Prognostic relevance of clinicopathological factors in sporadic and syndromic odontogenic keratocysts: A comparative study

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Conflict of interest
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Abstract

Background. Current evidence suggests that nevoid basal cell carcinoma syndrome (NBCCS)-associated odontogenic keratocysts (OKCs) exhibit more aggressive clinical behavior and a higher tendency to relapse. The prognostic efficacy of various markers in sporadic and syndromic OKCs is unclear, and so are the results of studies on the usefulness of immunohistochemistry in distinguishing syndromic from sporadic OKCs.

Objectives. This retrospective study aimed to compare the prognostic relevance of various clinicoradiological and histopathological features, as well as the immunoexpression of COX-2, Bcl-2, proliferating cell nuclear antigen (PCNA), p53, Ki-67, osteoprotegerin (OPG), receptor activator of nuclear factor κ B (RANK) and receptor activator of nuclear factor κ B ligand (RANKL), as well as RANKL/OPG balance between sporadic and syndromic OKCs, and to test their utility in distinguishing the 2 types of OKC.

Materials and methods. We compared the immunoexpression of the aforementioned markers between 31 sporadic and 12 syndromic OKCs, and tested clinicopathological findings and levels of immunostaining against recurrence.

Results. We found a significant association between NBCCS and OKC recurrence. There were significant differences in PCNA, p53 and OPG immunoexpression between sporadic and syndromic OKCs. We also found that recurrent sporadic OKCs were significantly larger and markedly more often associated with cortical perforation. Recurrent sporadic OKCs exhibited COX-2 upregulation, but we failed to demonstrate its prognostic relevance. Recurrent syndromic OKCs showed a markedly higher RANKL > OPG ratio.

Conclusions. The NBCCS-associated OKCs are significantly more prone to recur than their sporadic counterparts. Larger size and radiological signs of cortical perforation in sporadic OKCs may indicate a higher risk of recurrence. The COX-2 is upregulated in recurrent sporadic OKCs, whereas recurrent syndromic OKCs exhibit higher RANKL and lower OPG expression; however, these findings have no prognostic relevance. The immunoexpression of p53, PCNA and OPG may help to distinguish syndromic from sporadic OKCs.

Key words: oral surgery, odontogenic cyst, keratocystic odontogenic tumor, odontogenic keratocysts

Background

Odontogenic keratocyst (OKC) is a benign intraosseous lesion of the jaw with a tendency toward aggressive growth and a relatively high recurrence rate. There is no agreement among authors as to whether OKC should be considered a cyst or a neoplasm.[1,2] Although it has been recently reclassified as a cyst,[3] for over a decade OKC was defined by the World Health Organization (WHO) as an intraosseous tumor due to *PTCH* gene mutations and infiltrative growth.[4] The WHO consensus panel does not necessarily state that OKC is not neoplastic, but rather that currently there is a lack of evidence to justify its classification as a tumor.[3,5,6] These discrepancies may lead to confusion among clinicians, particularly since the lesion is the 3rd most common cyst of the jaw.[3,7] The results of a recent meta-analysis advocate the concept that OKC is prone to behave as an odontogenic tumor in view of its p53 expression.[7]

As many as 5–6.4% of all OKCs occur as part of the nevoid basal cell carcinoma syndrome (NBCCS).[8] The NBCCS is an autosomal dominant inherited disorder related to a germline mutation of the patched gene 1 (*PTCH1*), whose alteration results in a carcinogenic process activated by an altered cell cycle and cellular proliferation.[9] The OKC is one of the most common signs of NBCCS, along with basal cell carcinomas (BCCs), palmar pits, skeletal abnormalities, and calcified falx cerebri. Syndromic cases of OKC tend to be multiple and occur in younger patients.[3] It has been suggested that syndromic OKCs have more aggressive clinical behavior and a higher tendency to relapse.[10,11] An early recognition of NBCCS is of vital importance, as affected patients are prone to develop neoplasms, such as BCC, medulloblastoma and ovarian fibroma.[12]

As genetic confirmation of NBCCS is not routinely conducted due to its high cost, the diagnosis is currently based on a combination of major and minor clinical criteria.[13,14] Researchers have tried to elucidate whether immunohistochemistry may allow them to distinguish between sporadic and syndromic OKC, but their results are inconclusive. A recent meta-analysis determined that several markers of epithelial cell proliferation and apoptosis are indistinguishable between sporadic and syndromic OKCs; however, the quality assessment of the included studies revealed notable inconsistencies regarding the diagnostic criteria, including demographic and clinical characteristics, as well as the histopathological description of OKC.[12] On the other hand, various studies have demonstrated that immunohistochemistry is a valuable method to assess the biological profile and prognosis of OKC, as several markers, including Ki-67,[1] Bcl-2,[15] cyclin D1,[15] p53,[15] and proliferating cell nuclear antigen (PCNA),[15,16] may be consistent with the local aggressiveness and propensity for the recurrence of sporadic OKC cases. However, data on such correlations with regard to syndromic OKCs are scarce.

An association with NBCCS is not the only reason believed to be responsible for the higher recurrence of OKC, as some clinicoradiological features have also been shown to be negative prognostic factors of recurrence. In our previous reports, we demonstrated that larger size, multilocularity and cortical perforation may be related to a relapse in sporadic cases of OKCs. We did not, however, reveal any prognostic significance of immunoexpression of Bcl-2, cyclin D1, p53, osteoprotegerin (OPG), PCNA, receptor activator of nuclear factor κ B (RANK), and receptor activator of nuclear factor κ B ligand (RANKL) for OKCs not associated with NBCCS.[17,18] Hitherto, little is known about whether clinicoradiological and pathological features or expression of immunohistochemical markers are also associated with recurrence in cases of syndromic OKCs.

Objectives

We aimed to compare the prognostic relevance of clinicoradiological and histopathological features, as well as the immunoexpression of COX-2, Bcl-2, PCNA, p53, Ki-67, OPG, RANK, and RANKL, and RANKL/OPG balance, between sporadic and syndromic OKCs. The secondary objective was to test whether immunoexpression of the aforementioned proteins is useful in distinguishing between the 2 types of OKC.

Materials and methods

Study design and patients

Forty-three cases of OKC who were not lost to follow-up were selected for this retrospective study. Patients were treated with simple enucleation in the Chair of Oral Surgery at the Jagiellonian University Medical College, Kraków, Poland, between 1997 and 2015. The surgical technique did not include any adjunct procedures (e.g., Carnoy's solution, liquid nitrogen, peripheral ostectomy, or application of regenerative graft materials) and was standardized among board-certified specialists in oral surgery with at least 5 years of practical experience. Among the 43 subjects, 31 were diagnosed with sporadic OKC and 12 with syndromic OKC. The NBCCS cases were diagnosed using the features described by Kimonis et al.[13] and were subsequently found to be in conformity with the Consensus Statement from the First International Colloquium on Basal Cell Nevus Syndrome.[14] All cases were confirmed to meet the current WHO criteria for OKC (i.e., presence of a fibrous wall lined by a folded, thin, regular parakeratinized epithelium 5–8-cell layers thick, without rete ridges, with corrugation of the parakeratin surface and a well-defined, palisaded basal layer, hyperchromatic nuclei, and focal areas showing reversed nuclear polarity).[3] Ethical approval was obtained from

the Bioethical Committee of Jagiellonian University (approval No. 1072.6120.73.2019).

To evaluate the potential clinicoradiological, histopathological and immunohistochemical prognostic factors, we employed the methods used in our previous studies.[17,18] At the time of diagnosis, panoramic and occlusal radiographs were taken, supplemented in most cases with computed tomography (CT). Follow-up appointments took place quarterly in the 1st postoperative year, and semi-annually starting from the 2nd postoperative year. Upon appointments, panoramic radiographs were performed. In the event of any signs of recurrence (e.g., expansion of bone and/or radiographic radiolucent zone in the area of the previous surgery), CT scans were performed. The interval between surgery and the detection of relapse was defined as the recurrence period. Each relapse was confirmed to meet the 2017 WHO microscopic criteria for OKC.[3]

The following clinicoradiological features were measured: age, gender, follow-up period, evidence of recurrence, size of the cyst in panoramic radiographs (determined by multiplying the major and minor axes), outline of the cyst on radiography (uni- or multilocular), and cortical perforation observed on occlusal radiographs or CT. The following histopathological features were investigated: number of satellite (daughter) cysts, inflammation intensity and presence of epithelial dysplasia. The position of the cyst was divided into anterior maxilla, posterior maxilla, anterior mandible, and posterior mandible. The posterior surface of the second premolar served as the division line between the anterior and posterior parts of the jaw.

Specimen characteristics and assay methods

Formalin-fixed, paraffin-embedded archival blocks were sectioned (5 μm in thickness) and stained with hematoxylin and eosin (H&E). Slides were used to corroborate a diagnosis of OKC and to appraise its histopathological features using a light microscope (Olympus BX40; Olympus Corp., Tokyo, Japan). The inflammatory score was calculated by counting the inflammatory cells and categorized with the 4-grade scoring system according to Kuroyanagi et al.:[1] grade 0 – no inflammation, grade 1 – fewer than 15 cells, grade 2 – 15–50 cells, and grade 3 – more than 50 cells in 10 high-power fields (HPFs).

For immunohistochemical analysis, 3-μm thick tissue sections were used. They were deparaffinized with xylene, rehydrated in graded alcohol and washed in deionized water. Retrieval of antigen was accomplished with Heat-Induced Epitope Retrieval Buffer (Thermo Fisher Scientific, Fremont, USA) at pH 6 or pH 9 for 20 min at 95°C. Subsequently, sections were blocked by incubation with 3% H_2O_2 and protein block (Thermo Fisher Scientific), followed by the incubation of the slides in a humidified chamber with one of the following antibodies:

– rabbit monoclonal COX-2 (SP21; Cell Marque, Rocklin, USA; 1:200) at room temperature for 45 min;
– rabbit monoclonal Bcl-2 (E17; Cell Marque; 1:300) at 4°C overnight;
– mouse monoclonal p53 (DO7; Cell Marque; 1:100) at room temperature for 50 min;
– rabbit polyclonal PCNA (RB-9055-P0; Thermo Fisher Scientific; 1:700) at room temperature for 30 min;
– mouse monoclonal Ki-67 (SP6; Cell Marque; 1:150) at room temperature for 30 min;
– mouse monoclonal anti-RANK (ab13918; Abcam, Waltham, USA; 1:200) at 4°C overnight;
– rabbit polyclonal anti-RANKL (ab169966; Abcam; 1:400) at 4°C overnight;
– rabbit polyclonal anti-OPG (ab183910; Abcam; 1:400) at 4°C overnight.

Next, the sections were washed in Tris-buffered saline (TBS) and treated in compliance with the manufacturer's instructions using the Primary Antibody Amplifier Quanto system, followed by the HRP Polymer Quanto system (both from Thermo Fisher Scientific). Then, they were stained using a 3-3'-diaminobenzidine Quanto kit (Thermo Fisher Scientific). Finally, the sections were counterstained with hematoxylin, dehydrated and coverslipped for further analyses. With regard to Bcl-2, p53 and PCNA, tonsil tissue was used as a positive control. For RANK, RANKL and OPG, a case of central giant cell granuloma was used as a positive control. For the negative control, the section treatment was similar, but there was no primary antibody exposure. For COX-2, RANK, RANKL, and OPG, a cytoplasmic staining pattern was detected. For Bcl-2, a membranous-cytoplasmic pattern was detected, and for p53, Ki-67 and PCNA, a nuclear pattern was detected.

For COX-2, Bcl-2, RANK, RANKL, and OPG, a semi-quantitative analysis with a 4-grade scoring system was employed, as follows: grade 0 – negative reaction (no stained cells), grade 1 – staining of 1–25% of cells, grade 2 – staining of 26–50% of cells, and grade 3 – more than 50% of stained cells in the epithelium. For PCNA, Ki-67 and p53, a quantitative assessment was applied. The number of positive-staining nuclear cells was counted in 10 HPFs (×400 magnification), and the mean value was used for the analysis. All histopathological and immunohistochemical analyses were completed by a board-certified specialist in pathomorphology (B. D.).

Statistical analyses

The clinicopathological and immunohistochemical data sets were evaluated against the frequency of relapse. The results were reported as mean ± standard deviation (M ±SD) and median (1st quartile–3rd quartile (Q1–Q3)). In the case of qualitative data, the χ^2 test or Fisher's exact test (when the χ^2 test assumption of large expected value was not met) were employed to analyze the differences in clinicoradiological and pathological data sets. With

regard to quantitative data, the Mann–Whitney U test was used. Nonparametric tests were used because of deviations from normality in quantitative variable distribution, as was shown by the results of the Shapiro–Wilk test ($p < 0.05$). To evaluate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) as estimates of risk for recurrence potential, the Cox proportional hazard model for time-dependent variables was utilized. The p-values less than 0.05 were considered statistically significant. All analyses were performed using R software v. 4.1.3[19] (R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/) with the "survival" package.[20,21]

Results

The follow-up period for all analyzed OKCs was 104.09 ±46.97 months (110.71 ±52.28 months and 87 ±22.95 months for sporadic and syndromic lesions, respectively; U = 234, p = 0.123; Mann–Whitney U test). The recurrence period for the entire analyzed group was 77.14 ±31.88 months (69.82 ±36.72 months and 85.2 ±24.95 months for sporadic and syndromic lesions, respectively; U = 43.5, p = 0.434; Mann–Whitney U test). Of all the OKC cases, 48.84% recurred; however, syndromic cases were significantly more prone to recur than sporadic cases (83.33% compared to 35.48%; p = 0.013; Fisher's exact test). An association with NBCCS significantly increased the risk of OKC recurrence (HR: 9.091, 95% CI: 1.682–49.123; p = 0.01).

There were no gender differences between sporadic and syndromic OKCs, but the patients with syndromic OKCs were significantly younger than those with sporadic lesions (Table 1). Also, there were no significant differences between sporadic and syndromic OKCs in regard to radiological size of the lesion, locularity, cortical perforation, histological number of daughter cysts, inflammatory score, and epithelial dysplasia grading (Table 1). However, there were significant differences in PCNA, p53 and OPG immunoexpression between sporadic and syndromic lesions (Table 2). Details of immunoexpression of PCNA, p53 and OPG are shown in Fig. 1–3.

The clinicoradiological, histopathological and immunohistochemical characteristics of sporadic cases are presented in Table 2. Recurrent sporadic OKCs were significantly larger (p = 0.002) and markedly more often associated with cortical perforation (p = 0.038). In fact, sporadic lesions with radiological signs of cortical perforation were 6.8 times more likely to recur. With regard to immunostaining, recurrent OKCs had significantly increased the immunoexpression of COX-2. However, by using the Cox proportional hazard model, we did not find COX-2 significance to be a prognostic marker (Table 2). Details of COX-2 immunoexpression are shown in Fig. 4. Because of the fact that all of the non-recurrent sporadic OKCs were negative for epithelial dysplasia, the HR for this group was indefinite (Table 2).

The clinicoradiological, histopathological and immunohistochemical characteristics of the syndromic cases are presented in Table 3. Of the 12 syndromic cases, only 2 did not relapse. Among the analyzed factors, we did not find any significant differences between non-recurrent and recurrent lesions, with the exception of RANKL/OPG ratio, where RANKL predominance was particularly marked in recurrent OKCs (p = 0.015). Details of RANKL immunoexpression are shown in Fig. 5. Due to the small number of non-recurrent cases and the lack of non-recurrent cases for some groups, the HRs for several clinicoradiological (gender, cyst location and cortical perforation), histopathological (epithelial dysplasia) and immunohistochemical (RANK, RANKL, RANKL/OPG ratio) features were indefinite (Table 3). The complete characteristics of the recurrent sporadic and syndromic cases are presented in Table 4,5. Details of immunoexpression of Bcl-2, Ki-67 and RANK are shown in Fig. 6–8.

Discussion

The OKC is the main feature observed in patients with NBCCS. The possible reasons for its high recurrence rate have been a subject of debate for several decades. Authors have linked the recurrence rate to treatment methods[22,23] and association with NBCCS.[24] Recent evidence has attributed OKC relapse to some clinicoradiological features, such as association with dentition,[25] larger size, multilocularity, and cortical perforation,[17] or to some pathological features, mainly the presence of daughter cysts.[26] Moreover, the expression of various markers have been tested against the recurrence rate, and a few have proved to be significantly associated with cyst relapse.[1,15,16] In the present study, we endeavored to compare the clinicopathological features of sporadic and syndromic cases of OKC, and test whether the expression of some previously considered markers was related to recurrence.

Most authors agree that OKCs associated with NBCCS generally exhibit greater aggressiveness and higher propensity for recurrence.[27] The results of several studies support the existence of distinct biological behaviors of syndromic and sporadic OKCs.[28–32] However, only Titinchi and Nortje[24] demonstrated that there is a statistically significant higher recurrence rate in syndromic cases of OKC.[24] Our results indicate that there is a strong association between NBCCS and OKC recurrence. In fact, in the current study, the association of OKC with NBCCS increased the risk of relapse by 9.091 times. However, this observation should be considered with caution, as OKCs in patients affected with NBCCS are typically multifocal and tend to appear metachronously. Thus, it may be difficult to unequivocally determine whether the new lesion is, in fact, a recurrence of a previously treated cyst or a new development. Other authors have also pointed out that it may be difficult to correctly estimate the recurrence rate of syndromic cases of OKC

Table 1. Comparative clinicoradiological, histopathological and immunohistochemical characteristics of the sporadic and syndromic cases of OKC

Variables		Sporadic OKC (n = 31)	Syndromic OKC (n = 12)	Total (n = 43)	p-value
Gender	female male	13 (41.94%) 18 (58.06%)	5 (41.67%) 7 (58.33%)	18 (41.86%) 25 (58.14%)	$\chi^2 = 0.000$, p = 1***
Age at the time of diagnosis [years]	M ±SD median Q1–Q3	40.23 ±17.18 39 26.0–51.5	18.42 ±5.79 17 13.5–23.0	34.14 ±17.82 30 19–51	U = 329, **p < 0.001***
Cyst location	posterior maxilla anterior maxilla posterior mandible anterior mandible	2 (6.45%) 2 (6.45%) 20 (64.52%) 7 (22.58%)	5 (41.67%) 0 (0.00%) 5 (41.67%) 2 (16.67%)	7 (16.28%) 2 (4.65%) 25 (58.14%) 9 (20.93%)	p = 0.053**
Size of lesion [mm^2]	M ±SD median Q1–Q3	504.06 ±276.37 396 315–647	487.08 ±282.23 397.5 345–531.25	499.33 ±274.74 396 315–594	U = 182.5, p = 0.935*
Lesion type	unilocular multilocular	21 (67.74%) 10 (32.26%)	7 (58.33%) 5 (41.67%)	28 (65.12%) 15 (34.88%)	p = 0.723**
Cortical perforation	negative positive	22 (70.97%) 9 (29.03%)	6 (50.00%) 6 (50.00%)	28 (65.12%) 15 (34.88%)	p = 0.287**
Number of daughter cysts	M ±SD median Q1–Q3	0.77 ±1.57 0 0–0	7.08 ±9.89 0 0–11.5	2.57 ±6.02 0 0–1.75	U = 129.5, p = 0.081*
Inflammatory score	grade 0 grade 1 grade 2 grade 3	14 (45.16%) 9 (29.03%) 7 (22.58%) 1 (3.23%)	2 (16.67%) 3 (25.00%) 6 (50.00%) 1 (8.33%)	16 (37.21%) 12 (27.91%) 13 (30.23%) 2 (4.65%)	p = 0.084**
Epithelial dysplasia	negative low-grade high-grade	29 (93.55%) 1 (3.23%) 1 (3.23%)	9 (75.00%) 3 (25.00%) 0 (0.00%)	38 (88.37%) 4 (9.30%) 1 (2.33%)	p = 0.063**
PCNA	M ±SD median Q1–Q3	19.07 ±8.26 18.5 13.62–24	27.17 ±10.91 30.5 19.12–34	21.38 ±9.69 19.75 14–27.25	U = 97, **p = 0.022***
p53	M ±SD median Q1–Q3	11.8 ±6.59 11.25 7.0–17.5	17.08 ±5.93 17 14.12–19	13.31 ±6.78 12.25 9–18	U = 98.5, **p = 0.024***
Ki-67	M ±SD median Q1–Q3	13.92 ±6.69 12 10.50–17.38	13.2 ±3.7 12.75 11.25–14.25	13.71 ±5.96 12.25 10.50–17.38	U = 175, p = 0.9*
Bcl-2	grade 0 grade 1 grade 2 grade 3	10 (32.25%) 15 (48.39%) 5 (16.13%) 1 (3.23%)	0 (0.00%) 9 (75.00%) 3 (25.00%) 0 (0.00%)	10 (23.25%) 24 (55.81%) 8 (18.60%) 1 (2.33%)	p = 0.215**
COX-2	grade 0 grade 1 grade 2 grade 3	6 (19.35%) 5 (16.13%) 10 (32.26%) 10 (32.26%)	0 (0.00%) 5 (41.67%) 2 (16.67%) 5 (41.67%)	6 (13.95%) 10 (23.25%) 12 (27.91%) 15 (34.88%)	p = 0.26**
RANK	grade 0 grade 1 grade 2 grade 3	17 (54.83%) 12 (38.71%) 2 (6.45%) 0 (0.00%)	6 (50.00%) 4 (33.33%) 2 (16.67%) 0 (0.00%)	23 (53.48%) 16 (37.21%) 4 (9.30%) 0 (0.00%)	p = 0.694**
RANKL	grade 0 grade 1 grade 2 grade 3	1 (3.23%) 7 (22.58%) 9 (29.03%) 14 (45.16%)	0 (0.00%) 1 (8.33%) 3 (25.00%) 8 (66.67%)	1 (2.33%) 8 (18.60%) 12 (27.91%) 22 (51.16%)	p = 0.438**
OPG	grade 0 grade 1 grade 2 grade 3	3 (9.67%) 19 (61.29%) 3 (9.67%) 6 (19.35%)	1 (8.33%) 1 (8.33%) 6 (50.00%) 4 (33.33%)	4 (9.30%) 20 (46.51%) 9 (20.93%) 10 (23.25%)	**p = 0.001****
RANKL/OPG ratio	RANKL < OPG RANKL = OPG RANKL > OPG	1 (3.23%) 14 (45.16%) 16 (51.61%)	2 (16.67%) 4 (33.33%) 6 (50.00%)	3 (6.98%) 18 (41.86%) 22 (51.16%)	p = 0.367**

*Mann–Whitney U test, **Fisher's exact test, ***χ^2 test. Statistically significant values are bolded. Q1 – lower quartile; Q3 – upper quartile; M ±SD – mean ± standard deviation; OKC – odontogenic keratocyst; RANKL – receptor activator for nuclear factor κ B ligand; OPG – osteoprotegerin; PCNA – proliferating cell nuclear antigen; RANK – receptor activator of nuclear factor κ B.

Table 2. Comparative clinicoradiological, histopathological and immunohistochemical characteristics of non-recurrent and recurrent sporadic OKC cases with risk for recurrence

Variables		Non-recurrent sporadic OKC (n = 20)	Recurrent sporadic OKC (n = 11)	p-value	Cox proportional hazard model	
					HR (95% CI)	p-value
Gender	female (n = 13) male (n = 18)	9 (45.00%) 11 (55.00%)	4 (36.36%) 7 (63.64%)	p = 0.718**	1.00 1.432 (0.316–6.492)	0.642
Age [years]	M ±SD median Q1–Q3	40.5 ±16.02 46 28.5–51.25	39.73 ±19.94 36 26–55	U = 117, p = 0.788*	0.997 (0.955–1.042)	0.903
Cyst location	posterior maxilla (n = 2) anterior maxilla (n = 2) posterior mandible (n = 20) anterior mandible (n = 7)	1 (5.00%) 2 (10.00%) 13 (65.00%) 4 (20.00%)	1 (9.09%) 0 (0.00%) 7 (63.64%) 3 (27.27%)	p = 0.923**	1.00 NR 1.615 (0.14–18.581) 2.25 (0.149–33.933)	– – 0.7 0.558
Size of lesion [mm^2]	M ±SD median Q1–Q3	370.75 ±130.55 367.5 270–420	746.45 ±310.79 760 522–987.5	U = 34.5, **p = 0.002***	1.007 (1.002–1.012)	**0.005**
Lesion type	unilocular (n = 21) multilocular (n = 10)	15 (75.00%) 5 (25.00%)	6 (54.55%) 5 (45.45%)	p = 0.423**	1.00 2.5 (0.525–11.894)	0.25
Cortical perforation	negative (n = 22) positive (n = 9)	17 (85.00%) 3 (15.00%)	5 (45.45%) 6 (54.55%)	**p = 0.038****	1.00 6.8 (1.233–37.497)	**0.028**
Number of daughter cysts	M ±SD median Q1–Q3	1 ±1.73 0 0–1.5	0.36 ±1.21 0 0–0	U = 127, p = 0.201*	0.721 (0.387–1.341)	0.301
Inflammatory score	grade 0 (n = 15) grade 1 (n = 9) grade 2 (n = 7) grade 3 (n = 0)	10 (50.00%) 6 (30.00%) 4 (20.00%) 0 (0.00%)	5 (45.45%) 3 (27.27%) 3 (27.27%) 0 (0.00%)	p = 1**	1.00 0.9 (0.154–5.258) 1.35 (0.211–8.617) NR	– 0.907 0.751 –
Epithelial dysplasia	negative (n = 30) low-grade (n = 1) high-grade (n = 0)	20 (100.00%) 0 (0.00%) 0 (0.00%)	10 (90.91%) 1 (9.09%) 0 (0.00%)	p = 0.367**	indefinite	
PCNA	M ±SD median Q1–Q3	18.55 ±9.4 18 13.5–22.75	19.95 ±6.14 23 13.75–24.5	U = 89, p = 0.518*	1.021 (0.932–1.12)	0.65
p53	M ±SD median Q1–Q3	11.08 ±7 10.5 6–17	13.05 ±5.92 11.5 9.5–15.5	U = 86.5, p = 0.451*	1.048 (0.933–1.178)	0.427
Ki-67	M ±SD median Q1–Q3	12.61 ±5.23 12 10–15.75	16.18 ±8.48 16.5 10.5–18.5	U = 83, p = 0.365*	1.09 (0.961–1.237)	0.179
Bcl-2	grade 0 (n = 10) grade 1 (n = 15) grade 2 (n = 5) grade 3 (n = 1)	8 (40.00%) 9 (45.00%) 3 (15.00%) 0 (0.00%)	2 (18.18%) 6 (50.00%) 2 (18.18%) 1 (9.09%)	p = 0.362**	1.00 4 (0.379–42.177) 6 (0.422–85.248) 6 (0.422–85.248)	– – 0.249 0.186
COX-2	grade 0 (n = 6) grade 1 (n = 5) grade 2 (n = 10) grade 3 (n = 10)	5 (25.00%) 5 (25.00%) 7 (35.00%) 3 (15.00%)	1 (9.09%) 0 (0.00%) 3 (27.27%) 7 (63.64%)	**p = 0.042****	1.00 NR 0.857 (0.055–13.479) 4.667 (0.297–73.384)	– – 0.913 0.273
RANK	grade 0 (n = 17) grade 1 (n = 12) grade 2 (n = 2) grade 3 (n = 0)	13 (65.00%) 7 (35.00%) 0 (0.00%) 0 (0.00%)	4 (36.36%) 5 (45.45%) 2 (18.18%) 0 (0.00%)	p = 0.126**	1.00 3 (0.642–14.023) 3 (0.642–14.023) NR	– 0.163 0.163 –
RANKL	grade 0 (n = 1) grade 1 (n = 7) grade 2 (n = 9) grade 3 (n = 14)	1 (5.00%) 6 (30.00%) 6 (30.00%) 7 (35.00%)	0 (0.00%) 1 (9.09%) 3 (27.27%) 7 (63.64%)	p = 0.304**	NR 1.00 3 (0.239–37.672) 6 (0.565–63.676)	– – 0.395 0.137
OPG	grade 0 (n = 3) grade 1 (n = 19) grade 2 (n = 3) grade 3 (n = 6)	2 (10.00%) 13 (65.00%) 2 (10.00%) 3 (15.00%)	1 (9.09%) 6 (54.55%) 1 (9.09%) 3 (27.27%)	p = 0.635**	1.00 1.00 0.929 (0.071–12.136) 1.857 (0.293–11.756)	– – 0.955 0.511
RANKL/OPG ratio	RANKL < OPG RANKL = OPG RANKL > OPG	2 (10.00%) 9 (45.00%) 9 (45.00%)	1 (9.09%) 3 (27.27%) 7 (63.64%)	p = 0.24**	1.00 1.00 1.75 (0.376–8.14)	0.476

*Mann–Whitney U test, **Fisher's exact test. Statistically significant values are bolded. Q1 – lower quartile; Q3 – upper quartile; M ±SD – mean ± standard deviation; NR – no recurrence; HR – hazard ratio; 95% CI – 95% confidence interval; OKC – odontogenic keratocyst; RANKL – receptor activator for nuclear factor κ B ligand; OPG – osteoprotegerin; PCNA – proliferating cell nuclear antigen; RANK – receptor activator of nuclear factor κ B.

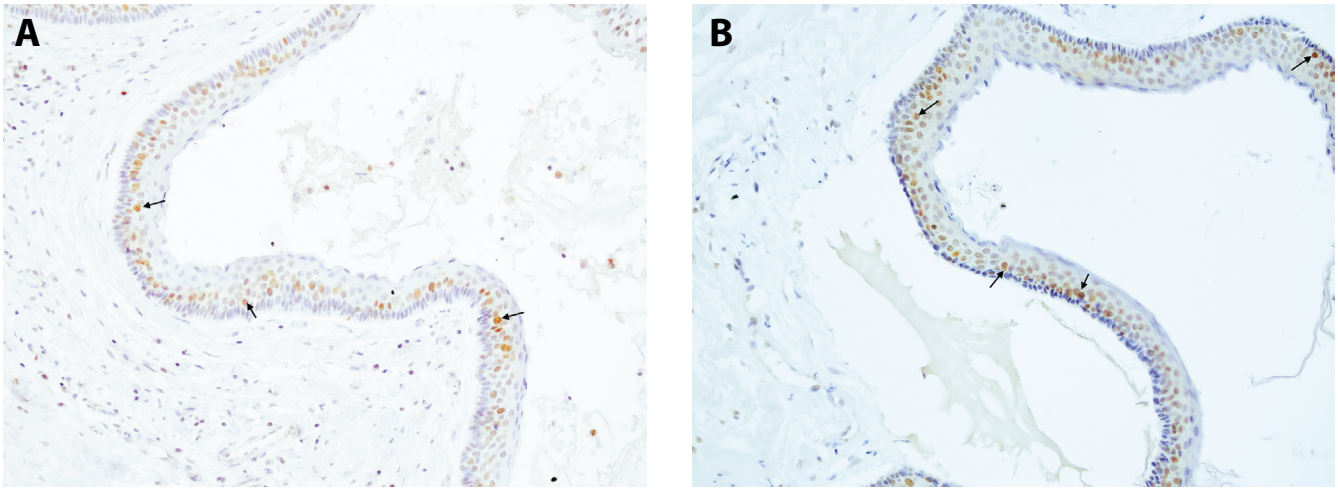

Fig. 1. Proliferating cell nuclear antigen (PCNA) – nuclear brown staining (arrows) in the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×200 magnification)

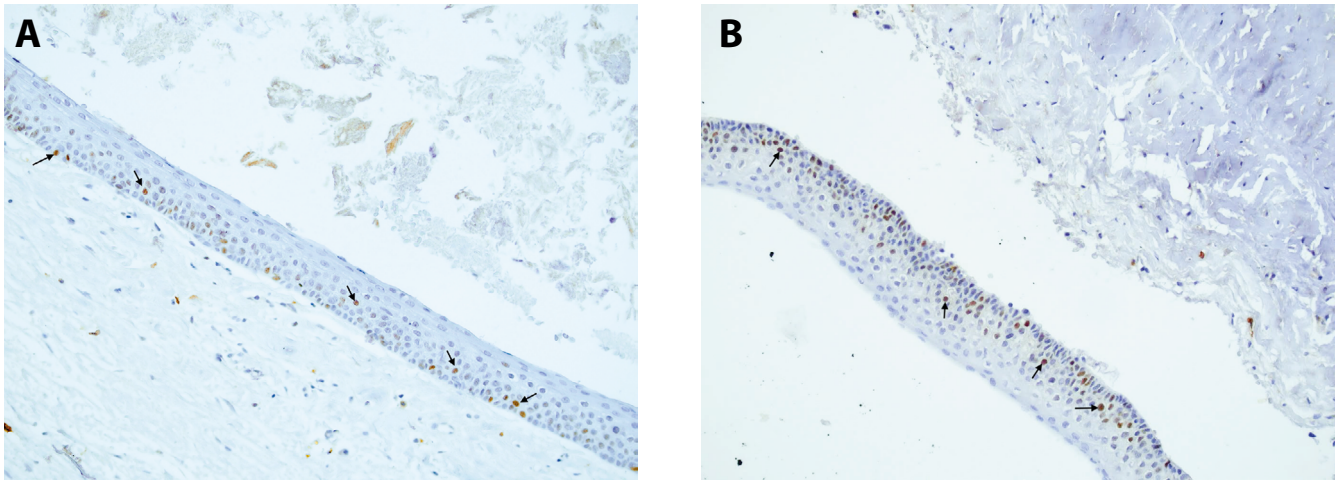

Fig. 2. p53 – nuclear brown staining (arrows) in the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×200 magnification)

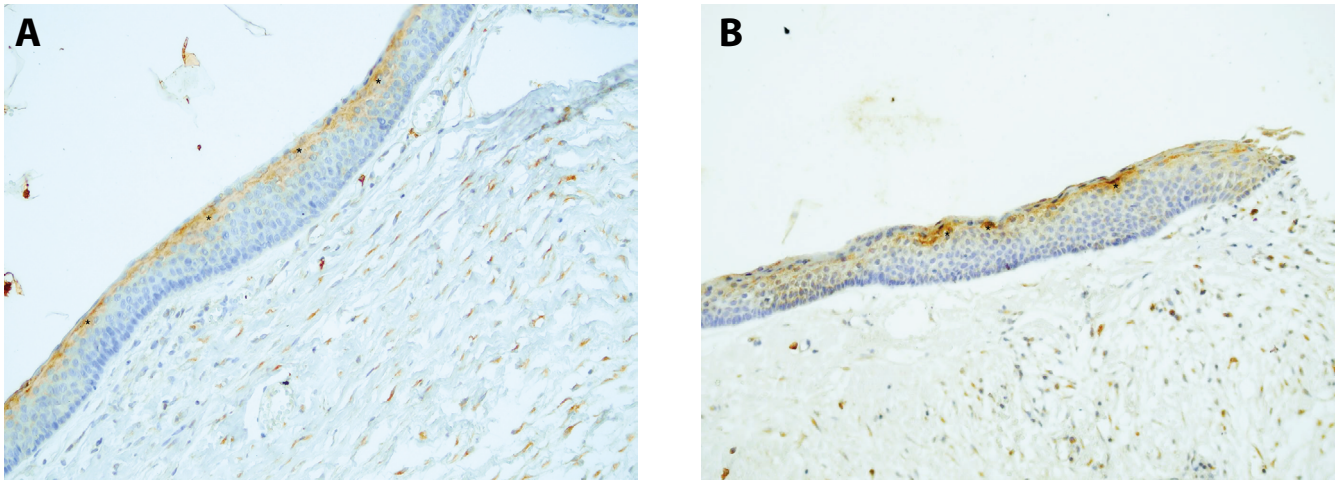

Fig. 3. Osteoprotegerin (OPG) – cytoplasmic brown staining in the superficial layer (asterisks) of the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×200 magnification)

for the above reasons.[24,33,34] Accordingly, it would appear reasonable to conduct further research into the molecular background of the distinct pathobiology of syndromic OKCs.

In our previous report, we demonstrated that larger size, multilocularity and radiological evidence of cortical perforation were correlated to a higher risk of recurrence

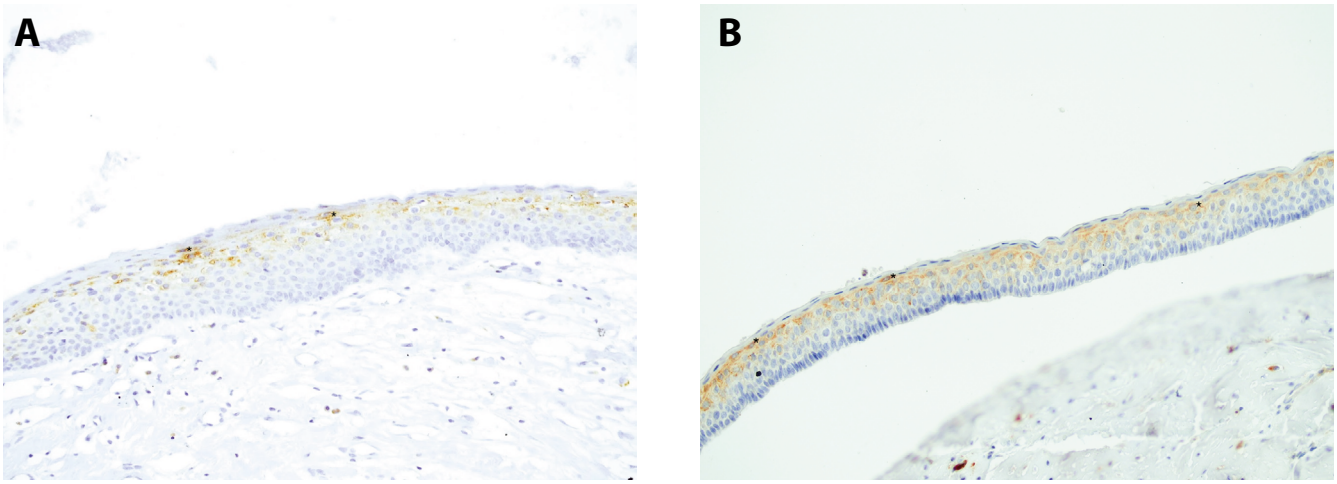

Fig. 4. COX-2 – cytoplasmic brown staining in the superficial layer (asterisks) of the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×200 magnification)

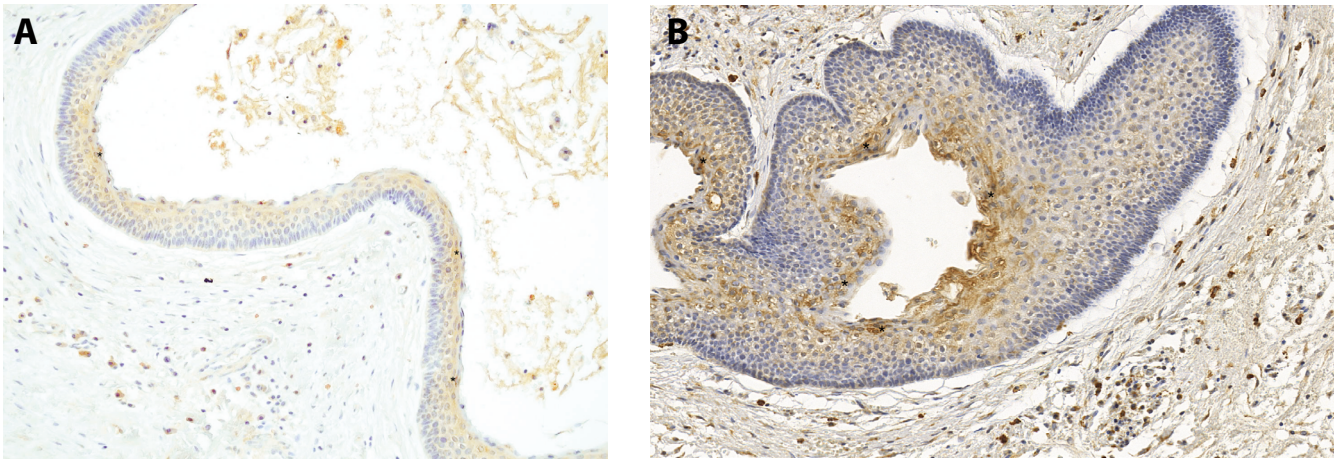

Fig. 5. Receptor activator for nuclear factor κ B ligand (RANKL) – cytoplasmic brown staining in the superficial layer (asterisks) of the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×50 magnification)

of sporadic OKCs.[17] The current results partially support these findings. The issue of cyst size and multilocularity have been addressed by many authors, and it has been shown that smaller lesions are usually unilocular, but with their expansion, they tend to become multilocular.[34,35] Larger lesions are more difficult to access, and epithelial residue may be a starting point of relapse. Recently, Fidele et al. suggested that the lining of larger OKCs is more thin and fragile and, thus, more prone to recur.[36] This is in contrast with smaller lesions, which are easier to enucleate in one piece. The authors also found a significantly higher recurrence rate for cysts whose diameter exceeded 4 cm.[36] With regard to syndromic cases, however, we did not find a significant influence of cyst size on recurrence rate, which may be due to the limited number of syndromic cases in the current series, as well as the fact that there might be disparate biological factors responsible for the relapse of NBCCS-associated counterparts of OKCs.

In the current study, sporadic OKCs with cortical perforation were 6.8 times more likely to recur. Our previous hypothesis[17] stating that this radiological sign is a significant prognostic factor has thus far been supported by the results of the study by Fidele et al.,[36] who found that cortical perforation, particularly combined with teeth involvement, is markedly associated with a high recurrence rate. The presence of either may lead to an incomplete surgical removal and subsequent relapse, since cortical perforation suggests the involvement of adjacent soft tissues, and close proximity to dentition (especially when the treatment plan entails teeth preservation) may be related to the involvement of the periodontal space. However, in the current study, we did not find significant differences in cortical perforation between recurrent and non-recurrent syndromic OKCs. In fact, all of the non-recurrent syndromic cases were negative for cortical perforation, which is why the HR for recurrence was indefinite. Interestingly, 50% of the syndromic cases exhibited cortical perforation (in contrast to 29.03% of the sporadic cases), which may be another reason for the high recurrence rate of NBCCS-associated cases. Contrary to the results of our previous report,[17] we did not demonstrate that multilocularity is associated with higher recurrence rates

Table 3. Comparative clinicoradiological, histopathological and immunohistochemical characteristics of non-recurrent and recurrent syndromic OKC cases with a risk for recurrence

Variables		Non-recurrent syndromic OKC (n = 2)	Recurrent syndromic OKC (n = 10)	p-value	Cox proportional hazard model	
					HR (95% CI)	p-value
Gender	female (n = 5) male (n = 7)	0 (0.00%) 2 (100.0%)	5 (50.00%) 5 (50.00%)	p = 0.47**	indefinite	
Age [years]	M ±SD median Q1–Q3	17 ±0 17 17–17	18.7 ±6.36 18 12.5–23.0	U = 9, p = 0.913*	1.062 (0.787–1.432)	0.695
Cyst location	posterior maxilla (n = 5) anterior maxilla (n = 0) posterior mandible (n = 5) anterior mandible (n = 12)	0 (0.00%) 0 (0.00%) 2 (100.0%) 0 (0.00%)	5 (50.00%) 0 (0.00%) 3 (30.00%) 2 (20.00%)	p = 0.621**	indefinite	
Size of lesion [mm^2]	M ±SD median Q1–Q3	397.5 ±31.82 397.5 386.25–408.75	505 ±308.38 412.5 315–543.75	U = 9.5, p = 1*	1.003 (0.992–1.014)	0.637
Lesion type	unilocular (n = 7) multilocular (n = 5)	1 (50.00%) 1 (50.00%)	6 (60.00%) 4 (40.00%)	p = 1**	1.00 0.667 (0.032–14.033)	0.794
Cortical perforation	negative (n = 6) positive (n = 6)	2 (100.0%) 0 (0.00%)	4 (40.00%) 6 (60.00%)	p = 0.455**	indefinite	
Number of daughter cysts	M ±SD median Q1–Q3	12.5 ±17.68 12.5 6.25–18.75	6 ±8.77 0 0–9	U = 13, p = 0.548*	0.936 (0.805–1.09)	0.395
Inflammatory score	grade 0 (n = 2) grade 1 (n = 3) grade 2 (n = 6) grade 3 (n = 0)	0 (0.00%) 1 (50.00%) 1 (50.00%) 0 (0.00%)	2 (20.00%) 2 (20.00%) 5 (50.00%) 1 (10.00%)	p = 1**	1.00 1.00 1.5 (0.071–31.575) 1.5 (0.071–31.575)	0.794
Epithelial dysplasia	negative (n = 9) low-grade (n = 3) high-grade (n = 0)	2 (100.0%) 0 (0.00%) 0 (0.00%)	7 (70.00%) 3 (30.00%) 0 (0.00%)	p = 1**	indefinite	
PCNA	M ±SD median Q1–Q3	26.5 ±9.9 26.5 23–30	27.3 ±11.6 30.5 18.75–35.00	U = 9.5, p = 1*	1.007 (0.872–1.164)	0.921
p53	M ±SD median Q1–Q3	16.25 ±2.47 16.25 15.38–17.12	17.25 ±6.49 17 13.38–20.75	U = 10.5, p = 1*	1.032 (0.784–1.36)	0.821
Ki-67	M ±SD median Q1–Q3	10.75 ±1.77 10.75 10.12–11.38	13.69 ±3.84 13 11.75–16.75	U = 4, p = 0.234*	1.38 (0.73–2.608)	0.321
Bcl-2	grade 0 (n = 0) grade 1 (n = 9) grade 2 (n = 3) grade 3 (n = 0)	0 (0.00%) 1 (50.00%) 1 (50.00%) 0 (0.00%)	0 (0.00%) 8 (80.00%) 2 (20.00%) 0 (0.00%)	p = 0.455**	NR 1.00 0.25 (0.01–5.985) NR	0.392
COX-2	grade 0 (n = 0) grade 1 (n = 5) grade 2 (n = 2) grade 3 (n = 5)	0 (0.00%) 1 (50.00%) 1 (50.00%) 0 (0.00%)	0 (0.00%) 4 (40.00%) 1 (10.00%) 5 (50.00%)	p = 0.621**	NR 1.00 1.5 (0.071–31.575) 1.5 (0.071–31.575)	0.794
RANK	grade 0 (n = 6) grade 1 (n = 4) grade 2 (n = 2) grade 3 (n = 0)	2 (100.0%) 0 (0.00%) 0 (0.00%) 0 (0.00%)	4 (40.00%) 4 (40.00%) 2 (20.00%) 0 (0.00%)	p = 0.636**	indefinite	
RANKL	grade 0 (n = 0) grade 1 (n = 1) grade 2 (n = 3) grade 3 (n = 8)	0 (0.00%) 1 (50.00%) 1 (50.00%) 0 (0.00%)	0 (0.00%) 0 (0.00%) 2 (20.00%) 8 (80.00%)	p = 0.091**	indefinite	
OPG	grade 0 (n = 1) grade 1 (n = 1) grade 2 (n = 6) grade 3 (n = 4)	0 (0.00%) 0 (0.00%) 1 (50.00%) 1 (50.00%)	1 (10.00%) 1 (10.00%) 5 (50.00%) 3 (30.00%)	p = 1**	1.00 1.00 1.00 0.429 (0.02–9.364)	0.59
RANKL/OPG ratio	RANKL < OPG RANKL = OPG RANKL > OPG	2 (100.0%) 0 (0.00%) 0 (0.00%)	0 (0.00%) 4 (40.00%) 6 (60.00%)	**p = 0.015****	indefinite	

*Mann–Whitney U test, **Fisher's exact test. Statistically significant values are bolded. Q1 – lower quartile; Q3 – upper quartile; M ±SD – mean ± standard deviation; NR – no recurrence; HR – hazard ratio; 95% CI – 95% confidence interval; OKC – odontogenic keratocyst; RANKL – receptor activator for nuclear factor κ B ligand; OPG – osteoprotegerin; PCNA – proliferating cell nuclear antigen; RANK – receptor activator of nuclear factor κ B.

Table 4. Clinicoradiological, histopathological and immunohistochemical characteristics of the recurrent sporadic cases of OKC

Patient No.	Age [years]	Sex	Location	Recurrence period [months]	Lesion type	Size of lesion [mm^2]	Cortical perforation	Inflammatory score	Number of daughter cysts	Epithelial dysplasia	PCNA [%]	p53 [%]	Ki-67 [%]	Bcl-2 [grade]	COX-2 [grade]	RANK [grade]	RANKL [grade]	OPG [grade]
1.	48	F	A-Mn	24	UL	950	(+)	2	0	(–)	10.5	6	10.5	1	2	0	2	1
2.	36	F	P-Mn	36	UL	760	(–)	0	0	(–)	13	13	16.5	2	2	0	3	1
3.	26	M	P-Mn	120	UL	450	(–)	2	0	(–)	14	25	36.5	2	3	1	2	3
4.	15	M	P-Mn	72	ML	1148	(+)	0	0	(–)	23	11.5	17	1	3	0	1	1
5.	26	F	P-Mx	108	UL	330	(–)	1	4	(–)	26	12	17.5	3	3	1	3	1
6.	19	M	A-Mn	108	ML	950	(+)	1	0	(+)	24	18	18.5	1	2	1	3	3
7.	62	F	P-Mn	108	UL	240	(–)	0	0	(–)	25	10	10	0	3	1	3	2
8.	36	M	P-Mn	36	ML	594	(+)	1	0	(–)	13.5	11	10.5	1	3	0	3	1
9.	73	M	A-Mn	24	ML	1025	(+)	1	0	(–)	27.5	7	10.5	0	3	2	3	1
10.	67	M	P-Mn	60	ML	1064	(+)	0	0	(–)	19	21	6	1	0	1	2	0
11.	29	M	P-Mn	72	UL	700	(–)	0	2	(–)	24	9	19.5	0	3	2	3	3

A-Mn – anterior mandible; P-Mn – posterior mandible; P-Mx – posterior maxilla; UL – unilocular; ML – multilocular; M – male; F – female; OKC – odontogenic keratocyst; RANKL – receptor activator for nuclear factor κ B ligand; OPG – osteoprotegerin; PCNA – proliferating cell nuclear antigen; RANK – receptor activator of nuclear factor κ B.

Table 5. Clinicoradiological, histopathological and immunohistochemical characteristics of the recurrent syndromic cases of OKC

Patient No.	Age [years]	Sex	Location	Recurrence period [months]	Lesion type	Size of lesion [mm^2]	Cortical perforation	Inflammatory score	Number of daughter cysts	Epithelial dysplasia	PCNA [%]	p53 [%]	Ki-67 [%]	Bcl-2 [grade]	COX-2 [grade]	RANK [grade]	RANKL [grade]	OPG [grade]
1.	25	F	P-Mx	96	UL	300	(–)	1	0	(–)	7.5	7.5	7.4	1	3	0	2	2
2.	12	M	P-Mn	24	UL	600	(+)	2	0	(+)	14	10.5	19.5	1	3	1	3	3
3.	14	M	P-Mx	96	UL	270	(–)	1	9	(+)	21	27	18	2	3	2	3	2
4.	19	F	A-Mn	72	ML	1320	(+)	2	0	(–)	18	22	11.5	1	1	1	2	1
5.	12	M	P-Mx	96	UL	375	(–)	0	0	(–)	33.5	17	10.5	1	1	1	3	0
6.	30	M	P-Mx	84	UL	360	(–)	2	9	(–)	31	13	18.5	2	3	2	3	3
7.	17	M	P-Mx	96	ML	450	(+)	2	19	(+)	41.5	17	13	1	1	0	3	2
8.	12	F	P-Mn	120	ML	550	(+)	3	0	(–)	35.5	17	13	1	1	0	3	2
9.	23	F	P-Mn	84	UL	300	(+)	0	0	(–)	30	14.5	13	1	3	1	3	3
10.	23	F	A-Mn	84	ML	525	(+)	2	23	(–)	41	27	12.5	1	2	0	3	2

A-Mn – anterior mandible; P-Mn – posterior mandible; P-Mx – posterior maxilla; UL – unilocular; ML – multilocular; M – male; F – female; OKC – odontogenic keratocyst; RANKL – receptor activator for nuclear factor κ B ligand; OPG – osteoprotegerin; PCNA – proliferating cell nuclear antigen; RANK – receptor activator of nuclear factor κ B.

of OKCs. The published results in this regard are ambiguous. On the one hand, Yagyuu et al. found a significantly higher propensity for relapse of multilocular OKC compared to its unilocular counterpart (recurrence rate 35.7% compared to 7.4%).[37] Similar results were demonstrated in a study by Tabrizi et al., who calculated the recurrence rate of multilocular OKC at 62.16%, compared to 5.5% for unilocular lesions.[38] On the other hand, de França et al. found a similar frequency of relapse between unilocular and multilocular OKCs (44.4% compared to 55.6%), and did not show that locularity of the cyst has any significant influence on relapse.[39] The ambiguity of our own results and of the published data suggests that, in essence, there may be a combination of several factors having an impact on OKC recurrence, including size, locularity and association with dentition. Thus, it seems reasonable that future research should focus on testing these factors – taken collectively or in different combinations.

The results of some previous studies suggest that COX-2 is overexpressed in OKC[40] and may serve as an important marker involved in local aggressiveness and recurrence of the lesion.[41,42] Herein, we showed that COX-2

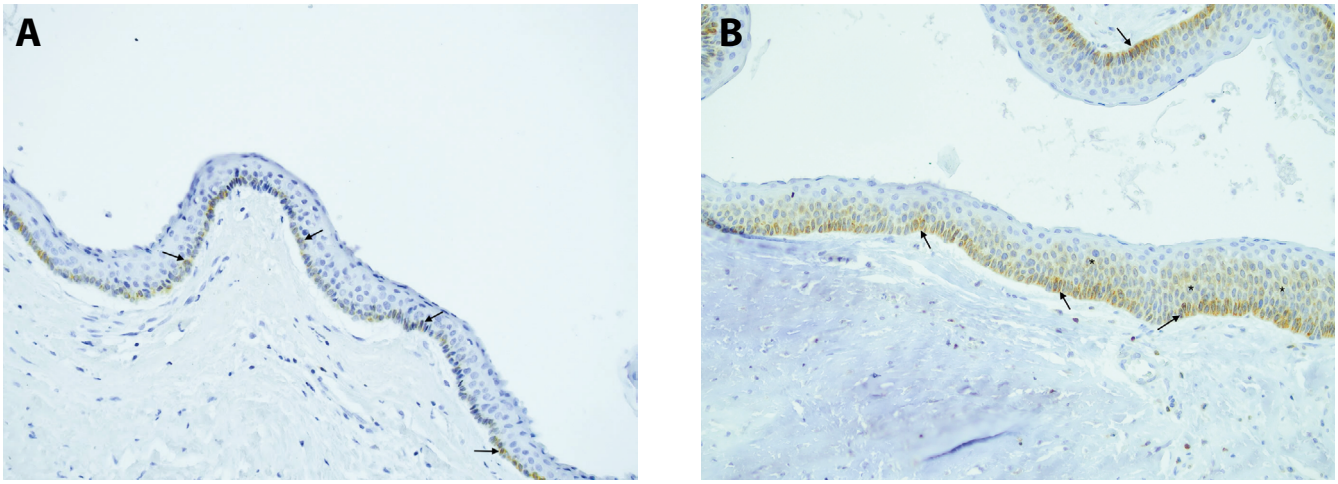

Fig. 6. Bcl-2 – membranous-cytoplasmic brown staining in the basal (arrows) and suprabasal (asterisks) layers of the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×200 magnification)

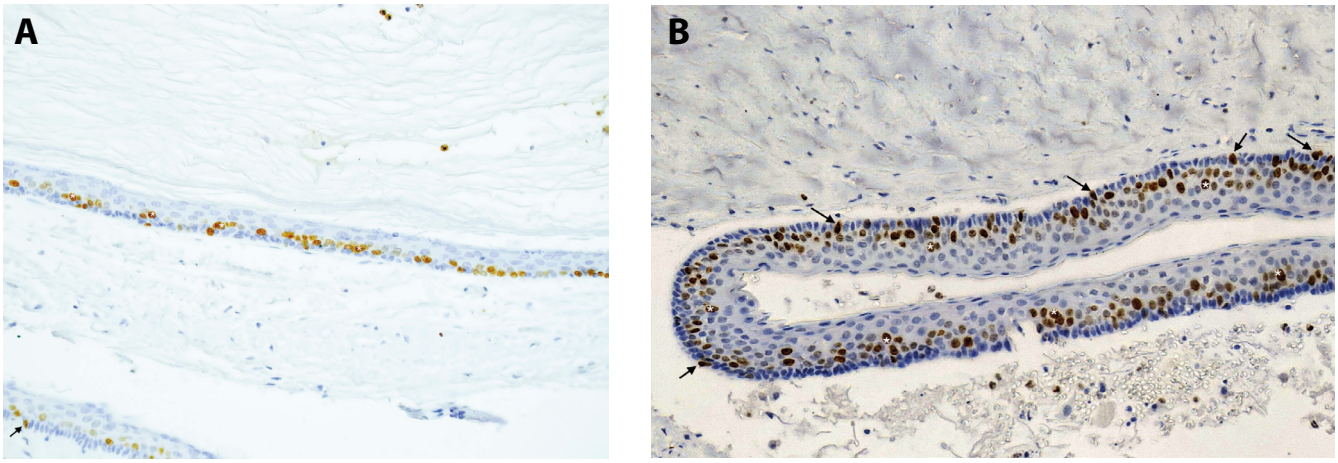

Fig. 7. Ki-67 – nuclear brown staining in the basal (arrows) and suprabasal/superficial (asterisks) layers of the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×60 magnification)

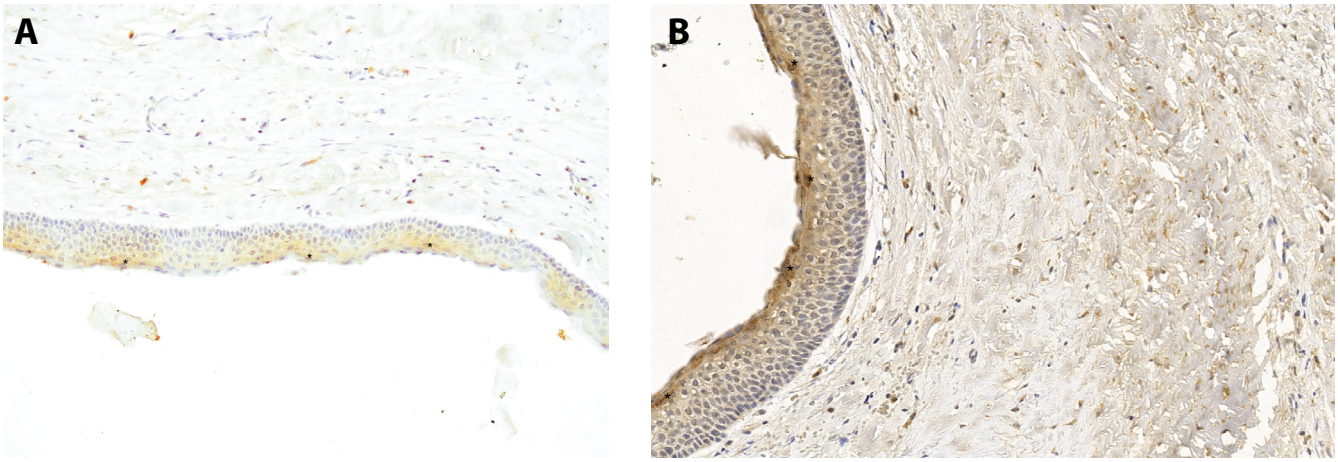

Fig. 8. Receptor activator of nuclear factor κ B (RANK) – cytoplasmic brown staining in the superficial layer (asterisks) of the epithelial lining of sporadic (A) and syndromic (B) odontogenic keratocyst (OKC) (×60 magnification)

immunostaining was significantly more pronounced in recurrent compared to non-recurrent sporadic cases of OKC. In fact, over 90% of recurrent cysts exhibited a moderate to strong reaction to COX-2, whereas 50% of the non-recurrent lesions showed a negative or merely weak stain. By juxtaposing these results with the evidence that overexpression of the *COX-2* gene alters adhesion, inhibits apoptosis and alters the response to growth regulatory

signals,[43,44] one might speculate that at least some of the recurrent OKCs are in fact neoplastic. However, in the current study, the immunoexpression of COX-2 had no prognostic relevance, as it did not influence the time to recurrence in a survival model; in this respect, it is consistent with our previous findings.[17] Although all of the syndromic cases exhibited COX-2 expression, we did not find any difference between recurrent and non-recurrent lesions in this regard. However, we found that 50% of the recurrent and 0% of the non-recurrent syndromic cases exhibited a strong reaction to COX-2. In light of this, it may be assumed that COX-2 upregulation is relevant to the growth and progression of OKCs. Previous studies have demonstrated that COX-2 plays an important role in the biological regulation of OKC epithelial lining,[41] and it has been shown to be partially involved in the mechanism of OKC progression.[39] The COX-2 is known to increase the level of Bcl-2, thus suppressing apoptosis.[44] Moreover, COX-2-dependent prostaglandins have been implicated in the induction of matrix metalloproteinases (MMPs), which are important in matrix degradation during tumor growth and invasion, and induction of angiogenesis.[45,46] Very few studies have elucidated an association between COX-2 immunoexpression and OKC recurrence. Furthermore, current evidence does not indicate that COX-2 has any prognostic relevance in OKC.[41] Nevertheless, our results hinting at COX-2 upregulation in recurrent sporadic cases coupled with data on its overexpression in OKCs suggest that COX-2 should be considered a potential marker to analyze the biological behavior of OKCs.[40] However, further investigation on larger samples is necessary. Recently, Alsaegh et al.[46] proposed that active human papillomavirus (HPV) infection may be associated with COX-2 expression in the odontogenic epithelium of OKC, and that HPV infection may have a role in the pathogenesis and aggressiveness of the cyst. However, this hypothesis has not been supported by any further research.

The RANKL and OPG are critical molecules for the control of osteoclastogenesis and pathophysiological bone remodeling.[27,47] In short, binding of RANKL to RANK leads to proosteoclast recruitment, and osteoclast activation and survival, whereas OPG is a decoy receptor for RANKL that blocks osteoclast formation by inhibiting RANKL from binding to RANK.[18,27,48] Not surprisingly, the vast majority of lesions in the current series exhibited RANKL > OPG or, to a lesser extent, a RANKL = OPG ratio, irrespective of the association with NBCCS. Many previous studies have demonstrated an elevated expression of RANKL in comparison with OPG in various osteolytic diseases, including aggressive odontogenic tumors like ameloblastoma, odontogenic myxoma and ameloblastic fibroma.[49–51] In our previous report, we showed that the majority of sporadic OKCs exhibited a RANKL > OPG ratio or, to a lesser degree, a RANKL = OPG ratio.[18] In the current study, we showed that there is a significantly increased expression of RANKL in recurrent syndromic lesions. In fact, 80% of the recurrent syndromic OKCs exhibited a strong reaction to RANKL, and 60% of the recurrent syndromic OKCs had a RANKL > OPG ratio. These findings suggest that there is an elevated osteoclast activity in recurrent OKCs. However, we are not certain whether it has any prognostic relevance. First, our previous[18] and current results do not show that the expression of RANK, RANKL or OPG, or RANKL/OPG balance in sporadic OKCs has any influence on the risk and time to recurrence in the survival model. It was also not possible to calculate the HR for syndromic cases in this regard. Finally, the results of some studies on RANKL/OPG ratio in OKC indicate that the majority of OKC cases exhibit a predominance of OPG over RANKL.[27,51] De Matos et al. demonstrated that the majority of OKC cases exhibited an OPG = RANKL or OPG > RANKL ratio and suggested that this may stem from the cystic but not solid architecture of OKCs.[51] However, the authors did not test their findings against recurrence. In turn, Nonaka et al., having achieved similar results of RANKL/OPG ratio, additionally found that there were significant differences in the balance of the 2 molecules between recurrent and non-recurrent cases.[27] However, their analysis addressed this issue only in regard to sporadic cases, whereas syndromic lesions were not divided into recurrent and non-recurrent. In view of the above, the current results and data from the published material indicate that there is a disturbance of the functional equilibrium in the RANKL/OPG system in both sporadic and syndromic OKCs; however, this dysregulation may be in either direction, and its prognostic significance is unclear.

A recent systematic review and meta-analysis failed to identify immunohistochemical markers that could accurately distinguish syndromic from sporadic cases of OKC.[12] In the current study, however, we have shown that there is a significant difference in the expression of PCNA, p53 and OPG between the 2 types of OKC. These 3 markers are associated with the expansion of the lesion. In addition, PCNA and p53 are directly related to cellular proliferation. Previous studies found that the proliferation of odontogenic epithelium in syndromic OKCs is significantly more common than in its sporadic counterpart; these conclusions were mainly based on the results of PCNA and p53 expression in the cystic epithelium.[16,52,53] Lo Muzio et al. found the overexpression of p53 protein in syndromic OKCs and, together with the increase of PCNA positivity, deduced that it constitutes a valid background for the existence of a more aggressive phenotype of lesions associated with NBCCS.[53] Recently, Slusarenko da Silva et al. in their systematic review and meta-analysis found that OKCs are more prone to express the p53 marker than clinically benign dentigerous cysts, but quite similar to clinically aggressive ameloblastomas.[7] This is another argument in favor of the concept that OKC is prone to behave as a destructive odontogenic tumor. The significantly higher immunoexpression of p53 revealed in syndromic cases in the present study may suggest that at least this subset of OKCs might be considered tumors rather than cysts.

We also found that there is a significant difference in the expression of OPG between sporadic and syndromic OKCs. The OPG upregulation may suggest decreased osteolytic activity, since OPG is an inhibitor of RANK/RANKL interaction, thus preventing osteoclastogenesis. In the current series, the majority of the lesions exhibited positive OPG immunoreaction; surprisingly, we found that most of the sporadic cases showed weak reactions, whereas in syndromic cysts the reaction was mainly moderate to strong. In light of the preceding considerations, we believe that OPG expression should be assessed in conjunction with RANKL expression, since the balance between both molecules regulates the process of bone resorption. As there was no significant difference in RANKL expression and RANKL/OPG balance, we do not think that our discovery of OPG upregulation in syndromic OKC cases indicates their decreased osteolytic activity.

Limitations

A weak point of this study is the small sample size, particularly in the subset of syndromic OKCs. Moreover, having only 2 cases of non-recurrent syndromic OKC prevented us from calculating the HR for recurrence potential with respect to certain variables. The shortcomings of this study preclude us from drawing any binding conclusions, but our results suggest that immunohistochemistry may play a role in discriminating syndromic from sporadic OKC cases and has some prognostic relevance. Contrary to expensive and not widely available genetic testing for NBCCS, immunohistochemistry is a cost-effective technique that can be routinely applied in fixed tissue samples and may offer the potential to distinguish syndromic from sporadic OKCs.[12] An early recognition of NBCCS is of vital importance, as patients with NBCCS have an elevated risk of developing malignancies, such as BCC or medulloblastoma, and OKCs associated with NBCCS are more aggressive and have an increased risk of recurrence.[27] In their meticulous meta-analysis on differences in immunoprofile between sporadic and syndromic OKCs, Kalogirou et al. pointed out that there is a remarkable inconsistency in the diagnostic criteria employed for the selection of patients with NBCCS in various studies.[12] There are no universally accepted criteria for the diagnosis of NBCCS, and, together with the retrospective nature of most studies and their incomplete histopathological description of OKC (reporting merely the presence of a parakeratinized epithelium, which is found in other odontogenic cysts, without mentioning pathognomonic characteristics), there is increased bias in most published evidence.[12]

Conclusions

In conclusion, the results of this study show that NBCCS-associated OKCs are significantly more prone to recur than their sporadic counterparts. Larger size and radiological signs of cortical perforation in sporadic OKCs may indicate a higher risk of recurrence. The significance of these features, together with the cyst's locularity and association with dentition, should be confirmed in large-scale studies, especially by testing them in different configurations. The COX-2 is upregulated in recurrent sporadic OKCs, but it has no prognostic relevance. Recurrent syndromic OKCs exhibited higher RANKL and lower OPG expression, but we failed to demonstrate their prognostic significance. The immunoexpression of p53, PCNA and OPG may distinguish syndromic from sporadic OKCs, but these results require a confirmation with a larger sample.

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