Proliferative and anti-apoptotic indices of unicystic ameloblastoma, odontogenic keratocyst, dentigerous cyst and radicular cyst

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Abstract

Objective: The aim of this study is to compare the proliferative capacity and antiapoptotic capacity of unicystic ameloblastoma (UA), odontogenic keratocyst (OKC), dentigerous cyst (DC) and radicular cyst (RC) by assessing the Ki-67 labeling index (LI) and Bcl-2 LI, respectively.

Materials and Methods: Formalin-fixed, paraffin-embedded tissue blocks of twenty-three histopathologically diagnosed UAs, 6 OKCs, 8 DCs and 10 RCs were selected from the archival specimens. Five micrometer thick sections of tissue blocks of the lesions were processed for immunohistochemical staining with Ki-67 and Bcl-2. The Ki-67 LI and Bcl-2 LI were determined for the cases with a positive reaction.

Results: Ki-67 expression was seen in 6 (26.1%) cases of UA, 4 (66.7%) OKC, 1 (12.5%) DC and 1 (10.0%) RC. There was a statistically significant difference between the mean Ki-67 LI of UA and OKC (P = 0.024). Bcl-2 expression was seen in 16 (69.6%) UA, 5 (83.3%) OKC, 5 (62.5%) DC and 5 (50.0%) RC. The mean Bcl-2 LI of UA was significantly higher than that of DC (P = 0.048). Furthermore, cases of OKC had significantly higher mean Bcl-2 LI compared to DC (P = 0.026) and RC (P = 0.049).

Conclusion: This study suggests that the Ki-67 LI may help in differentiating OKC from UA. The Bcl-2 LI may be useful in differentiating UA from DCs, and differentiating OKC from DC and RC.

Keywords: Bcl-2, Ki-67, odontogenic cysts, unicystic ameloblastoma

INTRODUCTION

Growth and development are an essential process for the sustenance of any species, and the process of cellular proliferation and division plays an important role in growth. This process is closely regulated under normal circumstances, and dysregulation of cellular proliferation can lead to the development of neoplasms.[1] Markers of cellular proliferation, such as Ki-67, are used in the assessment of the proliferative activity of both normal and neoplastic cells.[2] Ki-67 is a nuclear protein expressed only in dividing cells. Its expression in ameloblastoma and odontogenic cysts has been used as a diagnostic and prognostic tool and has also aided the understanding of the biological behavior of these pathological conditions.[3] Its relatively short half-life, rapid degradation and absence in quiescent cells make it a very useful and specific marker of cellular proliferation.[4]
Apoptosis refers to programmed cell death that normally occurs during development and aging. It may also occur as a protective mechanism in damaged cells. The process of apoptosis is closely regulated by several proapoptotic and antiapoptotic proteins. Bcl-2 is an antiapoptotic protein that inhibits programmed cell death without concurrent promotion of cellular proliferation. Overexpression of Bcl-2 favors cell survival and is a common finding in many human neoplasms. Its expression is useful in differentiating neoplasms and neoplastic cysts from non-neoplastic cysts.

Unicystic ameloblastoma (UA), odontogenic keratocyst (OKC), dentigerous cyst (DC) and radicular cyst (RC) are common cystic lesions in the jaws. UA is a distinct type of ameloblastoma and is regarded as a neoplastic cyst. DC and RC are non-neoplastic odontogenic cysts. The nature of OKC is yet to be fully elucidated, and it has been regarded by various authors to be a neoplastic cyst. However, it is presently considered by the WHO to represent a nonneoplastic cyst with the potential for aggressive behavior.

Efforts have been made to determine the biologic behavior of these odontogenic lesions using different markers. Variable biologic behavior has been reported for ameloblastoma, depending on the clinical type. The UA is said to have a less aggressive behavior and lower recurrence rate compared to the solid/multicystic type. OKC has a more aggressive biologic behavior compared to DC and RCs, which show an indolent behavior, together with a low recurrence rate.

Although many studies have evaluated the proliferative and antiapoptotic potentials of UA, OKC, DC, and RC, there appears to be the paucity of studies that have concurrently studied both the proliferative and antiapoptotic potential of these lesions. This study aims to compare the proliferative capacity and antiapoptotic capacity of UA, OKC, DC, and RC by assessing the Ki-67 labeling index (LI) and Bcl-2 LI, respectively.

**MATERIALS AND METHODS**

**Samples**

Formalin-fixed, paraffin-embedded tissue blocks of histopathologically diagnosed UAs, 6 OKCs, 8 DCs and 10 RCs were selected from the archival specimens of the Department of Oral Pathology/Medicine, University of Benin Teaching Hospital. Confirmation of the previous diagnosis was done by taking fresh sections from the tissue blocks stained with hematoxylin and eosin.

**Immunohistochemistry**

Five micrometer thick sections were made from the tissue blocks and mounted on silanized glass slides. Specimens were deparaffinized in xylene and hydrated by passing through descending grades of alcohol. Antigen retrieval was done by immersing in citrate buffer (for Ki-67) or tris-EDTA buffer (for Bcl-2) and incubating at 95°C in a water bath for 20 min. Human tonsillar tissue was used as a positive control.

The tissue area on the slides was marked with a hydrophobic pen. Endogenous peroxidase activity was blocked by applying 3% hydrogen peroxide solution in methanol (Dako, Denmark) and incubating at room temperature for 20 min, following which a serum-free protein block was added and slides incubated at room temperature for 5 min to prevent non-specific reactions.

For Ki-67, a 1:100 dilution of rabbit monoclonal anti-Ki-67 primary antibodies (Thermoscientific, Freemont, CA, USA; Clone SP6) was applied, and slides were incubated for 20 min at room temperature, according to the manufacturer’s instructions. For Bcl-2, a 1:40 dilution of mouse monoclonal anti-Bcl-2 primary antibodies (Dako, Denmark; Clone 124) was applied and slides incubated overnight at 4°C. Appropriately diluted horseradish peroxidase polymer detection solution (Dako, Denmark) was then applied, and the slides incubated in a humidified chamber at room temperature for 15 min, following which diaminobenzidine substrate solution (Dako, Denmark) was added to reveal the color of antibody reaction.

Counterstaining was done by immersing slides in Gill’s hematoxylin for 20 s. The slides were then rinsed in distilled water, dehydrated in ascending grades of alcohol, cleared in xylene and coverslipped using distyrene plasticizer in xylene as mountant.

**Interpretation of staining**

Stained tissue sections were evaluated for the presence, distribution, and intensity of staining. For Ki-67, a brown nuclear staining of the epithelial cells was considered as a positive reaction, whereas for Bcl-2, a brown cytoplasmic staining of the epithelial cells was considered as a positive reaction.

Distribution was determined as either being focal (involving <50% of the epithelium) or diffuse (involving >50% of the epithelium). The intensity was graded as follows: 0 = no staining; (+) = weak staining; (++) = moderate staining and (+++) = intense staining.
The Ki-67 LI and Bcl-2 LI were determined for the cases with a positive reaction. This was evaluated as the ratio of positively stained epithelial cells in five high power fields to the total number of epithelial cells, expressed as a percentage.

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 23 software (IBM Corp., CA, USA, 2015). The results were subjected to descriptive analysis and data presented as frequencies and percentages in the form of tables. Chi-square statistical test was employed to determine the association between lesion type and the immunohistochemical expression of markers. A parametric one-way ANOVA was used to compare the mean Ki-67 LI and mean Bcl-2 LI of the different lesions. A post hoc analysis (PHA) [Tukey test] was done to show the areas of significant differences. The level of statistical significance was set at 95% (P < 0.05).

RESULTS

Ki-67 expression was seen in 6 (26.1%) cases of UA, 4 (66.7%) OKC, 1 (12.5%) DC and 1 (10.0%) RC [Figure 1a-d and Table 1]. The mean Ki-67 LI was 1.3%, 7.7%, 1.7% and 15.3% for UA, OKC, DC and RC respectively. There was a statistically significant difference between the mean Ki-67 LI of UA and OKC (P = 0.024).

Immunohistochemical expression of Bcl-2 was seen in 16 (69.6%) UA, 5 (83.3%) OKC, 5 (62.5%) DC and 5 (50.0%) RC [Figure 2a-d and Table 2]. The mean Bcl-2 LI was 44.7%, 58.8%, 5.2% and 10.3% for UA, OKC, DC and RC respectively. The staining reaction in UA and OKC was seen mainly in the basal cells of the lining epithelium, whereas in DC and RC, the staining was seen in all the epithelial layers. The mean Bcl-2 LI of UA was significantly higher than that of DC (P = 0.048). Furthermore, cases of OKC had significantly higher mean Bcl-2 LI compared to DC (P = 0.026) and RC (P = 0.049) [Figure 3].

DISCUSSION

UA, OKC, DC and RC are common cystic lesions of the jaws that may show the considerable overlap of their diagnostic histopathologic features, especially in the presence of an ongoing inflammatory reaction.\(^{[15]}\) Assessment of the proliferative and antiapoptotic indices of these lesions may help in a better understanding of their nature and behavior, as well as in differentiating one from another.

There is a dearth of literature reporting on the percentage of UA's showing Ki-67 expression. This may be because the expression of Ki-67 is expected in all cases since UA is a neoplastic cyst. However, this study showed immunoreactivity to the Ki-67 antibody in only 26.1% of UA. The Ki-67 LI is regarded as a more useful parameter for assessing the proliferative activity.
Moreover, Distribution of Bcl-2 expression
1 (2.5) [17]
[2,18]
1 - 8
4 - 8 [19]
- 8
4 - 8 [20]
- 8
5 (66.7) [21]
- 8
5 (62.5) [22]
- 8
5 (50.0) [23]
- 8

It is relatively
1 8
4 - 8 [24]
1 8
8

4 (66.7)
5 (62.5)
5 (50.0)

In this study, Ki-67 positive cells in the odontogenic keratocyst were seen mostly in the suprabasal layer of the epithelium. This suggests that some of the proliferative capacity of the cystic lining of OKCs reside within this layer. The mean LI for Ki-67 expression in OKC in this study was 7.7%. This is less than that reported by other authors. Guler et al. reported a mean Ki-67 LI of 6.6%, while that reported by Kichi et al. was 7%. Guler et al. and de Vicente et al. found a mean Ki-67 LI of 7.43% and 17%, respectively, in DCs.

This study observed the immunohistochemical expression of Ki-67 in only 1 (12.5%) case of the DCs examined. Slootweg found expression of the Ki-67 protein in 14 (93.3%) of the 15 cases of DC studied, while Nadalin et al. found expression of Ki-67 in 54.5% of DCs in their study. The mean Ki-67 LI for DC was 1.7% in this study. This is slightly less than the 3.14% reported by Piattelli et al. Other authors have reported much higher values. Kim et al. and Rosenstein et al. both reported a mean Ki-67 LI of 6.6%, while that reported by Kichi et al. was 7%. Guler et al. and de Vicente et al. found a mean Ki-67 LI of 7.43% and 17%, respectively, in DCs.

There was the positive immunohistochemical expression of the Ki-67 antigen in 10% of the cases of RCs in this study. In their study, Ayoub et al. found expression of the Ki-67 antigen in 53.8% of RCs, while that reported by Nadalin et al. was 60%. Slootweg, however, found positive expression of Ki-67 in all (100%) the cases of RC examined. The mean Ki-67 LI for RCs in this study was 15.3%. This is similar to the 15.5% mean Ki-67 LI reported by De Vincente et al. for RCs. It is relatively higher compared to the mean Ki-67 LI of 12.17% seen by Guler et al. and 8.64% reported by Martins et al. in RCs. The expression of Ki-67 in RCs in this study was seen in all layers of the epithelium. A similar finding was reported by Slootweg. However, other authors have reported more Ki-67 expression in the basal layer of RCs.

Overall, this study observed a relatively lower immunohistochemical expression of Ki-67 in all the lesions compared to previous reports in the literature. This may have been because other similar studies either utilized a higher antibody concentration or employed a longer incubation time than the 20 min utilized in this study (recommended by the manufacturer). Moreover,
differences in the sensitivity of antibodies and detection kits by different manufacturers may have accounted for the low Ki-67 LI recorded in this study. The percentage expression of Ki-67 in OKC (66.7%) was higher than those of UA (26.1%), DC (12.5%) and RC (10.0%) in this study. However, this was not statistically significant. A one-way ANOVA test showed a significant difference in the mean Ki-67 labeling indices across the different group of lesions studied \((P = 0.020)\). Areas of significant differences could not be identified using PHA because only one case each of DC and RC showed positive expression of Ki-67. An independent samples \(t\)-test, however, showed the mean Ki-67 LI of the odontogenic keratocyst to be significantly higher than that of UA \((P = 0.024)\) in this study. This finding is similar to those of previous reports.\(^9,2,28\) Therefore, Ki-67 may be a useful immunohistochemical marker in differentiating OKC from UA. The higher mean Ki-67 LI in OKC observed in this study suggests a high growth potential of the epithelium, supporting the aggressive biologic behavior of this lesion, and its possible neoplastic nature.

The expression of Bcl-2 seen in 69.6% of UA in this study is less than the 88.2% reported by Florescu et al.\(^9\) and the 100% seen by other authors in cases of ameloblastomas.\(^10,11\) The expression of Bcl-2 in UA s in this study was seen mainly in the basal layer of the epithelial lining or the peripheral cells of the tumor islands. This is consistent with reports in the literature.\(^9\) The mean Bcl-2 LI of 44.7% seen in this study is less than that found by other researchers.\(^2,30\) Florescu et al.\(^9\) found a mean Bcl-2 LI of 67.7% in ameloblastomas, whereas that reported by Razavi et al.\(^30\) was 63%. The lower Bcl-2 expression and mean LI seen in UA s in this study may be because this study included only cases of UAs, whereas other authors studied ameloblastomas in general.\(^2,30\)

This study observed the immunohistochemical expression of the Bcl-2 protein in 83.3% of the OKC s examined. This is less than the 100% expression reported by some authors.\(^10,12,14\) Expression of Bcl-2 in OKCs in this study was seen mainly in the basal layer of the epithelium. Two cases also showed the expression of Bcl-2 involving the suprabasal layer. Most reports in the literature have shown Bcl-2 expression in OKCs restricted to the basal layer of the epithelium.\(^30\) Soluk-Tekkesin et al. found the expression of Bcl-2 in all the epithelial layers in OKC.\(^9\) This finding of Bcl-2 expression involving both basal and suprabasal epithelial layers in some OKCs may suggest greater clinical aggression in such cases. Staining for Bcl-2 was diffuse in all the positive cases. In addition, most of the positive cases showed intense staining for Bcl-2. The mean Bcl-2 LI for OKCs in this study was 58.8%. This is higher than the 32% reported by Kichi et al.\(^9\) These findings suggest that inhibition of apoptosis may play a role in the pathogenesis of OKC.

Immunohistochemical expression of Bcl-2 in DCs in this study was seen in 62.5% of the cases. This contrasts with the findings of many authors who recorded either complete or almost complete negativity of Bcl-2 expression in DCs.\(^10,11,31\) Some authors have, however, found positive expression of Bcl-2 in DCs. This was seen in 5%, 20% and 80% of the cases examined by Jahanshahi et al.,\(^12\) Kichi et al.,\(^11\) and Rahman et al.,\(^33\) respectively. The staining for Bcl-2 in DCs in this study was mild in all cases, and mostly focal (60.0%). This may suggest focal areas of aggressiveness with possible incipient ameloblastomatous change. The expression was not confined to any particular layer of the epithelium. The mean Bcl-2 LI observed for DCs in this study was 5.2%. This is more than the 1% reported by Kichi et al.,\(^11\) but slightly less than the 7.3% seen by Loyola et al.\(^14\)

This study observed positive immunohistochemical expression of Bcl-2 in 50% of the RCs examined, supporting what has been previously reported.\(^14,33\) The result of this study is, however, in contrast with other reports that found almost complete or complete lack of Bcl-2 expression in RCs.\(^18,11,31\)

This study observed the highest expression of Bcl-2 in OKC (83.3%) among the lesions studied, with that of RC (50.0%) being the least. This was, however, not statistically significant. The mean Bcl-2 LI for OKC was the highest in this study, closely followed by UA. The mean Bcl-2 labeling indices for DC and RC were low. The mean Bcl-2 LI for UA was significantly higher when compared with that of DC. Similar findings have previously been reported.\(^11\) Furthermore, OKC had a significantly higher mean Bcl-2 LI compared to DC and RC, similar to the reports of Soluk Tekkesin et al.,\(^9\) Piattelli et al.,\(^10\) and Vered et al.\(^11\) However, there was no significant difference between the mean Bcl-2 LI of UA and OKC in this study. Sindura et al.\(^8\) and Soluk Tekkesin et al.\(^9\) reported a similar finding. The findings of this study suggest that UA and OKC share similar biologic behavior, with a tendency toward aggressiveness, different from that shown by dentigerous and RCs.

**CONCLUSION**

This study suggests that the Ki-67 LI may help in differentiating OKC from UA. The Bcl-2 LI may be useful in differentiating UA from DCs, and differentiating OKC from DC and RC.
Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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