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MCM3 and Ki67 proliferation markers in odontogenic cysts and ameloblastoma

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ABSTRACT

Background: MCM3 is a marker of proliferation that has been used as a diagnostic or prognostic marker in many pathologic lesions.
Purpose: The purpose of this study is to investigate the proliferative activity of dentigerous cysts, odontogenic keratocyst and ameloblastoma using minichromosome maintenance 3 (MCM3) and compare it with Ki-67 proliferation marker.
Methods: In this cross-sectional study, 40 cases including 11 cases of dentigerous cyst, 14 odontogenic keratocyst, and 15 ameloblastoma were included. Immunohistochemical expression of MCM3 and Ki-67 were investigated and compared statistically using ANOVA, Chi-square, Tukey and Spearman's correlation tests.
Results: All subjects were positive for Ki-67 and MCM3. There was a significant difference in MCM3 and Ki-67 expression among all groups. Compared to Ki-67, MCM3 exhibited a higher level of expression. Ameloblastoma and odontogenic keratocyst revealed higher expression rate of both markers in comparison to dentigerous cysts.
Conclusion: The results showed MCM3 and Ki67 expression in the most common odontogenic cysts and tumors, especially more aggressive lesions. MCM3 might have a role in pathogenesis of these lesions and could be a reliable marker for assessing proliferation activity.

1. Introduction

Odontogenic cysts and tumors are heterogeneous group of osteodestructive lesions which represent a wide spectrum of clinical and biological behavior. They arise from remnants of tooth germ epithelial cells.[1] Dentigerous cyst (DC) and odontogenic keratocyst (OKC) are the most common developmental odontogenic cysts. DC exhibits slow growth, low recurrence rate and minimal invasiveness.[2] However, OKC is a unique cyst that, due to its locally aggressive nature and high recurrence rate, in a short time, had been reclassified as a cystic odontogenic tumor (Keratocystic odontogenic tumor) by World Health Organization (WHO).[2] Ameloblastoma, as the most common odontogenic tumor with clinical significances, is also a locally invasive tumor with tendency to recur after removal, in spite of its benign nature.[3] Previous studies have suggested that increased epithelial activity in OKC is responsible for the aggressiveness of this lesion in comparison with other odontogenic cysts.[3] Moreover, several factors in the cyst wall may be involved in biologic behavior of OKC.[4]

Cell proliferation plays a basic role in cell growth and the maintenance of tissue homeostasis, and also in several biological and pathological events, such as tumor development.[5,6] Identification of cell proliferation markers could be a useful diagnostic or prognostic tool to understand or predict clinical and biological behavior of many pathologic lesions.[7] Ki-67 is a well-known proliferation marker that is widely used in the diagnosis of several pathologic lesions, especially human tumors and malignancies.[8] Ki-67 is a nuclear non-histone protein, which is expressed in all active phases of cell cycle, including G, S, G2, M phases and disappears rapidly after mitosis. The half-life of detectable antigen is 1 h or less.[6] Odontogenic cysts and tumors show a low Ki-67 labeling index,[9] which may make assessing the proliferation activity difficult, especially in incisional biopsies. Minichromosome maintenance (MCM) proteins family consists of eight members, including MCM2-MCM7. They form a ring-shape complex that is involved in the initiation and elongation of DNA replication. They also prevent replication and maintain the genome integrity.[10,11] These proteins are expressed during all cell cycle phases in dividing cells, but are not detectable in quiescent cells (G0 phase). Because of MCMs expression in early G1 phase, in contrast to Ki-67, it is pertinent to study MCMs for evaluating tumor behavior.[5] Expression of MCM3 as a member of MCM2-7 complex has been established in several human neoplasms.[12,13] One study reported that MCM2 and MCM3 were more sensitive than Ki-67 for predicting the growth rate and biological

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behavior of ameloblastoma and ameloblastic carcinoma.[5] Guler et al. presented the expression of MCM2 in dental follicle and odontogenic cysts[6]; however, MCM3 has not been detected in OKC in Shahsavari et al.'s work.[14]

In view of the different behaviors of DC, OKC and ameloblastoma, and also controversial results of the few studies conducted on MCMs expression in odontogenic lesions, we endeavored to investigate the expression of MCM3 in these common lesions and compare it with Ki-67 proliferation marker.

2. Material and method

2.1. Patients and samples

In this cross-sectional analytical study, 40 cases including 11 DC, 14 OKC and 15 ameloblastomas (11 solid, 4 unicystic) were collected from the archive of Oral & Maxillofacial Pathology Department. All specimens had proper epithelial compartment and definite diagnosis. Patients' baseline data, regarding age and gender of the patients, were obtained from archived medical files.

2.2. Immunohistochemical staining

A 4μ thick section was prepared from formalin-fixed and paraffin-embedded blocks. After deparaffinization and rehydration, IHC was performed using the Envision labeled proxidase system (DAKO, Carpentaria, CA, USA). Antigen retrieval was done by DAKO cytomation target retrieval solution for 20 min, and endogenous proxidase activity was blocked by 3% H2O2. The tissue sections were first incubated with anti- MCM3 and anti-Ki-67 primary antibodies (1:100 and ready to use, respectively, DAKO Corporation, Denmark). Then, the sections were rinsed with phosphate-buffered saline (PBS), and incubated with Envision solution. 3,3 di-aminobenzidine (DAB) was used as chromogen, and finally the sections were counterstained with Myer's Hematoxillyne. Positive control was the basal cells of oral mucosa. Primary antibodies were replaced by PBS in negative control slides. Cells with brown nuclei were considered as positive staining for both antibodies. The stained cells were counted in at least 500 cells basal and parabasal cells of the cysts and also in peripheral cells of ameloblastic nests.[15] Five microscopic fields at x400 magnification were assessed and the percentage of positive cells in at least 500 cells was determined.[4]

2.3. Statistical analysis

ANOVA and Tukey tests were used to compare means. Chi-square and Spearman's correlation tests were also used for detecting any correlation between variables, at PV < 0.05 level.

3. Results

Out of 40 patients, 21 were males and 19 were females with mean age of 28.9 ± 18.6. The base line data of all study groups are illustrated in Table 1. All specimens were positive for MCM3 and Ki-67 expression. Both markers were expressed by basal and parabasal layers of the cysts (Figs. 1 and 2). Immunoreaction for Ki-67 and MCM3 was more prominent in basal layer of dentigerous cyst and parabasal layers of OKCs (Figs. 1 and 2). In ameloblastomas, the expression was mostly found in peripheral ameloblast-like and also in stellate reticulum (SR)-like cells (with lower rate) (Fig. 3). Overall, in all study groups, MCM3 exhibited a higher expression rate in compression with Ki-67. Table 2 illustrates the expression rate of both markers in all study groups.

Table 1
Baseline data of all study groups.

	Age (Mean ± SD)	M:F	Mandible: Maxilla
Dentigerous	27.8 ± 18.9	6:5	3:8
OKC	28.8 ± 18.4	7:7	10:4
Ameloblastoma	29.8 ± 12	8:7	13:2

OKC: odontogenic keratocyst.

ANOVA test exhibited a significant difference in all study groups in terms of the expression rate of both markers, ($p < 0.000$). Tukey test showed that Ki-67 and MCM3 expression were significantly higher in OKCs and ameloblastoma than in DCs ($p = 0.000$ and $p = 0.04$, respectively).

Although Ki-67 showed higher expression rate in OKC compared to ameloblastoma ($p = 0.000$), MCM3 was statistically similar in two groups ($p = 0.7$).

Spearman's correlation test revealed a weak positive correlation between two markers in all study groups (rho = 0.57 and $p = 0.002$).

Out of 27 cystic specimens, there were 8 cases of inflamed odontogenic cyst, and Chi-Square test revealed that there was no significant difference between inflamed and non-inflamed cases in the expression of both markers ($p > 0.05$).

4. Discussion

IHC is a suitable tool for detecting the difference of biological behavior of various pathologic lesions.[4,5] Owing to the crucial role of MCM3 in cellular proliferation, the present study evaluated and compared the immunohistochemical expression of Ki-67 and MCM3, one as a well-known and the other, as a new proliferation marker, respectively, in the most common odontogenic cysts and tumors. Previous studies showed a low Ki-67 expression in odontogenic lesions.[4,7,9,16] Now, it is assumed that this low expression may hinder the evaluation of proliferation activity, particularly when the size of the specimen is small. Recently, various MCM proteins have been used as novel prognostic or diagnostic proliferation markers in some human tumors, as well as odontogenic lesions.[5,13,17] Carreon- Barciage et al. demonstrated the higher levels of MCM2 and MCM3 markers compared with Ki-67 in ameloblastoma and ameloblastic carcinoma, and concluded that they were more sensitive markers and hence were more suitable for predicting biologic behavior of these tumors.[5] Although, in the present study, the sample size was small for determining a correlation between Ki-67 and MCM3, there was a positive but weak correlation between two markers. This study found MCM3 a reliable and even more sensitive proliferation marker than Ki-67, as shown in some other studies[4,5,18]

In the present study, while all samples expressed MCM3 and Ki-67, the latter expression was lower than the former in all groups. This finding was in agreement with previous results in oral squamous cell carcinoma (SCC), papillary thyroid carcinoma, salivary gland tumors and ameloblastic neoplasms.[5,10,12,13,17] In all these studies, the tumoral cells expressed more MCM3 than Ki-67. This finding can be explained by different expression of Ki-67 and MCM3 in the cell cycle. Ki-67 is expressed during late G1 to M phases and disappears quickly, whereas the expression of MCM3 is found in early G1 phase and may even be present in non-proliferating cells, waiting to enter the cell cycle.[5] This longer expression may facilitate assessing the proliferation activity.

In the present study, compared to DC, both Ki-67 and MCM3 proteins have exhibited higher expression rate in ameloblastoma and OKC. This result indicates higher proliferation activity of ameloblastoma and OKCs. As it is mentioned, these two entities have a high recurrence rate and aggressive behavior. Also, this finding supports the findings of other researchers. Guler et al. and Nadalin et al., have reported the lower proliferation activity of DC in comparison with OKC, using Ki-67 and MCM2 markers,[6,19] and also Nafarzadeh et al. have found lower expression of Ki-67 and PCNA in DC, compared to ameloblastoma.[16] Thosaporn et al. have evaluated proliferation activity in DC, OKC and orthokeratinized odontogenic cyst (OOC) using IPO-38 and