UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO *STRICTO SENSU* EM ODONTOLOGIA – NÍVEL MESTRADO

JESSICA LUANA DOS SANTOS

Avaliação da imunorreatividade da beta catenina, geminina e MCM2 em tumores queratocísticos odontogênicos esporádicos e associados à síndrome do carcinoma nevóide basocelular

Expression of beta catenin, geminin and MCM2 in sporadic keratocystic odontogenic tumor and associated with the nevoid basal cell carcinoma syndrome

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Orientadora: Prof. Dra. Ana Lúcia Carrinho Ayroza Rangel

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Avaliação da imunorreatividade da beta catenina, gemina e MCM2 em queratocistos odontogênicos esporádicos ou associados à síndrome do carcinoma nevoide basocelular: estudo colaborativo internacional

Dissertação apresentada ao Programa de pós-graduação stricto sensu em Odontologia em cumprimento parcial aos requisitos para obtenção do título de Mestra em Odontologia, área de concentração Odontologia, linha de pesquisa Patologia aplicada à clínica odontológica, APROVADO(A) pela seguinte banca examinadora:

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“Quando os ventos de mudança sopram, umas pessoas levantam barreiras, outras constroem moinhos de vento.”

Érico Veríssimo

SUMÁRIO

EXPRESSION OF BETA CATENIN, GEMININ AND MCM2 IN SPORADIC KERATOCYSTIC ODONTOGENIC TUMOR AND ASSOCIATED WITH THE NEVOID BASAL CELL CARCINOMA SYNDROME

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Resumo

Objetivo: O objetivo deste estudo foi avaliar a expressão de beta catenina, geminina e MCM2 em tumors odontogênicos queratocísticos (KCOTs) sindrômicos e esporádicos.

Material e Metódos: Dados clínicos de 40 casos de KCOTs (30 casos sindrômicos e 10 esporádicos) foram coletados e cortes histológicos foram imuno-histoquimicamente corados e avaliados para beta catenina, geminina e MCM2.

Resultados: Cistos satélites e pleomorfismo celular foram mais prevalentes nos casos sindrômicos. O padrão de marcação da beta catenina foi membranoso e sua reatividade avaliada em extensão não foi estatisticamente diferente entre os grupos de casos sindrômicos e esporádicos, no entanto, lesões sindrômicas apresentaram reatividade menos intensa para beta catenina do que os casos esporádicos. A reatividade da geminina e MCM2 em ambos os grupos foi nuclear. Nesses grupos, a marcação ocorreu predominantemente na camada parabasal. Não houve diferença estatística entre lesões sindrômicas e esporádicas para geminina, já a MCM2 apresentou maior média de células positivas em KCOTs esporádicos ($p= 0,011$).

Conclusão: Características histológicas mostraram evidências de maior agressividade em KCOTs sindrômicos, mas não houve achados que confirmem o maior potencial proliferativo de KCOTs sindrômicos utilizando beta catenina, geminina e MCM2.

Palavras-chave: Neoplasias maxilomandibulares; imuno-histoquímica; proliferação de células.

Abstract

Objective: The aim of this study was to investigate the beta catenin, geminin and MCM2 expression in sporadics and syndromics keratocystic odontogenic tumors (KCOTs).

Material and Methods: Clinical data from 40 cases of KCOTs (30 syndromic and 10 sporadic cases) were coleted and sections from they were immunohistochemically stained and assayed for beta catenin, geminin and MCM2.

Results: Sattelites cysts and cellular pleomorfism were more prevalent in syndromic cases. The beta catenin staining pattern was membranous and its reactive extension does not show statistical difference between syndromic and sporadic KCOTs, whereas the syndromic lesions showed less intense reactivity for beta catenin. The reactivity for geminin and MCM2 in both groups showed a nuclear staining pattern. In these groups, the nuclear staining occurred predominantly in the first suprabasal layer. There is no statistical difference in the geminin reactivity between the groups, whereas the means of MCM2 positive cells was higher in sporadic KCOTs than syndromic KCOTs group (p=0.011).

Conclusion: Histological features shows evidences of greater aggressiveness in syndromic KCOTs, but there is not significant evidence that ensures the higher proliferative potencial of syndromic KCOT using these markers.

Key words: Jaw neoplasms; immunohistochemistry; cell proliferation.

Introduction

According to World Health Organization (WHO), Keratocystic Odontogenic Tumor (KCOT) is a cystic benign tumor that arises from epithelial remnants of the dental lamina occurring sporadically or as a manifestation of Nevoid Basal Cell Carcinoma Syndrome (NBCCS). In syndromic patients has higher recurrence index and early ocurrence when compared to patients who have this cyst sporadically. This entity has undergone changes in its nomenclature and classification, being called odontogenic keratocyst until 2005, however, since this year the WHO entered it into the category of benign neoplasms of the head and neck due to its locally aggressive behavior and molecular findings consistent with neoplasms (Barnes et al. 2005)[1]. Wright et al. (2014)[2] argue that there is insufficient evidence to justify the reclassification to keratocystic odontogenic tumor.

Nevoid basal cell carcinoma syndrome (NBCCS) or Gorlin Syndrome (GS) is a rare autosomal dominant disorder in which patients may present multiple basal cell carcinomas over the body, especially in areas exposed to ultraviolet radiation, as well multiple KCOTs in the jaws. Others manifestations can be noticed[3-5].

The cell proliferation plays an important role in many biological and pathological events such as tumors and cysts. The proliferative potential can be assessed by immunohistochemistry using antibodies against specific proteins associated with the cell cycle, such beta catenin, geminin and MCM2 (minichromosome maintenance-2) . The beta catenin is a protein related to Wnt signaling pathway, which regulates proliferation and cellular differentiation. The Wnt signaling pathway controls a variety of developmental processes, regulation of cell proliferation, morphology, motility and differentiation of various organs, including teeth. The Wnt signaling is controlled by different levels of beta catenin and its activation induces cytoplasmic accumulation and nuclear translocation of beta

catenin. Dysregulation in the beta catenin levels probably plays a critical step in tumorigenesis in a variety of cancers[6,7].

Geminin is a protein that acts controlling the cell division. Its function is to prevent re-licensing after initiation. When a cell enters in the cell cycle, intracellular mechanisms are activated so that after primary cell division does not start a new mitotic process. The concentration of geminin is not constant during the cell cycle. Their presence is not noticed during the G1 phase, when the cell becomes able to continue the cycle. The concentration increases during the phases S, G2 and M. This increase in concentration is intended to prevent new mitotic process at the end of the cycle, however, mutations in this protein may lead in an uncontrolled cell proliferation[8].

The MCM2 is involved in DNA replication control. The MCM2 expression begins early in G1 and is maintained throughout the cell cycle. The MCM2 is also expressed in proliferating cells without being necessarily synthesizing DNA at the time of fixing the material and this makes their expression greater than short-lived cell proliferation markers, as the Ki-67[9].

There are many studies about the reactivity of immunohistochemical markers in odontogenic keratocysts and its clinicopathologic correlation; however, the results do not define a standard of marking that distinguishes these sporadic cysts from NBCCS associated cysts[10-13].

This study aims to investigate the immunoreactivity of beta catenin, geminin and MCM2, proteins related to cell proliferation in syndromic and sporadic KCOTs, since there is a lack of studies with these markers in these types of lesions.

Materials and Methods

Specimens

A total of 40 paraffin embedded blocks of KCOT (30 were from NBCCS patients and 10 sporadic lesions) were retrieved from the files of *Centro Clínico de Cabeza y Cuello* (Guatemala) (21 syndromic KCOTs); State University of Western Paraná (Brazil) (4 syndromic KCOTs), Federal University of Rio de Janeiro (Brazil) (2 syndromic KCOTs) and Piracicaba Dental School (Brazil) (3 syndromic KCOTs and 10 randomly selected sporadic KCOTs).

The KCOT were obtained from 22 patients, 12 of them carriers of nevoid basal cell carcinoma syndrome. Data of the patient's age at the moment of diagnosis, gender, lesion location and radiographic characteristics were collected.

Histopathology and immunohistochemistry

The slides stained with hematoxylin and eosin (HE) were evaluated for the presence of epithelial islands, buddings, satellites cysts, orthokeratin, cellular pleomorfism, inflammation and ameloblastoma-like sites.

The immunohistochemical reactions were performed as described by Rumayor et al. (2015)[14]. Antigen retrieval was performed in a pressure cooker with citrate buffer (pH 6.0) for beta catenin and MCM2 and EDTA/Tris (pH 9.0) for geminin. Adequate positive control was obtained for each antibody. A descriptive analysis of histopathological features and immunohistochemical findings was performed for all the markers. Detailed data about the antibodies are present in the **Table 1.**

The immunohistochemical staining of beta catenin, geminin and MCM2 was independently evaluated by one experienced observer without prior knowledge of the clinical parameters or patient conditions. Using a microscope (Leica DM500 Microsystems, Switzerland) coupled to the digital camera (Leica ICC50HD, Leica Microsystems, Switzerland) the reactivity of the beta catenin was performed using two semi-quantitative scores systems, the first aiming to evaluate the extent of reactivity in the epitelial line in each of 10 fields evaluated. For this, was considered "score 0" for non-reactive, "score 1" for reactivity 5 to 25% of the epithelial extension field, "score 2" for reactivity 25 to 50% of the epithelial extension, "score 3" for reactivity 50 to 75% and "score 4" reactivity 75 to 100% of the epithelial extension of the focus field. The intensity of staining was evaluated in the second score system, as follows: 0 (no reactivity), 1 (weak), 2 (moderate) and 3 (strong). The analysis for immunostaining of geminin and MCM2 was performed by counting the epithelial cell nuclei in ten consecutive fields per slide (magnification, x400): labeling index (LI;%) = (number of positively staining nuclei / number the total cells counted). All processes were performed using the Leica Application Suite program - LAS 4.2.0 (Leica Microsystems, Switerzland). The current study was approved by the Ethical Committee of State University of Western Paraná.

Statistical Analysis

Shapiro-Wilk Test was used to check the normality of the quantitative data and the homogeneity of variance was performed using the F Test. The Chi-Square Test for Independence was applied to analyse the variables “gender” and “anatomical location”, followed by Yates’s Corretion Continuity. In order to compare the histopathological data,

Chi Square Test For K Proportions was used followed by Marascuillo Procedure. The T Test was used to analyze the differences between groups only in geminina, since it was the only that presented data normality. Beta catenin and MCM2 were tested using the Mann Whitney U Test. The significance level was $p \leq 0.05$. These tests were performed by XLSTAT® program (Addinsoft, 2015 – França-Paris).

Results

Clinical Data

Thirty KCOTs cases affected 12 patients with NBCCS, 5 females (44.66%) and 7 males (58.33%), whereas in the group of sporadic KCOTs, 5 cases (50%) affected the female patients and 5 (50%) male. There was no statistical significance in gender analysis (p=0.969). Considering the 12 patients with syndromic KCOTs, 7 (58.33%) had more than one lesion over a lifetime: 1 patient had 4 synchronous lesions, 4 patients had 3 synchronous lesions and 2 patients had two synchronous lesions.

In the group of syndromic lesions, the age at the diagnosis moment ranged from 9 months to 59 years with a mean of 16.81 years ± 14.66 years. Patients with sporadic KCOTs showed variation of the age 13-71 years, mean age of 38.44 ± 21.92 years. The mean age of non-syndromic patients was conducted from ages available in 9 cases, one case showed no description of age, so was excluded from this analysis. Ten (83.33%) of 12 syndromic patients with KCOTs developed lesions until 20 years old, while only 2 patients with sporadic KCOTs (20%) were younger than 20 years in the diagnosis time **(Figure 1)**.

Considering the anatomical site of the syndromic KCOTs, 13 occurred in maxilla (43.33%) and 17 in the mandible (56.66%). Of them, it was possible to obtain more detailed information of the location in 22 cases, of which 12 lesions (54.54%) were located in the posterior mandible, 6 lesions (27.27%) in the posterior region of the maxilla, 3 (13%) in the anterior region of the maxilla and 1 (4.5%) in the anterior region of the mandible. In the 10 cases of non-syndromic KCOTs, 7 affected the posterior region of the mandible (70%), 2 (20%) were generically described as "mandible". The syndromic KCOTs were more prevalent in maxilla when compared with the sporadic group (p=0.009), but into the syndromic KCOTs group there is not predilection.

The radiographic data was available in 19 of 30 KCOTs syndromic: 14 lesions (73.68%) had standard unilocular radiolucent and 5 (26.31%) were multilocular radiolucent pattern. In the sporadic KCOTs group, 6 lesions had some information about the radiographic pattern: 4 of them (66.66%) were described as radiolucent and 2 described as mixed pattern (33.33%). There was no description about the morphology observed by imaging the sporadic KCOTs group.

Histopathological Features

The KCOTs showed cystic cavities lined by parakeratinized stratified squamous epithelium with 5-8 cells thick and the corneal surface layer was corrugated. The basal layer of the epithelium was composed by cubic or columnar cells arranged in a palisade pattern. There was epithelial detachment of connective tissue capsule and in some areas it was observed epithelial buddings into the cystic capsule. In addition, it was identified the presence

of keratin filaments into the lumen in some cases. Cystic capsule was composed by fibrovascular tissue, with the presence of chronic inflammation in some cases, fibroangioblastic proliferation; foamy macrophages, satellites cysts, epithelial islands areas and ameloblastoma-like sites. Satellites cysts and cellular pleomorfism were more prevalent in syndromic cases **(Table 2)**.

Immunohistochemical Assessment

Beta catenin labeling pattern presents in cell membrane and its reactivity of extesion does not show statistical significance between syndromic and sporadic KCOTs groups (p=0.537) respectively. The analysis of intensity of beta catenin showed that score 0 and score 1 was more prevalent in syndromic KCOTs group, the score 2 was more prevalent in sporadic KCOTs and there was statistical equivalence in the score 3 **(Table 3)**. The reactivity for geminin and MCM2 in both groups showed a nuclear staining in basal and suprabasal layers, with the exception of luminal layer. In these groups, the nuclear staining occurred predominantly in the first suprabasal layer. The mean of positive geminin cells was 3.47% in syndromic KCOTs group while in sporadic KCOTs group was 4.17% ($p=0.386$) whereas the means of MCM2 positive cells was higher in sporadic KCOTs group than syndromic KCOTs group (p=0.011) (**Table 4**). Sites with the presence of inflammation were more reactive, however, these areas were not considered for statistical analysis.

It was observed that the reactivity of beta catenin, geminin and MCM2 in epithelial islands and satellites cysts showed the same staining pattern of cystic epithelium **(Figure 2)**. The areas of epithelial buddings and high cellularity showed no or occasional reactivity. The

Figure 3 shows representative images of immunohistochemical reactions of geminina and MCM2 and **Figure 4** shows cystic epithelia images in syndromic and sporadic KCOTs reactive for beta catenin.

Discussion

Previous studies suggest that sporadic KCOTs and syndromic KCOTs share a common pathogenesis associated with mutations in the gene patched-1 (PTCH), with consequent anomalous activation of the Sonic Hedgehog signaling pathway[3]. Despite this statement, the KCOTs associated with NBCCS have high growth potential, infiltration, recurrence and tend to occur as multiple lesions when compared to sporadic KCOTs. By consequence, research supports the existence of a distinct biological behavior between the two injuries. Studies evaluating the expression of proteins associated with proliferative activity of the epithelium, as well as its relationship with the stroma, have been conducted in order to demonstrate its aggressive potential[10, 15, 16].

The mean age at diagnosis of 12 syndromic patients of this study was 16.81 years, approaching the analysis of Kimonis et al. (1997)[17], Amorim et al. (2004)[16] and Gonzales-Alva et al. (2008)[19] found that as average age: 17.1; 15.2; 19.5 years, respectively. In the group of sporadic KCOTs, the mean age was higher (38.44 years). These data are similar to study of Jones et al. (2006)[20] in which the mean age was 41.3 years, however differ from Amorim et al. (2004)[18] in which the mean age of the patients with sporadic lesions was 19.8 years. Also with respect to age, this study showed that 83.33% of syndromics KCOTs developed in individuals belonging to the first and second decades of life, similar analyzes of Ahn et al. (2004)[7], Oda et al. (1999)[21]. In the group of sporadic lesions, there was a bimodal

distribution, with 60% of the lesions occurring until the third decade of life and 40% occurred after the fifth decade of life, which is in agreement with findings by Oda et al. (1999)[21], Jones et al. (2006)[20] and Mendes et al. (2010)[12] who demonstrated the same bimodal pattern.

In this study 7 patients (58.33%) of 12 NBCCS patients have more than one lesion throughout life. However, 4 of 5 patients with single lesions presented at the first biopsy moment, less than 13 years old, it can be assumed that the development of other injuries can still occur in their lifetime. In the study of Woolgar et al. (1987)[22], 55 (91.66%) of 60 NBCCS patients showed 2 or more KCOTs throughout their lives.

Still referring to the 12 syndromics patients, 7 (58.33%) were males and 5 (41.66%) females. In the study by González-Alva et al. (2008)[19] a higher prevalence in females was observed (63.6%). In the sporadic KCOTs group of this study, the distribution of lesions between men and women was equivalent, which also differs from research Zhao et al. (2002)[23] that presented predominance of males (65.91%) of a 484 non-syndromic patients.

Anatomically, the majority of the syndrome and sporadic lesions in this study affected the jaw (56,66% and 90%, respectively). In addition, in 22 of 30 syndromic lesions was possible to obtain more detailed data of the location, in which 12 lesions (54%) were located in the posterior mandible and 1 (4%) in the anterior region of the mandible. Regarding the sporadic lesions, 7 of them (70%) contained information that had occurred in the posterior mandible, 2 (20%) were described as the generic location “jaw” and 1 case had not described its location.

These data are similar to others found in the literature as the study of Woolgar et al. (1987)[22] in which the mandible bone, more particularly the posterior region was the most

affected in 66% of syndromic KCOT. This data is also similar to other studies that have found the following percentages of mandibular prevalence: 70.5% of their sample that included sporadic and syndromic KCOTs in GONZÁLES-ALVA et al. (2008)[19]; 90% of sporadic KCOTs and 80% of syndromic KCOTs of Amorim et al. (2004)[17]; 85% of the sample of sporadic and syndromic KCOTs of Mendes et al. (2011)[12] and 80% of sporadic KCOTs and 70% of syndromic KCOTs of KADLUB et al. (2013)[24].

The radiographic pattern unilocular radiolucent was observed in 73.68% of syndromic KCOTs with information available. In the sporadics cases, also with available information, 66.66% had radiolucent pattern and 33.33% was reported with mixed pattern, however, in 1 sporadic case there was no description. Most authors report that the unilocular pattern is prevalent in KCOTs [23, 25, 26].

Among the histopathological characteristics investigated, the cellular pleomorfism and satellite cysts were more frequent in syndromic KCOTs than sporadic KCOTs. In addition, epithelial islands along the cystic capsule seem more frequent in syndromic cases, but without statistical significance. Only 2 syndromic cases showed areas ameloblastoma-like, while the same feature was not observed in any sporadic case. Payne (1972)[27] compared the histopathologic findings of KCOTs, including recurrent cases, non-recurrent and NBCCS associated KCOTs, and others non-keratinized cystic lesions and demonstrated that the presence of satellite cysts, epithelial islands and inflammation was more frequent in cases associated with NCBBS. These authors also did not find the presence of areas ameloblastoma-like in any sporadic KCOTs.

Many studies about the expression of proteins related to cell proliferative activity, suppressor tumor genes and oncogenes have been conducted in order to elucidate the nature of neoplastic KCOTs. In this context, some researchers have analyzed the expression of proteins such as p53[10, 12, 19, 28,-32], ki-67[6, 33- 35], p63[36-39]. However, studies involving markers as beta catenin, geminin and MCM2 are used in other types of lesions, not odontogenic.

The beta-catenin is a protein related to Wnt signaling pathway, which regulates cellular proliferation and differentiation. The findings of Ahn et al. (2008)[7] indicate that aberrations in Wnt signaling by beta catenin mutations may play a crucial role in the development and differentiation of the odontogenic epithelium of calcifying odontogenic cyst[40]. Leonardi et al. (2013)[41] conducted a study comparing the activity of beta catenin in syndromic and sporadic KCOTs. In their study, immunostaining in sporadic KCOTs was restricted to the basal and suprabasal layers, while syndromic KCOTs were positive for beta catenin in all layers. The team suggested that the expression of beta catenin is related to inhibition of apoptosis and this interaction may develop significant role in the growth and recurrence of KCOTs. Similar to their study, our research also showed variation in the staining pattern among the various lesions evaluated. It was observed that both syndromic and sporadic KCOTs showed reactivity consistent to the beta catenin protein. However, considering the score system adopted, the lowers scores (0 and 1) of intensity were more prevalent in syndromic cases.

The geminin is another protein that acts in the cell division control. The geminin concentrations fluctuate a lot during the cell cycle in which its presence is noticed after the G1 phase. The concentration of geminin increases during S, G2 and M phases, however,

changes in this protein can cause uncontrolled cell proliferation[11]. According Sundara Rajan, et al. (2014)[42] there is no established criteria in the literature to evaluate the positivity of geminina. In the study published by these authors, 63.9% of the sample was positive for geminin, however, the work was referring to breast tumors. In our study, only 1 case was negative for anti-geminina, being the sporadic KCOTs group. Gouvea et al. (2012)[43] conducted an immunohistochemical study in a sample of 21 patients with proliferative verrucous leukoplakia and they found a lot of variation in reactivity rates (3-40%), and in general, this oscillating rate even within similar degrees of epithelial dysplasia.

In our study, the immunohistochemical profile was strictly nuclear staining in epithelial cells. Areas with increased cellularity and cellular atypia showed low positivity rates, however, the first suprabasal layer adjacent to these areas showed most cells positive for geminina. The positivity rate for geminina was relatively low, being the means 3.47% and 4.17% in syndromic KCOTs and sporadic KCOTs respectively, without statistical difference.

The proliferative potential can be assessed by immunohistochemistry using antibodies against specific proteins associated with the cell cycle, such as MCM2. The MCM2 protein plays an important role in many biological and pathological events, such as in the pathogenesis of cysts and tumors, furthermore participates in the cell proliferation process[44]. The MCM2 is involved in DNA replication and controlling expression begins early in the G1 phase and is maintained throughout the cell cycle. The MCM2 is also expressed in proliferating cells without being in DNA synthesis activity at the time of fixation of the material collected and this makes its expression is greater than cell proliferation markers

short-lived, as the Ki-67. Gouvea et al. (2012)[43], in a study using the proliferative verrucous leukoplakia, demonstrated that the MCM2 positivity rate was higher in tissues with the highest degree of epithelial dysplasia, indicating constant proliferative process. In our study, as well as geminin, the staining was also confined to the nucleus of epithelial cells in areas adjacent to the areas of increased cell proliferation. The positivity index for MCM2, was higher in sporadic KCOTs than syndromic KCOTs ($p=0.011$).

In summary, this study aimed to analyze and compare the clinical, histopathological and immunohistochemical cases of syndromic and sporadic KCOTs from different institutions in Brazil and abroad and understanding the role of proteins associated with proliferation /cell cycle (beta catenin, geminina and MCM2) in an attempt to associate its expression with the biological behavior of KCOTs. In this study, the histological features show evidences of greater aggressiveness as, for example, most cellular pleomorphism rate and higher satellites cysts index. However, in this study, there is not significant evidence that makes sure of the higher proliferative potencial in syndromic KCOT using these markers. Further larger studies are needed to obtain more precise estimates of the sensitivity and specificity of these markers in these types of lesions.

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Figures legends

Figure 1. Distribution of patients according to the decade at diagnosis time.

Figure 2. Immunohistochemical expression of beta catenin in an epithelial island from a syndromic KCOT (IHC, ×400).

Figure 3. A- Immunohistochemical expression of geminin in a syndromic KCOT. B- Immunohistochemical expression of geminin in a sporadic KCOT. C- Immunohistochemical expression of MCM2 in a syndromic KCOT. D- Immunohistochemical expression of MCM2 in a sporadic KCOT (IHC, ×400).

Figure 4: A- Immunohistochemical expression of beta catenin in a syndromic KCOT. B- Immunohistochemical expression of beta catenin in a sporadic KCOT

Table legends

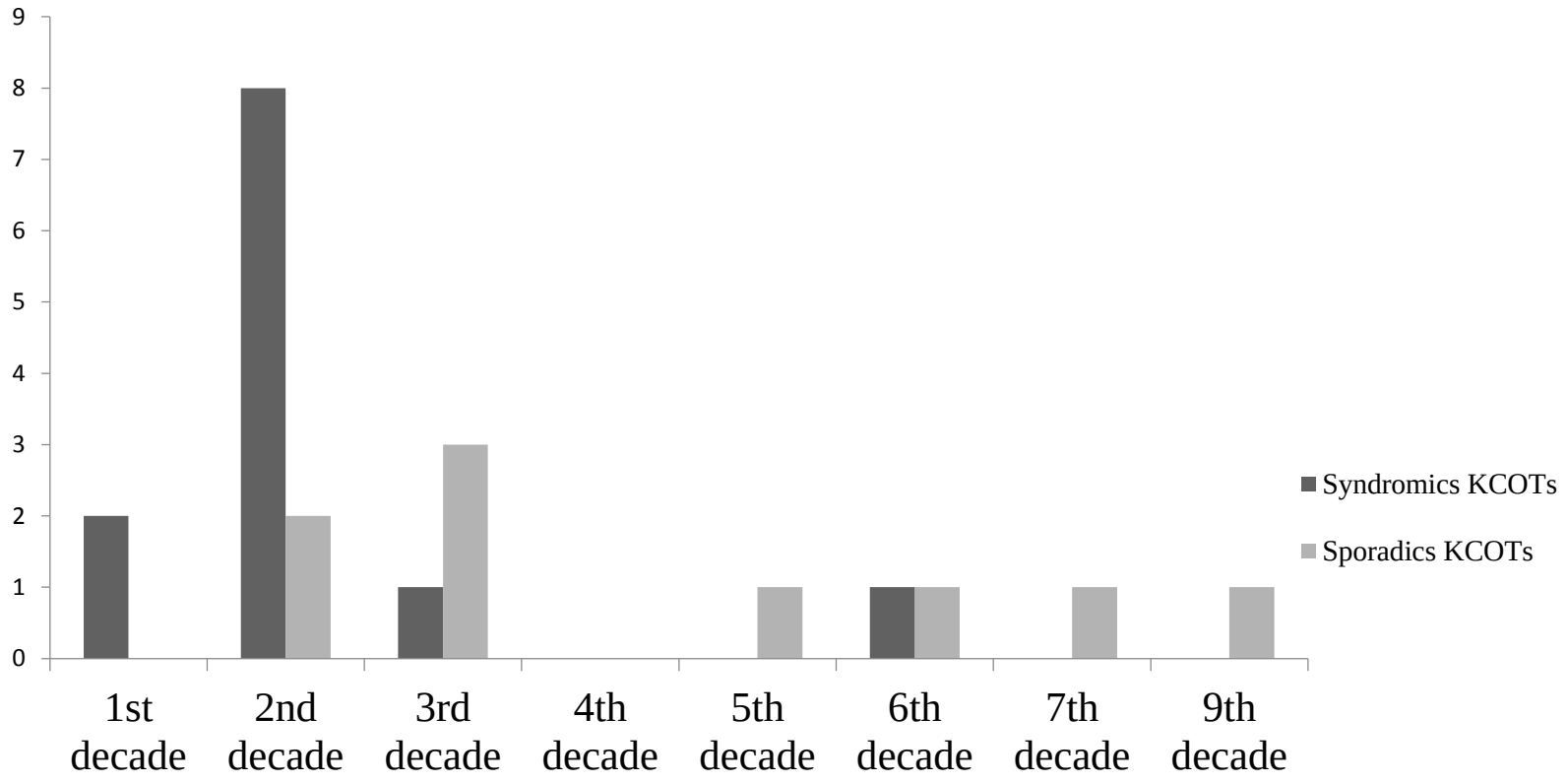
Table 1. Antibodies used for the immunohistochemical analysis.

Table 2. Occurrence of histopathological features in syndromic and sporadic KCOTs.

Table 3. Immunohistochemical analysis of beta catenin (semi-quantitative scoring systems of intensity of reactivity).

Table 4. Reactivity means for beta catenin (extension), geminin and MCM2.

Figure 1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Syndromics KCOTs
Sporadics KCOTs

Figure 2

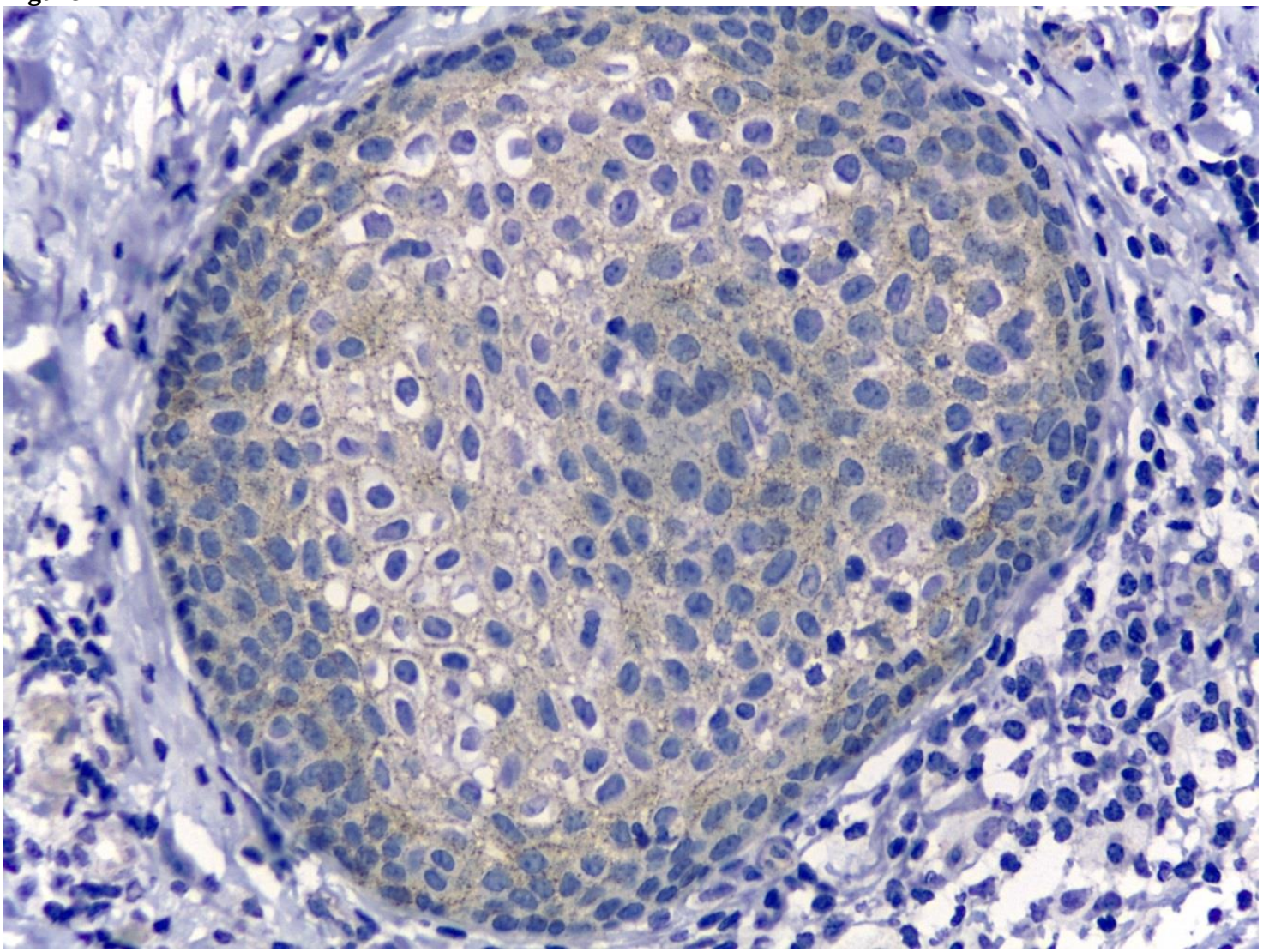

Figure 3

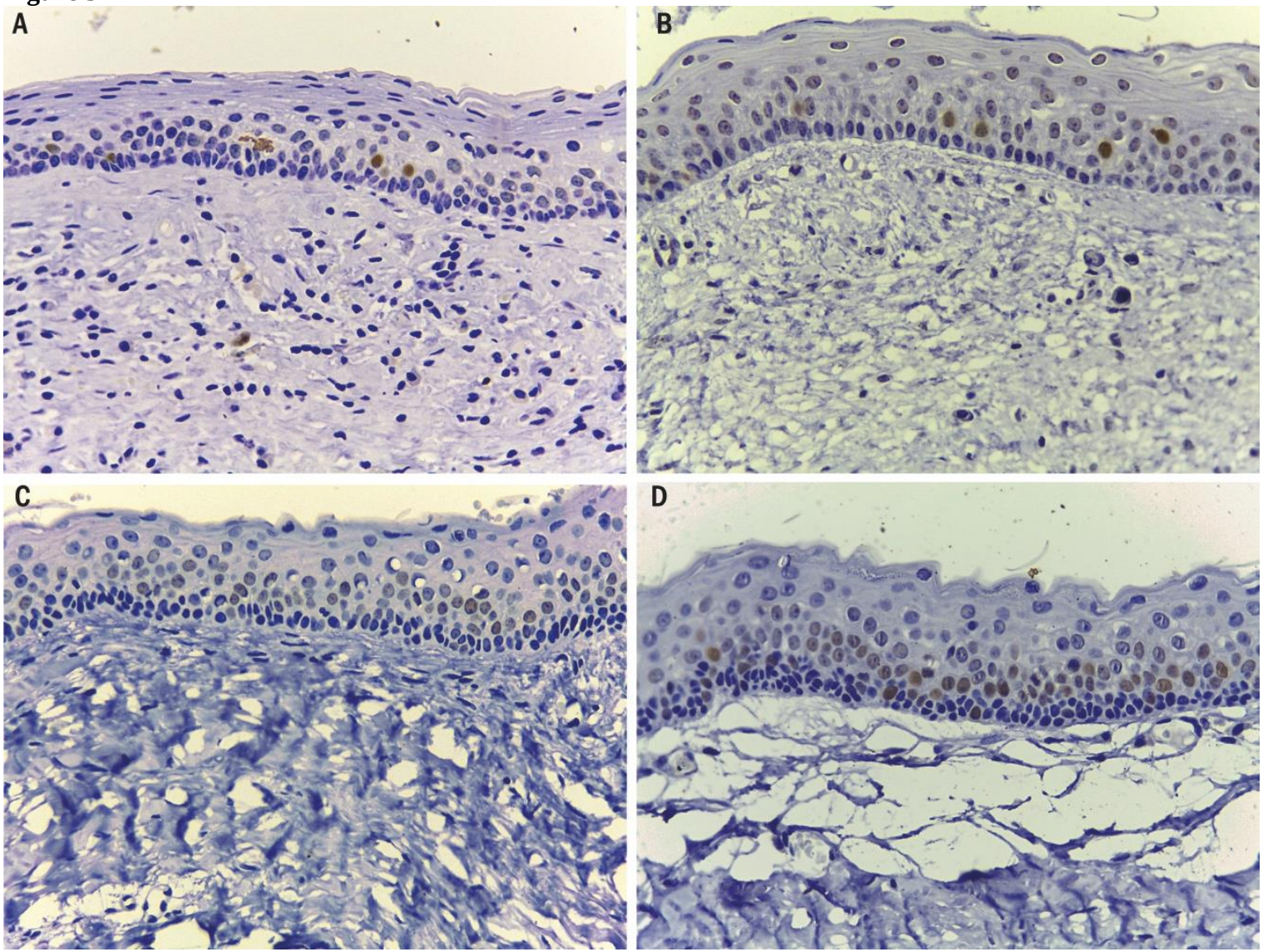

Figure 4

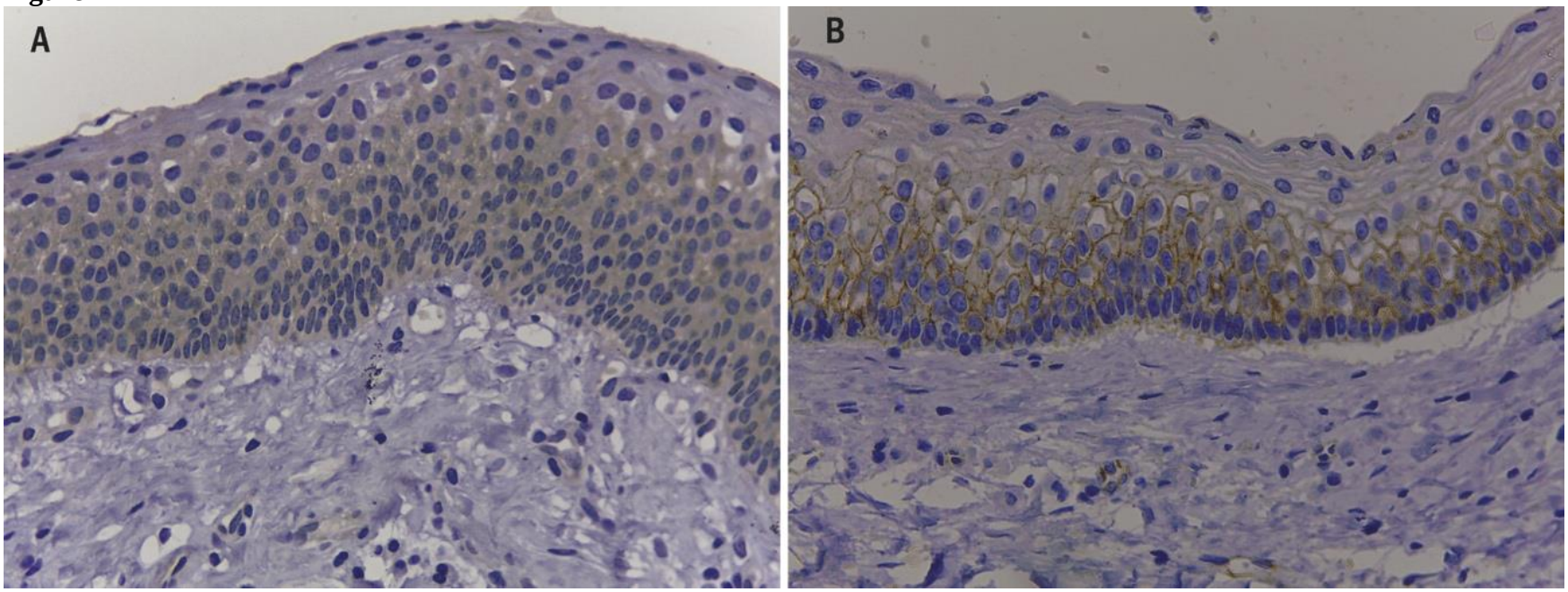

Table 1

Primary Antibody	Clone	Dilution	Source
Beta catenin	17C2	1:50	Novocastra®, Nussloch,Germany
Geminin	EM6	1:50	Novocastra® Nussloch,Germany
MCM2	CRCT2.1	1:30	Novocastra® Nussloch,Germany

Table 2

Histopathological Features	**Syndromics KCOTs (n=30)**	**Sporadics KCOTs (n=10)**	***p value***
Epithelial Islands	15 (50%)	4 (40%)	0,583
Buddings	16 (53,33%)	7 (70%)	0,356
Satellites Cysts	14 (46,66%)	0 (0%)	0,007*
Orthokeratin	1 (3%)	0 (0%)	0,559
Cellular Pleomorphism	11 (36,66%)	0 (0%)	0,025*
Inflammation	22 (73,33%)	7 (70%)	0,839
Ameloblastoma-like sites	2 (6,66%)	0 (0%)	0,402

*Results with statistical significance ($p<0,05$).

Table 3

	Intensity				***p value***
	Score 0	*Score 1*	*Score 2*	*Score 3*	
Sporadics KCOTs (n=100)	4 * (4%)	12 * (12%)	37 * (37%)	47 (47%)	0,003
Syndromics KCOTs (n=300)	30 * (10%)	64 * (21,33%)	67 * (22,33%)	139 (46,33%)	

*Results with statistical difference (p<0,05).

Table 4

Relative Frequence (%)	**Syndromics KCOTs** Mean	**Sporadics KCOTs** Mean	***p value***
Beta catenin (extent)	87%	96%	0,348
Geminin	3,47%	4,17%	0,386
MCM2	3,85%	9,29%	0,011*

*Results with statistical significance ($p<0,05$).

Oral Surgery Oral Medicine Oral Pathology Oral Radiology

For Authors

Authors Informations

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Pesquisador: ANA LUCIA CARRINHO AYROZA RANGEL

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Comentários e Considerações sobre a Pesquisa:

O estudo proposto apresenta pertinência e valor científico Não há restrições quanto ao objeto ou metodologia do estudo.

Considerações sobre os Termos de apresentação obrigatória:

Todos os documentos necessários foram apresentados.

Recomendações:

Sem recomendações

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Conclusões ou Pendências e Lista de Inadequações:

Sem pendências

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

As solicitações feitas foram atendidas pela pesquisadora.

CASCAVEL, 04 de Dezembro de 2014

Assinado por:
João Fernando Christofoletti
(Coordenador)

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