Expressions of bax, bcl-2 and Ki-67 in Odontogenic Keratocysts (Keratocystic Odontogenic Tumor) in Comparison with Ameloblastomas and Radicular Cysts

Odontojen Keratokistlerin (Keratokistik Odontojen Tümör) bax, bcl-2 ve Ki-67 Ekspresyonlarının Ameloblastom ve Radiküler Kistlerle Karşılaştırılması

Merva SOLUK TEKKEŞİN¹, Sevcihan MUTLU², Vakur OLGAÇ¹

Department of ¹Tumor Pathology and ²Oncologic Cytology, Istanbul University, Institute of Oncology, İSTANBUL, TURKEY

ABSTRACT

Objective: The aim of the study was to determine the apoptotic features and proliferation potential of odontogenic keratocysts compared with ameloblastomas and radicular cysts by analysing the role of bax, bcl-2, and Ki-67.

Material and Method: The study material consisted of 20 odontogenic keratocysts, 20 radicular cysts, and 20 ameloblastomas. Immunohistochemically, bax, bcl-2 and Ki-67 were applied. The positive cells were evaluated in both neoplastic/nonneoplastic odontogenic epithelium and connective tissue cells.

Results: Ameloblastoma showed stronger bcl-2 expression than odontogenic keratocysts and radicular cysts. Bcl-2 expression in the whole thickness of epithelium and connective tissue of odontogenic keratocyst was significantly higher than radicular cyst. The expression of bax in the epithelium of radicular cyst was significantly higher than odontogenic keratocyst and ameloblastoma. The lining epithelium of odontogenic keratocyst showed stronger Ki-67 expression than ameloblastoma and radicular cyst.

Conclusion: The proliferation potential of the epithelium and the overexpression of various anti-apoptotic proteins in odontogenic epithelial tumors are quite significant for their clinical behaviour. High expressions of bcl-2 and Ki-67 in odontogenic keratocysts accord with their aggressive clinical behaviour and a high recurrence rate.

Key Words: Bax protein, bcl-2 genes, Ki-67 antigen, Odontogenic cysts, Squamous odontogenic tumor

ÖZ

Amaç: Bu çalışmanın amacı, odontojen keratokistlerin apoptotik özellikleri ve proliferasyon potansiyellerini analiz etmek için bax, bcl-2 ve Ki-67 analiz sonuçlarının ameloblastom ve radiküler kistlerle karşılaştırılmasıdır.


Anahtar Sözcükler: Bax proteinleri, bcl-2 genleri, Ki-67 antijeni, Odontojenik kistler, Skuamöz odontojenik tümör

INTRODUCTION

The odontogenic keratocyst used to be classified as odontogenic developmental cysts arising from dental lamina or remnants of the dental lamina (1). Tendency to recur following surgical treatment, high epithelial turnover rate, aggressive clinical behaviour, and a relationship to Gorlin-Goltz Syndrome (Nevoid basal cell carcinoma syndrome; NBCCS) indicate that odontogenic keratocyst
might be a benign tumor. Therefore, odontogenic keratocysts have been re-classified in the 2005 edition of the WHO Classification of Head and Neck Tumors. They were included in the odontogenic tumors classification, named keratocystic odontogenic tumor (2,3). But the question of ‘whether odontogenic keratocyst is a neoplasm or not’ is still controversial.

Apoptosis, also known as programmed cell death or physiologic cell death, plays an important role in the development and maintenance of homeostasis within all multicellular organisms. Bcl-2 family proteins have inhibitory and stimulatory effects. Bcl-2, bcl-xL, bcl-w are anti-apoptotic members of this family whereas bax, bak, bok are pro-apoptotic members (4). Overexpression of bcl-2 has been shown in various tumors and diseases (5). Expression of bcl-2 protein was also reported in many stages of tooth development (6).

A number of immunohistochemical studies have explored the various proliferation markers such as p53, Ki-67, PCNA, and apoptosis markers such as bax, bcl-2 to elucidate any relationship between histological features and biological potential of odontogenic keratocysts (7). Generally, these studies included epithelial cell examination.

In this study, the role of the apoptosis-related factors and proliferative activity both in lining epithelium of odontogenic keratocysts, radicular cysts, tumor islands of the ameloblastomas and connective tissue cells of all groups were examined.

MATERIAL and METHODS

Samples selection

A total of 60 cases diagnosed as odontogenic keratocyst, radicular cyst and ameloblastoma were selected from our tissue block archive between January 2005 and January 2008. The cases were reviewed and the most diagnostic cases were selected. They were consisted of 20 odontogenic keratocysts (9 women, 11 men; mean age 46.5), 20 radicular cysts (8 women, 12 men; mean age 29.05) and 20 ameloblastomas (10 women, 10 men; mean age 31.2).

Odontogenic keratocyst cases associated with NBCCS and orthokeratotic odontogenic keratocysts were excluded from the study.

Immunohistochemistry

For immunohistochemistry, the paraffin blocks were cut serially into approximately 5 µm thick sections on charged slides. They were deparaffinized with xylene and ethanol. Histostain-Plus Bulk Kit (Zymed 2nd Generation, LAB-SA Detection System, 85-9043) was used in the study. For antigen retrieval, the sections were microwaved four times for 5 min in citrate buffer (pH=6.0). Endogenous peroxidase activity was blocked by incubating the sections with 3% H₂O₂. To prevent non-specific reactions, sections were incubated with block solution. Bax antibody at a dilution of 1:50 (Santa Cruz Biotechnology, Inc., B-9, Mouse, Monoclonal, sc-7480), bcl-2 ready to use (Thermo Scientific, Mouse, Monoclonal MS-123-R7) and Ki-67 at a dilution of 1:50 (Zymed Laboratories, Mouse, Monoclonal, Clone 7B11) were used as primary antibodies. Slides were incubated for 120 min with Ki-67 and bax, and overnight with bcl-2. Negative control sections treated with phosphate-buffered antibodies were confirmed to be unstained. The secondary antibody was reacted for 25 min. AEC (Zymed Laboratories, 00-2007, Lot No: 319293A) chromogen was used to visualize the reaction. Finally, the sections were counterstained with Mayer’s haematoxylin and evaluated by a light microscope.

Evaluation methods

The specimens were examined in Olympus BX60 microscope attached to a colour video camera (Olympus Analysis Five). The positive-stained cells were evaluated in the epithelium and connective tissue cells. These cells were counted in 5 contiguous and consecutive microscopic high-power fields in both epithelial and connective tissue components. In the stroma, the endothelial, round and fusiform cells were counted. The number of positive cells was divided into the total number of cells counted in the whole area. The result was multiplied by 100 to find the percentage of positive cells.

All calculations were performed by the SPSS 16.0 (Statistical Package for Social Science). Comparative analysis of data was performed by using the Mann-Whitney U-test for two independent samples. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

The study group was consisted of 27 women (45%) and 33 men (55%) with a mean age of 35.5 (range 7 - 69).

All odontogenic keratocysts occurred in the mandible that the posterior region and ramus were the most frequent anatomic site with 15 cases (75%), followed by the premolar region with 4 cases (20%). Most ameloblastomas were located in the mandible with 19 cases (95%), of which 15 cases (75%) occurred in the posterior site and ramus. Only one case was in the maxilla molar region (5%). 14 cases of radicular cysts occurred in the maxilla (70%) with the most common site of presentation in the anterior maxilla (45%).

Immunohistochemical findings in the epithelial tumor island/epithelium of odontogenic keratocysts, radicular cysts and ameloblastomas

The results are summarized in Figure 1A-C. The Ki-67 immunopositive cells in odontogenic keratocysts were
Bcl-2 expression in the whole thickness of epithelium of odontogenic keratocysts (Figure 3A) was significantly higher than radicular cysts (p<0.001; Figure 3B). Ameloblastomas expressed stronger bcl-2 staining than odontogenic keratocysts and radicular cysts (p<0.001). Bcl-2 detected mainly in the peripheral layer whereas only a few cells were positively stained in the central layer of epithelial tumor islands in ameloblastomas (Figure 3C). The expression of bax in the epithelium of radicular cysts (Figure 4A) was significantly higher than odontogenic keratocysts and ameloblastomas (p<0.001). Bax expression did not show any statistically significant difference between odontogenic keratocysts (Figure 4B) and ameloblastomas (Figure 4C). Both of these groups showed weak immunoreactivity for bax.

Immunohistochemical findings in the connective tissue cells of odontogenic keratocysts, radicular cysts and ameloblastomas

Reactivities for bax, bcl-2, Ki-67 in the connective tissue cells of odontogenic keratocysts, radicular cysts and ameloblastomas were observed. The results are summarized in Figure 5A-C. Radicular cysts demonstrated higher number of Ki-67 positive cells than odontogenic keratocysts and ameloblastomas (p<0.001). There was no significant difference in the Ki-67 expression between connective tissue cells of odontogenic keratocysts and ameloblastomas. A higher number of bcl-2 was found in odontogenic keratocysts as compared to radicular cysts (p<0.001). There was no significant difference in expression of bcl-2 between odontogenic keratocysts and ameloblastomas, as well as between radicular cysts and ameloblastomas. All groups showed weak bax expression in the connective tissue cells and no significant differences between groups were observed.

DISCUSSION

There are a few studies in the literature to examine bax, bcl-2, and Ki-67 expressions both in odontogenic epithelium and connective tissue cells of oral lesions, so
Figure 2: Representative pictures of odontogenic lesions stained with Ki-67. Immunoreactivity was observed particularly (A) in the suprabasal layers of the epithelium of odontogenic keratocyst (x200), (B) in the basal layer of radicular cyst (x200), and (C) in the peripheral layer of tumor islands of ameloblastoma (x200).

Figure 3: Representative pictures of odontogenic lesions stained with bcl-2. Bcl-2 positive cells were observed (A) in all layers of the lining epithelium and connective tissue cells of odontogenic keratocyst (x200), (B) in radicular cyst, a small number of bcl-2 immunopositive cells were observed (x200), (C) ameloblastoma showed strong bcl-2 positive cells (x100).
analizimiz, odontogenic keratokistler, radikuler cystler ve ameloblastomların klinik özelliklerini, genetik mutasyonları ve patolojik改變を比較することに重視されています。Bcl-2 белки genetik mutasyonları ve protein düzeyleri, bu tıbbi durumları daha iyi anlamak için önemlidir.

Özellenen ve klinik özellikler:

- **Odontogenic Keratokist:** Bu tip cystler, çok sayıda epitel hücre ve prosentüllerin oluşturulmasıyla karakterizedir. Ki-67 ve Bax markaları, odontogenic keratokistlerin proliferasyon duygusunu gösterir.
- **Radikuler Cyst:** Bu cystler, çok sayıda fibroblast hücre ve epiteliyal hücrenin oluşturduğu bir yapıya sahiptir. Ki-67 ve Bax markaları, radikuler cystlerin proliferasyon duygusunu gösterir.
- **Ameloblastoma:** Bu tumor, çok sayıda epiteliyal hücre ve prosentüllerin oluşturulmasıyla karakterizedir. Ki-67 ve Bax markaları, ameloblastomların proliferasyon duygusunu gösterir.

Bu çalışma, odontogenic keratokistler, radikuler cystler ve ameloblastomların klinik özelliklerini, genetik mutasyonları ve patolojik改变を比較することに重視しています。Bcl-2 белки genetik mutasyonları ve protein düzeyleri, bu tıbbi durumları daha iyi anlamak için önemlidir.
In the present study, low expression of bcl-2 was found in the lining epithelium of radicular cysts whereas odontogenic epithelium of odontogenic keratocysts and ameloblastomas showed very strong positive staining for bcl-2. Piattelli et al. evaluated the bcl-2 protein expression in dentigerous cysts, radicular cysts and odontogenic keratocysts (15). They found that bcl-2 expression were shown in the basal layer of all odontogenic keratocyst cases, on the contrary the other types of cysts showed almost complete negativity for bcl-2. However, bcl-2 expression in radicular cysts was determined in other studies (16,17). In our study, expression of bcl-2 was found in the whole thickness of the epithelium of odontogenic keratocysts and predominantly in the peripheral cell layer of the tumor island of ameloblastomas. These results were similar to that found by Mitsuyasu et al. and Sandra et al. (18,19). Bcl-2 positive cells were higher in the stroma cells of odontogenic keratocysts and ameloblastomas than radicular cysts. This could lead to aggressive growth pattern of odontogenic keratocysts and ameloblastomas.

Bax expression was weaker than bcl-2 reactivity in odontogenic keratocysts and ameloblastomas and there was no distinct difference in bax reactivity of these two groups. In lining epithelium of radicular cysts, bax expression was observed very strongly which implies increased apoptotic activity as compared with odontogenic keratocysts. All groups showed almost complete bax negativity in stromal cells.

Our findings indicate that odontogenic keratocysts have much more pro-apoptotic protein than anti-apoptotic protein, considering that bcl-2 was found very higher than bax in all samples of odontogenic keratocysts.

In 2006, Kolár et al. analysed the expression of some apoptosis and proliferation markers in NBCCS-associated odontogenic keratocysts, sporadic odontogenic keratocysts and other odontogenic cysts (20). The results of odontogenic keratocysts associated with NBCCS had a different immunophenotype from sporadic odontogenic keratocysts and the results of both types were distinguishable from other odontogenic cysts. Odontogenic keratocyst cases with NBCCS were not included in this study not to confuse the results. Because NBCCS is an autosomal dominant disease, and is known as the patients with NBCCS have the gene mutation which underlies this disease has been mapped to chromosome 9q22.3-q31 (7,21).

Vered et al., in 2009, published a study on the expression of PTCH, SMO, GLI-1 and bcl-2 in odontogenic keratocysts, ameloblastomas and other odontogenic cysts (22). They found that the results of odontogenic keratocysts were similar to ameloblastomas but different from the other

---

Figure 5: Graphics of immunoexpression of A) Ki-67, B) bcl-2, and C) bax in connective tissue cells of ameloblastomas, odontogenic keratocysts, and radicular cysts.
odontogenic cysts. The authors suggested that their results supported the notion of odontogenic keratocyst having a neoplastic nature. Bcl-2 results in the study were almost the same comparing with our results.

In conclusion, the results of our study suggest that odontogenic keratocysts have a high proliferative and survival activity, and they might be one of the reasons why odontogenic keratocysts have a high recurrence rate. The proliferation potential of the epithelium and the overexpression of various anti-apoptotic proteins in odontogenic epithelial tumors are quite significant for their clinical behaviour. We have also shown that connective tissue cells were not as important as epithelial cells in the biological behaviour of these odontogenic lesions. High expressions of bcl-2 and Ki-67 in the lining epithelium of odontogenic keratocysts accord with their aggressive clinical behaviour and a high recurrence rate. Although these results support that odontogenic keratocyst is a neoplasm, there are not enough genetic studies. Further studies on genomic changes may help for better understanding of the pathogenesis of odontogenic keratocysts.

ACKNOWLEDGEMENT
The present work was supported by the Research Fund of Istanbul University. Project No: 2521

REFERENCES