reliable classification, if it is a cyst or a tumor, seeking to define the best treatment and prognosis for this lesion. Some studies try to determine the nature of OKC through the cell proliferation index using Ki-67. But almost all papers present different methodologies, as well as different results. Therefore, this study analyzed and compared these articles and their methodologies to know how the PI was calculated.

Large variations of PI values can be observed in the selected articles, from 10% to 51.43% as mean, and from 3.5% to 12% as median. This can be explained by the different methods used in each of the analyzed papers.

The magnification is a methodological variable. Most authors used x400, others did the count at x200 or x100, which can impact the total number of visualized cells. This difference can be observed in Tables 1 and 2, where the authors indicate the number of cells that were evaluated by field.

The number of analyzed fields and how they were chosen are also factors that may have impact on the calculation of the percentage of positive cells: some authors select random, continuous or representative fields. Any parameter that changes the total number of evaluated cells might influence the PI, i.e., the higher the number of analyzed fields, the higher the number of cells. In addition, all tissues have fields that are considered a "hotspot", where greater immunostaining occurs; thus, depending on the selection of fields, this may also influence the result. When papers mentioned representative fields, the criteria used for such selection was not explained, so we considered this feature as "hotspot".

The location of immunopositive cells is also an important feature. Some authors analyzed all epithelial layers: basal, suprabasal and superficial, while others analyzed only the basal and suprabasal layer. This variable may also have impact on the total number of evaluated cells.

Considering the papers that calculated the PI as mean, the indexes varied from 10% to 51.43% and the mean of these results is 20.70%. This shows a discrepancy among the results, mainly in the PI obtained by Kadlub *et al.*[28] (51.43%). However, we can verify that most values are very close, ranging from 10% to 25% (in 18 of 22 papers); therefore, the influence of the variables mentioned above may not be statically significant.

Focusing our analysis on Ledderhof *et al.*[27] and Kim *et al.*,[21] we find that they used the same magnification (x200) and the same number of analyzed fields, and that cell number and the PI of both was similar: 18.64% and 18.41%, respectively.

Despite such variance on OKC PI, it is interesting to note that according to the WHO's classification of odontogenic cysts and tumors (2017),[3] when using Ki-67, the PI of some benign odontogenic tumors is low, like primordial odontogenic tumor (less than 2%) and dentinogenic ghost cell tumor (less than 5%).[3] Therefore, further exploration of OKC's cell proliferation index are needed to actually verify the nature of this lesion.

Another example of how these variables may influence immunohistochemical results can be observed on Table 3: Amaral *et al.*[6] and Mateus *et al.*[19] used the same number of analyzed samples, magnification, fields and the same epithelial layers, so they found the same PI for OKC.

A high variation in the number of sample was also observed, from 3 to 52 among all papers. However, some articles showed similar PI using different number of sample, but with similar quantities of evaluated cells, showing that the number of sample may not have significant impact on the results.

For example, Kim *et al.*[21] and Ledderhof *et al.*[27] used the same magnification and number of analyzed fields, but a different number of sample, and achieved 18.41% and 18.64%

PI, respectively. Amaral *et al.*,[6] Mateus *et al.*[19] and Metqud & Grupta[7] used the same magnification, number of analyzed fields and epithelial layers, but as in the last example, with different sample numbers. Their PI were 9.83%, 9.83% and 10.91%, respectively.[6,7,19] Both of these examples showed similar PI, which implies that the number of samples does not interfere on this calculation.

The main limitations of this systematic review are that the collected data are not the same across all studies, as well as the impossibility of performing a quantitative analysis of these results. Therefore, comparing the different studies using Ki-67 to evaluate the biological behavior of OKC is very difficult. Further and thorough research on these methodological variables are needed to confirm if they significantly influence PI results, from the statistical point of view, but many authors do not provide all relevant information of their methodology. For future researches, we suggest a standard evaluation of the proliferative activity and calculation of the index of these lesions, using the same magnification, number of cells and evaluating the entire epithelium.

Conclusion

From the results of this review, it is clear that most studies involving the immunohistochemical analysis with Ki-67 antigen for evaluating the proliferative activity in OKC have unclear and non-standardized methodologies; therefore, the standardization of immunohistochemical analyses is necessary to improve and optimize future research, associating this antibody with the nature of these lesions.

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Mini Curriculum and Author's Contribution

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