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Stromal Myofibroblasts in Correlation With Inflammation and Impacted Tooth in Odontogenic Cysts: An Immunohistochemical Comparative Study

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Research Article

Keywords: myofibroblasts, odontogenic cysts, impacted tooth, inflammation, immunohistochemistry

Posted Date: July 24th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3179661/v1

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Abstract

Aim

Stromal myofibroblasts may act favoring growth and progression of odontogenic lesions, notably odontogenic keratocyst (OKC). While little explored the associated inflammation, it has been shown that OKC associated with an impacted tooth has a tendency toward rapid growth. Whether this finding is associated with the differential expression of myofibroblast immunomarkers, is unknown.

Materials and methods

Thirteen cases of each, pericoronal follicle (PF), inflamed dentigerous cyst (iDC), noninflamed DC (nDC), OKC associated with (OKC-A) or not associated (OKC-N) with impacted tooth, were evaluated through vimentin, α-SMA, calponin and h-caldesmon immunomarkers The data obtained were analyzed using the Shapiro-Wilk, Kruskal-Wallis and Dwass-Steel-Critchlow-Fligner Multiple Comparisons tests, with $p < 0.05$ being considered statistically significant.

Results

All cases were vimentin positive, with few cases exhibiting mild expression. All PFs were α-SMA negative. OKCs showed significant higher expression of α-SMA than DCs ($p < 0.05$). With similar expression between DC groups, the α-SMA expression was higher in OKC-A than OKC-N ($p = 0.882$). Also, the mean age of OKC-A was significantly lower than OKC-N patients. Only 4 cases (1 iDC, 3 OKC-N) showed mild expression of calponin. All cases were h-caldesmon negative.

Conclusions

Our results suggest lack of myofibroblastic differentiation in PFs. Inflammation does not seem to influence the myofibroblast population in DCs. Although not significant, a higher expression of α-SMA can explain the clinicopathological features of OKC-A patients. Calponin seems not to be a reliable marker for myofibroblasts in these cases.

Clinical relevance

Lack of myofibroblastic differentiation seems to be consistent with the nature and function of PF. OKC (still, OKC-A than OKC-N) presents higher α-SMA expression than DC, which can help to explain its distinctive growth potentials.

Introduction

The pericoronal follicle (PF) is a structure present in unerupted or impacted teeth, composed of fibrous connective tissue and epithelial remnants of the odontogenic epithelium, which can be predecessors for the development of odontogenic lesions [1]. Among them, the most cited are dentigerous cyst (DC), odontogenic keratocyst (OKC) and ameloblastoma [2–4]. However, the proliferative potential of the odontogenic epithelium in PF is still not fully understood [5]. There are three studies showing α-SMA expression in PFs [6, 7 8]. There is one study showing vimentin expression in PFs [7] and are no studies showing calponin and h-caldesmon expression in PFs.

The DC, associated with the crown of unerupted teeth, is the most common developmental odontogenic cyst [9, 10]. The cystic formation may be due to an accumulation of fluid between the enamel organ's reduced epithelium and the impacted tooth's crown, or to inflammatory conditions, which occur mainly in children [11]. Histologically, a non-inflamed wall of loose fibrous tissue is seen, often with a slightly myxoid appearance, lining epithelium with an approximate thickness of 2 to 4 cell layers, and if DC is associated with inflammation, histology shows epithelial hyperplasia with adjacent cholesterol crystals [12].

The OKC is the third most common odontogenic cyst, often presenting an aggressive local clinical behavior and with higher risk of recurrence [13, 14]. Approximately 35% of OKC cases are associated with an unerupted tooth, making the differential diagnosis with DC essential [12, 15, 16]. The histological characteristics show a capsule composed of collagenous connective tissue, lined by a parakeratinized epithelium and palisaded basal layer with a thickness of 5 to 8 cell layers [12].

The stromal connective tissue plays a fundamental role in the maintenance of epithelial tissues, with the fibroblast being their main constituent cell [13, 14]. Studies carried out in the last decades revealed that the modulation of fibroblasts towards the myofibroblastic phenotype is essential for connective tissue remodeling during normal and pathological healing processes [7, 15]. The presence of these cellular components may play a critical role in the growth and behavior of odontogenic cystic lesions [6, 10, 14, 17, 18]. While the α-SMA imunomarker is routinely used, the calponin expression is little explored [19]. In fact, it has been demonstrated that expression of myofibroblast markers is higher in OKC than DC [10, 13, 17, 20–22]. Furthermore, an interesting study assessing OKCs associated with and not associated with an impacted mandibular third molar, has shown that age of patients and area of cysts are smaller and larger, respectively, in OKCs associated with impacted tooth [23]. In these cases, the differential expression of myofibroblastic markers is unknown.

Therefore, this study aims to evaluate the immunoexpression of vimentin, α-SMA, calponin and h-caldesmon in PFs (control group), DCs (with and without inflammation) and OKCs (with and without impacted tooth association).

Materials and methods

Sample selection

Tissue specimens fixed in formalin and embedded in paraffin were obtained from the archives of the Laboratory of Microscopy, Histology, Immunopathology and Genetic Analysis, Oral Pathology Service, Ribeirão Preto Dental School (FORP/USP). This study was approved by the Human Research Ethics Committee (Protocol number 59880222.4.0000.5416).

Sixty-five cases were selected for this retrospective study, which were diagnosed with PF, DC and OKC by histopathology and clinical correlation, according to the 2017, 2022 WHO criteria. Patients received treatment between 2015 and 2022. The samples were divided into groups: PFs (n = 13), which were associated with an unerupted third molar; DCs (n = 26) composed of inflamed DC (iDC, n = 13) and noninflamed DC (nDC) (n = 13); and sporadic, single OKCs (n = 26), without association with impacted tooth (OKC-N, n = 13) and with association with impacted tooth (OKC-A, n = 13).

Inclusion criteria were cases of PF, DC and OKC that present complete clinical information, with the availability of material archived in paraffin, sufficient for histopathological confirmation and for new histological sections, which will be used in the immunohistochemical reactions. Exclusion criteria were any case with lack of complete clinical information, paraffin blocks with insufficient quality and quantity to carry out the analysis of the study, and any case associated with genetic syndromes.

Histopathological Analysis

From the existing material in the paraffin blocks, two new 5 µm-thick sections were made, which were stained with hematoxylin-eosin (H&E) and subsequently analyzed for the morphological description of the conventional sections and confirmation of the diagnosis.

Immunohistochemical (IHC) analysis

For IHC analysis, consecutive (serial) histological sections of 3-µm were placed on organosilane-coated slides (Sigma-Aldrich, St. Louis, MO). The deparaffinization using xylene solution was performed and the rehydration of the sections occurred through the passage in ethanol solution with serial concentrations. The antigen retrieval involved the immersion of the sections in citrate buffer (target retrieval solution, pH6). After, the sections were submitted to the immunohistochemistry technique by streptavidin–biotin-peroxidase method (Universal LSAB™ + Kit/HRP, Dako, Carpinteria, CA, USA) to evaluate the presence of stained cells using the primary antibodies. (Table 1).

Table 1
List of primary antibodies used in this study.

Antibody	Dilution	Clone	Supplier
α-SMA	1:1000	1A4	DakoCytomation, Glostrup, Denmark
calponin	1:800	BSB-20	Bio SB, Goleta, USA
h-caldesmon	1:800	BSB-19	Bio SB, Goleta, USA
vimentin	1:1000	Vim 3B4	DakoCytomation, Glostrup, Denmark
a-SMA, Alpha smooth muscle actin;			

Analysis of immunohistochemistry reactions

Immunoexpression of vimentin, α-SMA, calponin and h-caldesmon markers was analyzed in connective tissue by counting the total number of positive cells in 10 representative fields of each case. Images were obtained using the Leica LAS X Image Manage program. Areas with higher immunostaining density were selected and the percentage of positive cells recorded.

For PFs and cystic lesions, counting was done beneath the lining epithelium. Counting was performed using binocular microscope with x10 eyepiece and x40 objective. The immunostained cells were identified in each of the ten fields chosen. The α-SMA, calponin and h-caldesmon positive cells in the blood vessel wall served as internal control. Those cells in close relationship with blood vessels were not counted. All other positively stained cells in each field were counted and recorded. The mean number of immunostained cells was also calculated.

The percentage of vimentin, α-SMA, calponin and h-caldesmon positive cells in each group was also calculated through the number of positive and negative cases.

The distribution of immunostained cells from each group was also registered, whether in the superficial part (subepithelial) and/or in the deeper part of the connective tissue stroma [13].

Statistical analysis

For statistical analysis, Jamovi Software Version 2.3 (Jamovi, Sydney, Australia) was used. The normal distribution of data was assessed using the Shapiro-Wilk test. The vimentin and α-SMA percentual of expressions were compared among PF, iDC, nDC, OKC-A OKC-N groups using the Kruskal-Wallis test. The intergroup comparisons were analyzed using the Dwass-Steel-Critchlow-Fligner Multiple Comparisons test. A P value less than 0.05 was considered statistically significant.

Results

Clinicopathological findings

The clinical features of the cyst are summarized in Table 2. The mean age of the patients was described with the age range of each analyzed group. The mean age between the iDC (p = 0.018) and nDC (p = 0.789) groups was statistically significant as well as the mean age of OKC-A was significantly lower than OKC-N patients (P < 0.05).

Table 2
Clinicopathological features of all cases included in this study.

Clinical features	PF	iDC	nDC	OKC-A	OKC-N
No. cases	13	13	13	13	13
Age (mean)	19.2 (range 13–30)	32.1 (range 9–68)	33.1 (range 9–66)	20.5 (range 14–34)	42.5 (range 18–78)
Male	6	9	10	10	10
Female	7	4	3	3	3
Site					
Maxilla	10	4	3	3	0
Mandible	3	9	10	10	13

PF, pericoronal follicle; iDC, inflamed dentigerous cyst; nDC, noninflamed DC; OKC-A, keratocyst odontogenic associated with impacted tooth; OKC-N, keratocyst not associated impacted tooth; OKC-A vs OKC-N (age): $p < 0.05$

α-SMA expression

All PF samples did not show α-SMA expression. Moderate to strong staining was observed in 11/13 iDCs, 12/13 nDCs, 10/13 OKC-As and 12/13 OKC-Ns. There were no significant differences in α-SMA expression between iDC and nDC, as well as OKC-A and OKC-N. However, it is necessary to comment that while a similar α-SMA expression between iDC and nDC was observed, a higher number of α-SMA-positive cells in OKC-A than OKC-N (p = 0.882) was detected (Table 3).

Table 3
Vimentin and α-SMA expression in the cases assessed by group

Markers	PF	iDC	nDC	OKC-A	OKC-N
α-SMA					
n	0	11	12	10	12
Mean ± SD	0	6.00 ± 3.61	6.75 ± 4.81	33.5 ± 29.2	22.1 ± 16.8
Vimentin					
n	9	11	13	11	11
Mean ± SD	27.3 ± 36.5	65.5 ± 31.3	60.0 ± 28.6	51.4 ± 36.7	28.3 ± 27.0
α-SMA, alpha-smooth muscle actin;					
Values are expressed as the means ± standard deviation (%)					

Only when comparing DC and OKC groups, the difference was statistically significant ($p < 0.05$) (Figs. 1 and 2).

In addition to myofibroblast detection, this marker also highlighted the vessel walls.

Calponin expression

Calponin expression was observed only in 4 cases. One case in the iDC group and 3 cases in the OKC-N group, showed a percentage of expression in the cystic capsule below 10%, not providing enough data to consider the statistical analysis (Fig. 1).

This marker mainly highlighted the vessel walls.

H-caldesmon expression

The h-caldesmon was expressed only by vascular smooth muscle cells (Fig. 1).

Vimentin expression

Vimentin expression, in moderate to strong staining, was observed in 9/13 PFs, 11/13 iDCs, in 13/13 nDC, 11/13 OKC-A and 11/13 OKC-N (Table 3). Regarding the homogeneous expression of vimentin between the groups, no statistical differences were found (Figs. 1 and 2).

Discussion

Stromal cells maintain tissue homeostasis through control over cell size, function, and response. Changes in the stromal microenvironment result in the presence of cellular components capable of modifying the extracellular matrix in pathological conditions [20, 24, 25]. OKC has specific histopathological characteristics, presenting aggressive behavior and a tendency to relapse compared to

other types of cysts [26–28]. In a study in the literature by Tsukamoto et al. [23] differences in age, area and peripheral shape of OKC associated and not associated with a lower third molar were evaluated, showing that the mean age of patients in the associated group was lower than that of the non-associated group, while mean area of the cysts in the group associated OKC was higher than in the non-associated group, suggesting that an impacted tooth and its dental follicles may affect the occurrence and proliferation of an OKC. In another study evaluating DC and OKC associated with a lower third molar, the mean age of patients in the OKC group was lower than that of the DC group and the mean area of the OKC group was greater than DC, with no correlations reflected between the ages and areas in the DC and OKC groups. These findings suggest that both cysts do not develop gradually during the period when follicles are formed but appear at various random times during a patient's life [29]. In the present study, the mean age of patients in all groups was evaluated, finding a statistical difference between the iDC and nDC, OKC-A and OKC-N groups ($p < 0.05$). These findings demonstrate an association with existing studies in the literature.

MFs are differentiated fibroblasts that express α-SMA and have intermediate characteristics between conventional fibroblasts and smooth muscle cells [30] being able to remodel and degrade the extracellular matrix by secreting matrix metalloproteinases, driving the invasive growth of lesions odontogenic cyst [31]. The few studies that investigated the presence of MFs in odontogenic lesions suggest that the increase in the number of these cells contributes to the progression of the lesion and more aggressive biological behavior [13, 17, 21, 24, 25, 32, 33]. Anusai et al. [10] reported that epithelial and stromal cell interactions are fundamental in controlling the growth and clinical behavior of odontogenic cystic lesions and that MFs are seen in greater numbers in OKC than in DC. These immunohistochemical findings are also observed in other studies in the literature [13, 17, 20–22] and corroborate the results of this present study, in which we found statistical differences between the groups of DC (iDC and nDC) and OKC (OKC -A and OKC-N) ($p < 0.05$), agreeing that growth and aggressive biological behavior is greater in OKC compared to DC.

The first authors to investigate the presence of MFs in odontogenic lesions were Lombardi and Morgan [7]. These authors evaluated the expression of α-SMA and vimentin in odontogenic cystic lesions and in control groups, including PF, in which there was no significant expression of α-SMA. However, the study by Oliveira Ramos et al., [34] found high expression of MFs using the α-SMA in cases of PF, associating it with the probability of bone remodeling that occurs during tooth formation and eruption. De Souza-Neto et al. [6] also evaluated the expression of α-SMA in PF and found the myofibroblast phenotype in all cases used. In the current study, however, no expression of MFs was found in cases of PF, only marking of vascular smooth muscle cells. The lack of myofibroblast differentiation in PF appears to be consistent with the nature and function of this structure.

Gratzinger et al. [19] mention calponin as a primary actin-bound protein of tissues containing smooth muscles and present some studies in which it exhibits characteristics like α-SMA. Furthermore, these authors did not find positive cases of calponin for DC and OKC. In our study, only 1 case of DC and 3 cases of OKC were positive for MF using calponin, considering that calponin does not seem to be a

reliable marker for myofibroblasts in these cases. No further studies were found that evaluated calponin expression in PF, DC, or OKC. Likewise, no studies were found with the expression of h-caldesmon in these cystic lesions.

Vimentin is a class II intermediate filament expressed primarily in mesenchymal tissue [35]. To date, only two studies provide data on vimentin in odontogenic cystic lesions [7, 35]. In this study, it was observed that all groups presented positive staining for vimentin, with the iDC group having the highest amount of expression, followed by nDC, OKC-A, OKC-N and PF. This can be explained by the presence of the inflammatory infiltrate, increasing the number of these cells. However, statistically, there is no difference between all groups compared ($p > 0.05$). In the study by Loreto et al., [14] in which the presence of fibroblasts in OKC variants was also evaluated, it is suggested that the different biological behavior of these lesions is not due to differences in the number of these cells, but to different metabolic activity. Thus, this study concludes that the lack of myofibroblastic differentiation appears to be consistent with the nature and function of the PF. OKC and DC show α-SMA expression, since in OKC (still, OKC-A greater than OKC-N) there is greater expression of the myofibroblastic phenotype than in DC. This may help to explain its distinctive growth potentials and aggressiveness.

Declarations

Funding:

Jorge Esquiche León have received research Grants (2015/22586-2; 2016/11419-0; 2016/14636-2; 2022/07479-9 and 2022/12760-9) from State of São Paulo Research Foundation (FAPESP) and research grants (304241/2021-0) from National Council for Scientific and Technological Development (CNPq). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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NOTES

Compliance with Ethical Standards

Ethics approval: This study was reviewed and approved by the Research Ethics Committee of the São Paulo State University Julio de Mesquita (Protocol number: 59880222.4.00005416) and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Author contributions: All authors were involved in drafting the article or revising it critically for important intellectual content.

Consent to participate: Informed consent was obtained from all the participants included in the clinical trial.

Competing interests: The authors declare no competing interests.

References

1. Villalba L, Stolbizer F, Blasco F, Mauriño NR, Piloni MJ, Keszler A (2012) Pericoronal follicles of asymptomatic impacted teeth: a radiographic, histomorphologic, and immunohistochemical study. Int J Dent 2012:935310. https://doi.org/10.1155/2012/935310
2. Glosser JW, Campbell JH (1999) Pathologic change in soft tissues associated with radiographically 'normal' third molar impactions. Br J Oral Maxillofac Surg 37(4):259–260. https://doi.org/10.1054/bjom.1999.0061
3. da Silva Baumgart C, da Silva Lauxen I, Filho MS, de Quadros OF (2007) Epidermal growth factor receptor distribution in pericoronal follicles: relationship with the origin of odontogenic cysts and tumors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 103(2):240–245. https://doi.org/10.1016/j.tripleo.2005.11.009
4. Saravana GH, Subhashraj K (2008) Cystic changes in dental follicle associated with radiographically normal impacted mandibular third molar. Br J Oral Maxillofac Surg 46(7):552–553. https://doi.org/10.1016/j.bjoms.2008.02.008
5. de Oliveira MG, Lauxen IS, Chaves AC, Rados PV, Sant'Ana Filho M (2011) Odontogenic epithelium: immunolabeling of Ki-67, EGFR and survivin in pericoronal follicles, dentigerous cysts, and keratocystic odontogenic tumors. Head Neck Pathol 5(1):1–7. https://doi.org/10.1007/s12105-010-0216-0
6. Sousa-Neto ES, Cangussu MC, Gurgel CA, Guimarães VS, Ramos EA, Xavier FC, Cury PR, Carneiro Júnior B, Leonardi R, Dos Santos JN (2016). Interaction of stromal and microvascular components in keratocystic odontogenic tumors. J Oral Pathol Med 45(8):557–564. https://doi.org/10.1111/jop.12400
7. Lombardi T, Morgan PR (1995) Immunohistochemical characterization of odontogenic cysts with mesenchymal and myofilament markers J Oral Pathol Med 24(4):170–176. https://doi.org/10.1111/j.1600-0714.1995.tb01160.x
8. de Oliveira Ramos G, Costa A, Meurer MI, Vieira DS, Rivero ER (2014) Immunohistochemical analysis of matrix metalloproteinases (1, 2, and 9), Ki-67, and myofibroblasts in keratocystic odontogenic tumors and pericoronal follicles. J Oral Pathol Med 43(4):282–288. https://doi.org/10.1111/jop.12131

9. Morgan PR (2011) Odontogenic tumors: a review. Periodontol 2000 57(1):160–176. https://doi.org/10.1111/j.1600-0757.2011.00393.x
10. Anusai VV, Shylaja S, Suvarna M, Ramanand OV, Reddy ES, Vamshi VR (2021) Immunohistochemical evaluation of myofibroblasts in odontogenic keratocyst, dentigerous cyst and different clinical variants of ameloblastoma: A comparative study. Dent Res J (Isfahan) 18:36.
11. Huang G, Moore L, Logan RM, Gue S (2019) Histological analysis of 41 dentigerous cysts in a pediatric population. J Oral Pathol Med 48(1):74–78. https://doi.org/10.1111/jop.12776
12. El- Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ (2017) WHO Classification of Head and Neck Tumours. 4ª ed. Wiley, Lyon, pp 234–235.
13. Syamala D, Suresh R, Janardhanan M, Savithri V, Anand PP, Jose A (2016) Immunohistochemical evaluation of myofibroblasts in odontogenic cysts and tumors: A comparative study. J Oral Maxillofac Pathol 20(2):208–213. https://doi.org/10.4103/0973-029X.185898
14. Loreto C, Polizzi A, Filetti V, Pannone G, Dos Santos JN, Venezia P, Leonardi R, Isola G (2022) Expression of Matrix Metalloproteinases 7 and 9, Desmin, Alpha-Smooth Muscle Actin, and Caldesmon, in Odontogenic Keratocyst Associated with NBCCS, Recurrent and Sporadic Keratocysts. Biomolecules 12(6):775. https://doi.org/10.3390/biom12060775
15. Bilodeau EA, Hunter KD (2021). Odontogenic and Developmental Oral Lesions in Pediatric Patients. Head Neck Pathol 15(1):71–84. https://doi.org/10.1007/s12105-020-01284-3
16. Odell EW, Muller S, Tilakaratne WH (2022) Chap. 8: Odontogenci and maxillofacial bone tumours. In: WHO Classification of Tumours Editorial Board. Head and neck tumors. Lyon (Frence): International Agency for Research on Cancer; forthcoming. (WHO classification of tumours series, 5th ed.; vol. 9). ed. Wiley, https://publications.iarc.fr.
17. Pereira JDS, de Oliveira Nóbrega FJ, Vasconcelos RG, de Souza Martins Câmara A C, de Souza LB, Queiroz LMG (2018). Myofibroblasts and mast cells: influences on the biological behavior of odontogenic lesions. Ann Diagn Pathol 34:66–71. https://doi.org/10.1016/j.anndiagpath.2014.09.003
18. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA (2002). Myofibroblasts and mechano-regulation of connective tissue remodeling. Nat Rev Mol Cell Biol 3(5):349–363. https://doi.org/10.1038/nrm809
19. Gratzinger D, Salama ME, Poh CF, Rouse RV (2008) Ameloblastoma, calcifying epithelial odontogenic tumor, and glandular odontogenic cyst show a distinctive immunophenotype with some myoepithelial antigen expression. J Oral Pathol Med 37(3):177–184. https://doi.org/10.1111/j.1600-0714.2007.00613.x
20. Ahmad MA, Gupta AA, Mhaske SA, Saawarn S, Ashok S, Mandora D, Jain M (2022) Expression of alpha-smooth muscle actin in odontogenic cysts and tumors. Exp Oncol 44(3):249–253. https://doi.org/10.32471/exp-oncology.2312-8852.vol-44-no-3.18676
21. Nadalin MR, Fregnani ER, Silva-Sousa, YT, da Cruz Perez DE (2012) Presence of myofibroblasts and matrix metalloproteinase 2 in radicular cysts, dentigerous cysts, and keratocystic odontogenic

tumors: a comparative immunohistochemical study. J Endod 38(10):1363–1367. https://doi.org/10.1016/j.joen.2012.05.020

22. Annegowda VM, Devi HU, Rao K, Smitha T, Sheethal HS, Smitha A (2018) Immunohistochemical study of alpha-smooth muscle actin in odontogenic cysts and tumors J Oral Maxillofac Pathol 22(2):188–192. https://doi.org/10.4103/jomfp.JOMFP_31_18
23. Tsukamoto G, Makino T, Kikuchi T, Kishimoto K, Nishiyama A, Sasaki A, Matsumura T (2002). A comparative study of odontogenic keratocysts associated with and not associated with an impacted mandibular third molar Oral Surg Oral Med Oral Pathol Oral Radiol Endod, *94*(2), 272–275. https://doi.org/10.1067/moe.2002.126888
24. Vered M, Shohat I, Buchner A, Dayan D (2005) Myofibroblasts in the stroma of odontogenic cysts and tumors can contribute to variations in the biological behavior of lesions. Oral Oncol 41(10):1028–1033. https://doi.org/10.1016/j.oraloncology.2005.06.011
25. Santos PPA, Nonaka CFW, Freitas RA, Pereira Pinto L, Souza LB (2017) Immunohistochemical analysis of myofibroblasts, TGF-β1, and IFN-γ in epithelial odontogenic lesions J Oral Pathol Med 46(5):365–370. https://doi.org/10.1111/jop.12494
26. Rubini C, Artese L, Zizzi A, Fioroni M, Ascani G, Goteri G, Stramazzotti D, Piccirilli M, Iezzi G, Piattelli A (2011) Immunohistochemical expression of vascular endothelial growth factor (VEGF) in different types of odontogenic cysts. Clin Oral Investig 15(5):757–761. https://doi.org/10.1007/s00784-010-0433-7
27. Kaczmarzyk T, Kisielowski K, Koszowski R, Rynkiewicz M, Gawełek E, Babiuch K, Bednarczyk A, Drozdzowska B (2018) Investigation of clinicopathological parameters and expression of COX-2, bcl-2, PCNA, and p53 in primary and recurrent sporadic odontogenic keratocysts. Clin Oral Investig 22(9):3097–3106. https://doi.org/10.1007/s00784-018-2400-7
28. Hoyos Cadavid AM, Kaminagakura E, Rodrigues MFSD, Pinto CAL, Teshima THN, Alves FA (2019) Immunohistochemical evaluation of Sonic Hedgehog signaling pathway proteins (Shh, Ptch1, Ptch2, Smo, Gli1, Gli2, and Gli3) in sporadic and syndromic odontogenic keratocyst. Clin Oral Investig 23(1):153–159. https://doi.org/10.1007/s00784-018-2421-2
29. Tsukamoto G, Sasaki A, Akiyama T, Ishikawa T, Kishimoto K, Nishiyama A, Matsumura T (2001) A radiologic analysis of dentigerous cysts and odontogenic keratocysts associated with a mandibular third molar. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 91(6), 743–747. https://doi.org/10.1067/moe.2001.114157
30. Hinz B, Lagares D (2020) Evasion of apoptosis by myofibroblasts: a hallmark of fibrotic diseases. Nat Rev Rheumatol 16(1):11–31. https://doi.org/10.1038/s41584-019-0324-5
31. Roy S, Garg V (2013) Evaluation of stromal myofibroblasts expression in keratocystic odontogenic tumor and orthokeratinized odontogenic cysts: A comparative study. J Oral Maxillofac Pathol 17(2):207–211. https://doi.org/10.4103/0973-029X.119789
32. Dandena VK, Thimmaiah SY, Kiresur MA, Hunsigi P, Roy S, Rashmi M (2017) A comparative study of odontogenic keratocyst and orthokeratinized odontogenic cyst using Ki67 and α smooth muscle

actin. J Oral Maxillofac Pathol 21(3):458–459. https://doi.org/10.4103/jomfp.JOMFP_71_17

33. Pinisetti S, Tadi D, Manyam R (2022) Myofibroblasts in Odontogenic Cysts and Tumors: An Immunohistochemical Study. J Microsc Ultrastruct 11(1):68–73. https://doi.org/10.4103/jmau.jmau_64_21
34. de Oliveira Ramos G, Costa A, Meurer MI, Vieira DS, Rivero ER (2014) Immunohistochemical analysis of matrix metalloproteinases (1, 2, and 9), Ki-67, and myofibroblasts in keratocystic odontogenic tumors and pericoronal follicles. J Oral Pathol Med 43(4):282–288. https://doi.org/10.1111/jop.12131
35. Sudhakara M, Rudrayya SP, Vanaki SS, Bhullar RK, Shivakumar MS, Hosur M (2016) Expression of CK14 and vimentin in adenomatoid odontogenic tumor and dentigerous cyst. J Oral Maxillofac Pathol 20(3):369–376. https://doi.org/10.4103/0973-029X.190904

Figures

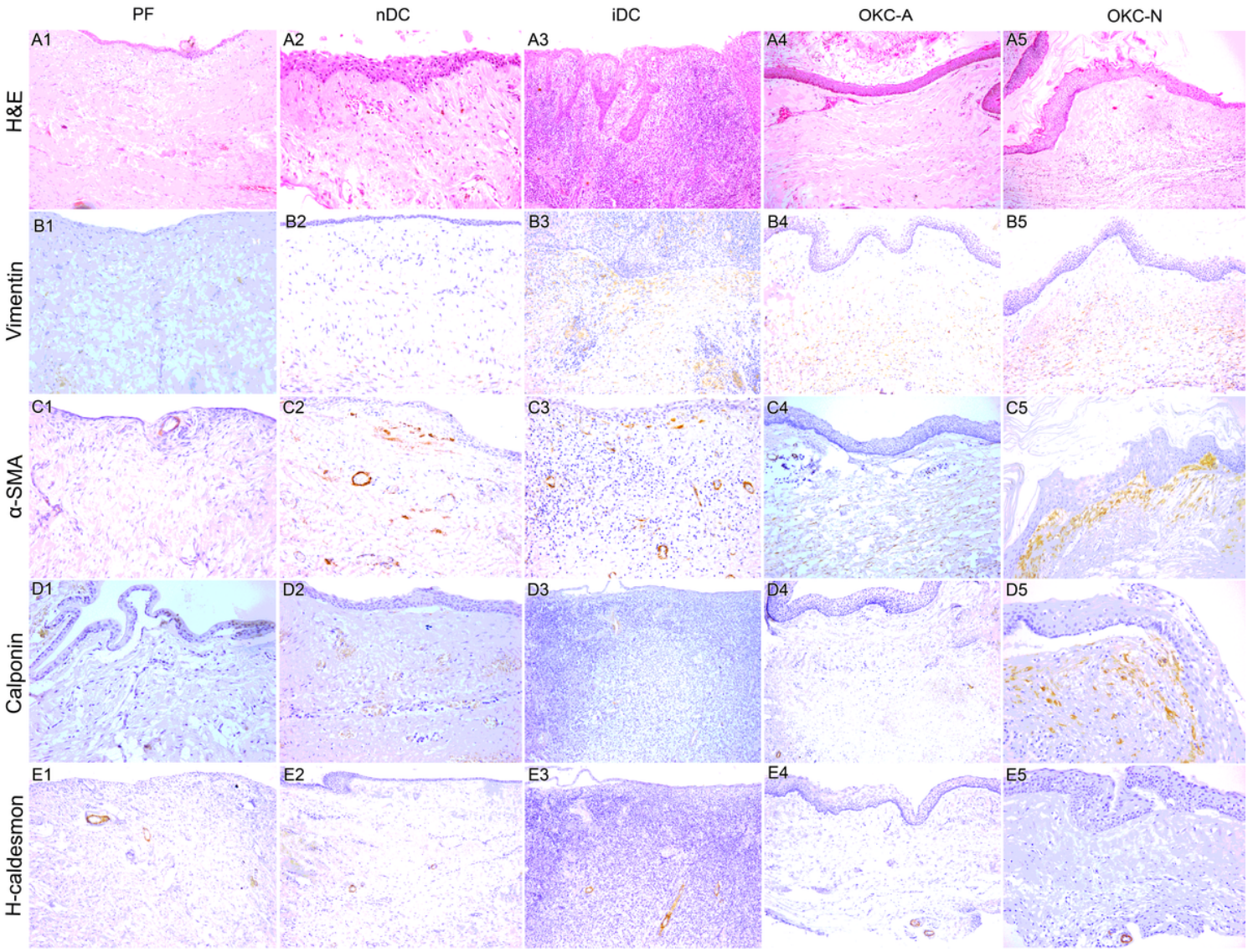

Figure 1

Histopathological and immunohistochemical analysis through consecutive (serial) tissue sections of pericoronal follicle (PF) (**A1-E1**), non-inflammatory dentigerous cyst (nDC) (**A2-E2**), inflamed dentigerous cyst (iDC) (**A3-E3**), odontogenic keratocyst associated with impacted tooth (OKC-A) (**A4-E4**) and odontogenic keratocyst not associated with impacted tooth (OKC-N) (**A5-E5**). In line **B**, IHC for vimentin showed expression in moderate to strong staining in both groups, not finding statistical differences. In **C**, α-SMA expression was absent in all PF samples (**C1**), while the remaining groups exhibited myofibroblast expression, with moderate to strong staining (**C2 - C5**). Calponin expression was only observed in 4 cases, one case in the iDC group (**D2**) and 3 cases in the OKC-N group (**D5**), showing a percentage of expression in the cystic capsule below 10%. In addition to detecting myofibroblasts, α-SMA and calponin also highlighted vessel walls (**C** and **D**). The h-caldesmon (**E**) was expressed only by vascular smooth muscle cells. (Figures A2, C2, C3, C5, D1, D2, D5 and E5 are at 20x magnification; the other figures are at 10x original magnification).

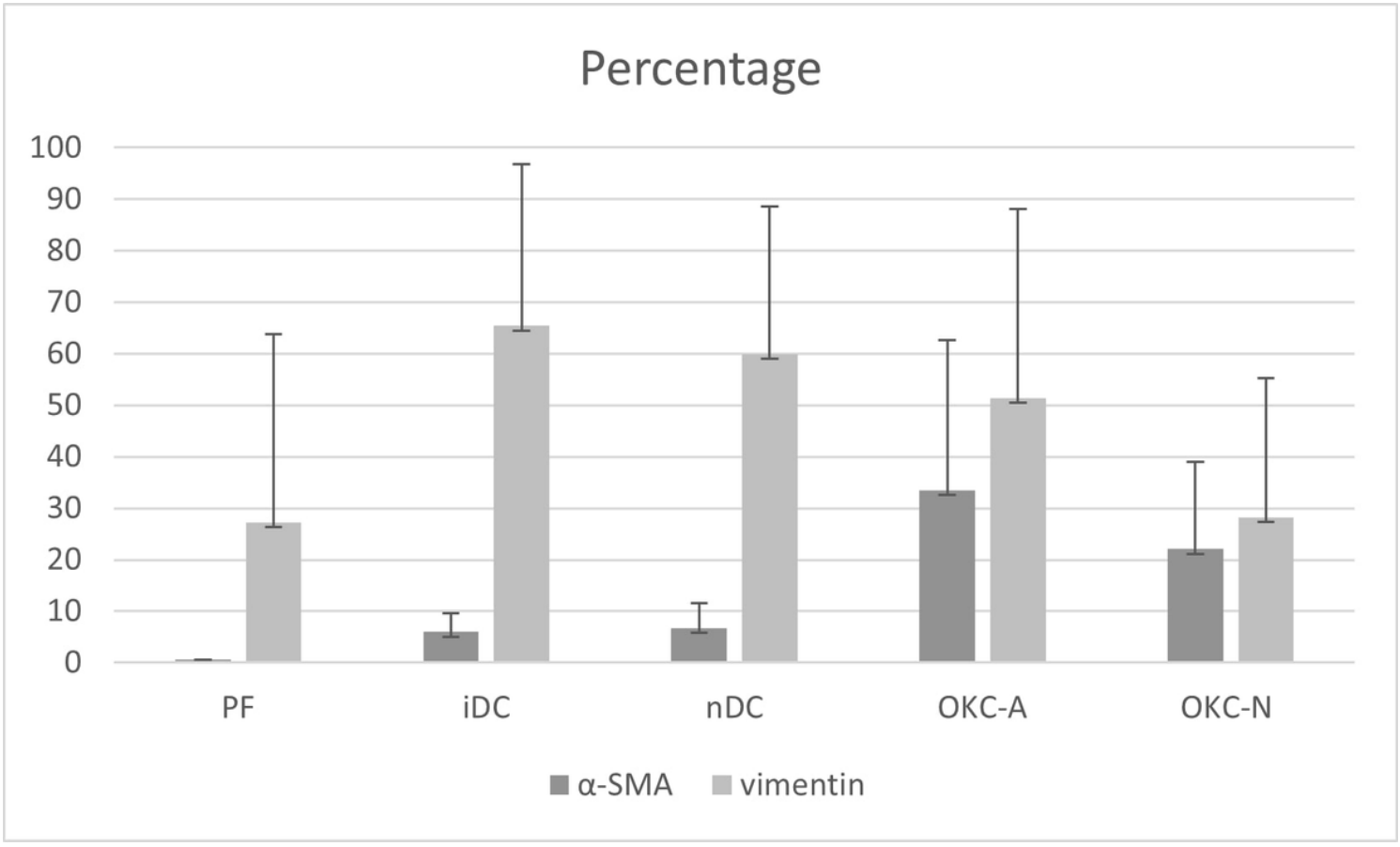

Figure 2

Graphic showing the differential expression of vimentin and α-SMA in the PFs, DCs and OKCs.

Abbreviations: PF, pericoronal follicle; iDC, inflamed dentigerous cyst; nDC, noninflamed dentigerous cyst; OKC-A, keratocyst odontogenic associated with impacted tooth; OKC-N, keratocyst not associated impacted tooth; α-SMA, alpha-smooth muscle actin.