ORIGINAL ARTICLE

Investigation of clinicopathological parameters alongside with p53 expression in primary and recurrent keratocysticodontogenic tumours

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Abstract

**Background:** Keratocysticodontogenic tumor (KCOT) is one of the most common odontogenic cysts and has a high recurrence rate after various treatment methods. Some studies have been conducted to identify the predictive factors of recurrence. In this study, the clinicopathological and immunohistochemical features of primary and recurrent KCOTs are analyzed, including immunohistochemically expression of p53 protein in cyst-lining epithelial cells in order to find more markers to predict the specific behaviour and greater tendency for recurrence. **Methods:** In this descriptive analytic study, a total of 78 archived specimens of KCOTs, including 52 primary KCOT with no registered recurrences to date and 26 recurrent KCOT were selected. The clinical data and histopathological features of the epithelial lining and connective tissue wall were analyzed. Immunohistochemical staining intensity distribution (SID) score for p53-positive cells were calculated for both groups. Results were analyzed by T-test, Chi-square, and Fisher’s exact test. **Results:** There were no statistically significant differences between primary and recurrent cases in terms of age (\(P = 0.181\)), gender (\(P = 0.744\)), and anatomical location (\(P = 0.294\)). In the histopathological assessment, epithelial budding (\(P = 0.001\)), daughter cysts (\(P = 0.013\)), and odontogenic rests (\(P = 0.036\)) were significantly more common in recurrent KCOTs. Immunohistochemical staining for p53 show statistically significant difference between the two groups (\(P = 0.041\)). **Conclusion:** In this study, some predictive factors of recurrence of KCOTs such as epithelial budding, daughter cyst and odontogenic rests were found. Furthermore, the evaluation of p53 expression in KCOT at the time of diagnosis was helpful for the prediction of recurrence.

**Keywords:** Keratocystic odontogenic tumour, odontogenic keratocyst, p53

INTRODUCTION

The keratocysticodontogenic tumour (KCOT) is a developmental odontogenic cyst that originates from epithelial rests of dental lamina. It was introduced by Philipsen in 1956 for the first time.\(^1\) This lesion is one of the most common odontogenic cysts.\(^2\) In the recent classification, the World Health Organization (WHO) considered this lesion as a benign neoplasm and suggested the term “keratocystodontogenic tumor (KCOT)” due to aggressive behaviour, high recurrence rate, and chromosomal studies.\(^3\) Furthermore, the sporadic cases of KCOTs and the patients with multiple KCOTs in nevoid basal cell carcinoma syndrome (NBCCS) have patched gene (PTCH) mutations.\(^4,5\)

Keratocysticodontogenic tumour account for 11.2% of developmental odontogenic cysts and 1.8 to 21.5% of jaw cysts.\(^6,7\) This lesion can occur at any age, but most cases have been observed in the second and third decades of life.\(^7,9\) KCOT is mostly frequent in males.\(^7,10\) The posterior body of the mandible is the most common site for occurrence of this lesion.\(^11\) Radiographically, the KCOT appears as unilocular or multilocular well-defined corticated border radiolucency with or without root resorption.\(^12\) Cortical bone destruction and invasion into adjacent soft tissue is sometimes seen.\(^13,14\)
Histologically, the epithelial lining consists of a relatively uniform layer, usually corrugated surface of parakeratinized stratified squamous epithelium 8-12 cells thick, and often without rete ridge formation. The basal cell layer shows a palisaded pattern with nuclear hyperchromatism and reversed polarity. However, in some cases basal budding, odontogenic rests, and daughter cysts have also been observed in fibrous connective tissue wall. The cyst wall is often thin, loose, and without inflammatory cells infiltration.

According to results of previous studies, KCOT has a high recurrence rate after various treatment methods. Previous studies have demonstrated the recurrence rates of 0 to 62%, and the highest rate of recurrence has been 5 years after treatment. As for the special biological behaviour of the KCOT, many immunohistochemical studies have been performed using different markers of proliferation and apoptosis. They have indicated that the epithelial cells of KCOTs have high proliferation activity. The p53 protein is a tumour-suppressor gene that affects G1 phase of the cell cycle to arrest cell proliferation, to repair damaged DNA, or to induce apoptosis. The results of previous studies have shown that p53 protein is expressed more frequently and intensely in the KCOTs in comparison with other odontogenic cysts.

Gadbail et al have suggested that the quantitative and qualitative expression of p53 protein can be used as a prediction marker of aggressive behavior in odontogenic lesions, including KCOT. Despite the large number of studies on the specific clinical behaviour, histopathological parameters of KCOT and p53 expression performed, few studies on the relation between these parameters and recurrence rate of this lesion was done.

The purpose of this study was to retrospectively analyze the clinical and histopathological features of recurrent and non-recurrent KCOTs and immunohistochemically investigate the expression of p53 protein in cyst-lining epithelial cells in order to find more markers to predict the specific behavior and greater tendency for recurrence.

MATERIALS AND METHODS

Patients and tissue selection
In this descriptive analytic study, all patients diagnosed with KCOT and recorded in the Department of Oral and Maxillofacial Pathology, Dental School at Isfahan University of Medical Sciences during 1988-2012 were retrieved. The clinical data including age, sex, and location of lesions were obtained. Patients with nevoid basal cell carcinoma syndrome-associated KCOT (NBCCS), and the patients without all clinical information were excluded.

In total, 370 cases of OKC were detected from the archives, 78 of whom were selected to be used in this study, including 52 primary KCOT with no registered recurrences to date and 26 recurrent KCOT. All selected primary samples had a minimum of 5 years follow-up. The study protocol was approved by ethics committee of Isfahan University of Medical Sciences. The microscopic haematoxylin-eosin stained slides of all cases were reviewed by two oral pathologists to confirm the diagnosis. After that, all samples were randomly numbered.

Histopathological study
For morphological assessment, all haematoxylin-eosin stained slides were blindly examined by two oral pathologists by light microscopy (Olympus BX41TF, Tokyo, Japan). The histological criteria of epithelial lining (epithelial folding, epithelial budding, thickness of keratin layer, corrugated surface, intercellular edema, and reversed polarity of basal cells layer) and connective tissue wall of KCOTs (level of collagen fibers, odontogenic rest and daughter cysts) were evaluated.

Immunohistochemical study
For the detection of p53 expression by immunohistochemical staining, 3-5 μm sections were cut from the paraffin-embedded specimens. The tissue sections were deparaffinized with xylene, and rehydrated with graded ethanol. For antigen retrieval, the sections were heated in microwave oven at 96°C for 15 min in citrate buffer (pH 6.0), and then cooled in the room temperature for 20 min. Endogenous peroxidase activities were blocked by incubation with 3% H₂O₂ in methanol for 20 min and the sections were washed with phosphate-buffered saline (PBS). The sections were incubated with the lyophilized monoclonal anti-p53 (NCL-P53-DO1, Novacastra, Germany) in a 1:50 dilution for 60 min. After that, the immunocomplexes were treated with post-primary block and then detected by Novolink polymer (Novacastra, Germany) for 30 min, followed by washing in PBS. The immunoreactivity was visualized by diaminobenzidine (DAB) (DAKO, Denmark).

In the final stage, the sections were
counterstained with hematoxylin. The result was considered positive when the nuclei of cells were stained brown. Breast carcinomas with known antigen reactivity were used as the positive control. The negative control was based on staining by omitting the primary antibody.

Assessment of immunohistochemical staining
To analyze immunohistochemical staining, all slides were examined by two oral pathologists in a blinded manner with light microscopy (Olympus BX41TF, Tokyo, Japan), and cells were counted at 400× magnification in 10 randomly selected fields.

The immunoreactive cells of epithelial lining of KCOTs were evaluated using the semi-quantitative scale: 0 (negative: without immunostained cells), +1 (<25% immunostained), +2 (25 to 50%), and +3 (>50%). Furthermore, staining intensity was evaluated on the following scores: 0 (without immunostained cells), +1 (very low staining), +2 (low), +3 (moderate), and +4 (high).27,28

Staining intensity distribution (SID) score was calculated by multiplying the distribution (proportion of stained cells) by staining intensity.29

Statistical analysis
The data obtained from clinical, histopathological, and immunohistochemical studies were analyzed by the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA) using T-test, Chi-square, and Fisher’s exact tests. A P-value <0.05 was considered statistically significant.

RESULTS
Clinical findings
The 78 cases in this study included 47 males (60.3%) and 31 females (39.7%) with a male to female ratio of 1.93:1, showing a male predominance of cases. The primary cases of KCOTs comprised of 32 males (61.5%) and 20 females (38.5%). There were 15 males (57.7%) and 11 females (42.3%) in the recurrent group of KCOTs. In relation to gender, no statistically significant difference was observed between two groups of the study (P = 0.744).

The mean age for the recurrent group of KCOT was found lower than that of the primary KCOTs, this difference was not statistically significant (P = 0.181). Some differences in the age distribution between the two groups of study are shown in Figure 1.

The majority of all selected cases occurred in the mandible with a mandibular to maxillary ratio of 2.25:1. The difference in distribution between the two groups by location in the jaws was not statistically significant (P=0.294) (Table 1).

Histopathological findings
The histopathological findings for the recurrent and primary KCOTs are shown in Table 2. Some histopathological criteria of epithelial lining such as keratin layer thickness (P=0.712), epithelial folding (P=0.508), reversed polarity of basal cell layer (P=0.414) and corrugated surface (P=0.174) had no significant difference between the two groups (Fig. 2A, 2B, 2C).

The greatest identified difference between recurrent and primary KCOTs was the

FIG. 1: Age distribution of primary and recurrent KCOTs based on percentage of frequencies
Mean age of total cases: (mean ± SD = 30.51 ± 15.24).
Mean age of primary KCOTs: (mean ± SD = 32.15 ± 16.10).
Mean age of recurrent KCOTs: (mean ± SD = 27.23 ± 13.04)
presence of epithelial budding (Fig. 2D), which was statistically significant ($P=0.001$). With regard to the presence of intercellular oedema (Fig. 2E) in the epithelial lining, no statistically significant difference was observed between the two groups ($P=0.335$). The majority (65.4%) of 78 cases had a mixed connective tissue wall (Fig. 2F). However, the differences between two groups were not statistically significant ($P=0.259$). Histopathological features such as odontogenic rests ($P=0.036$) and daughter cysts ($P=0.013$) in the cyst wall showed statistically significant differences between the two groups (Fig. 2I, 2J).

**Immunohistochemical findings**

p53 expression was observed in 77.5% of keratocysticodontogenic tumours (Figure 3). The greatest SID score for p53 staining was found in recurrent KCOT (Table 3). The difference between primary and recurrent KCOTs was statistically significant ($P=0.041$).

**DISCUSSION**

The present study was performed on 78 KCOT, 26 being recurrent and 52 primary cases. Many studies have investigated the prediction factors of recurrence rates and special behaviour of OKCs in terms of the clinicopathological, immunohistochemical and radiological features, and treatment methods. In this study, the clinicopathological features and immunohistochemical findings of recurrent and primary cases of KCOT were examined.

In most of the recurrent patients, the recurrence of KCOTs was 1 (46.1%) or 2 (26.9%) years after treatment, that was similar

**TABLE 1: Location distribution of primary and recurrent KCOTs**

<table>
<thead>
<tr>
<th>Site</th>
<th>Recurrent n (%)</th>
<th>Primary n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior region</td>
<td>0 (0)</td>
<td>5 (9.6)</td>
<td>5 (6.4)</td>
</tr>
<tr>
<td>Premolar region</td>
<td>3 (11.5)</td>
<td>3 (5.8)</td>
<td>6 (7.7)</td>
</tr>
<tr>
<td>Posterior region (molar, angle, ramus)</td>
<td>17 (65.4)</td>
<td>26 (50)</td>
<td>43 (55.1)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (76.9)</td>
<td>34 (65.4)</td>
<td>54 (69.2)</td>
</tr>
<tr>
<td>Maxilla</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior region</td>
<td>3 (11.5)</td>
<td>7 (13.5)</td>
<td>10 (12.8)</td>
</tr>
<tr>
<td>Premolar region</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Posterior region (molar, tuberosity)</td>
<td>3 (11.5)</td>
<td>11 (21.2)</td>
<td>14 (17.9)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (23)</td>
<td>18 (34.7)</td>
<td>24 (30.7)</td>
</tr>
</tbody>
</table>

**TABLE 2: Histopathological characteristics of primary and recurrent KCOTs**

<table>
<thead>
<tr>
<th>Histopathological feature Subtypes</th>
<th>Recurrent n (%)</th>
<th>Primary n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial lining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratin layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thin</td>
<td>24 (92.3)</td>
<td>46 (88.5)</td>
<td>70 (89.7)</td>
</tr>
<tr>
<td>Thick</td>
<td>2 (7.7)</td>
<td>6 (11.5)</td>
<td>8 (10.3)</td>
</tr>
<tr>
<td>Corrugated surface</td>
<td>20 (76.9)</td>
<td>32 (61.5)</td>
<td>52 (66.7)</td>
</tr>
<tr>
<td>Epithelial folding</td>
<td>11 (42.3)</td>
<td>18 (34.6)</td>
<td>29 (37.2)</td>
</tr>
<tr>
<td>Epithelial budding</td>
<td>9 (34.6)</td>
<td>2 (3.8)</td>
<td>11 (14.1)</td>
</tr>
<tr>
<td>Intercellular edema</td>
<td>10 (38.5)</td>
<td>26 (50)</td>
<td>36 (46.2)</td>
</tr>
<tr>
<td>Reversed polarity</td>
<td>25 (96.2)</td>
<td>46 (88.5)</td>
<td>71 (91)</td>
</tr>
<tr>
<td>Cyst wall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loose</td>
<td>6 (23.1)</td>
<td>6 (11.5)</td>
<td>12 (15.4)</td>
</tr>
<tr>
<td>Dense</td>
<td>3 (11.5)</td>
<td>12 (23.1)</td>
<td>15 (19.2)</td>
</tr>
<tr>
<td>Mixed</td>
<td>17 (65.4)</td>
<td>34 (65.4)</td>
<td>51 (65.4)</td>
</tr>
<tr>
<td>Odontogenic rests</td>
<td>7 (26.9)</td>
<td>4 (7.7)</td>
<td>11 (14.1)</td>
</tr>
<tr>
<td>Daughter cysts</td>
<td>7 (26.9)</td>
<td>3 (5.8)</td>
<td>10 (12.8)</td>
</tr>
</tbody>
</table>
FIG. 2: Histopathological features of KCOT (hematoxylin-eosin staining) (A) Folded epithelium (original magnification ×100), (B) Reversed polarity of basal cell nuclei (×400), (C) Corrugated surface of epithelial lining (×100), (D) Basal budding (×400), (E) Intercellular edema (×400), (F) Mixed, (G) Loose, (H) Dense connective tissue wall (×10), (I) Odontogenic rests (×400), (J) Daughter cysts (×100).
to the results of other studies. As the results indicated, all the patients in second and third decades of life were most commonly affected. This finding is quite similar to those of earlier studies. Furthermore, the mean age of recurrent patients was less than 30 years. Although this result was not significant ($P > 0.05$), it is in agreement with other studies, which reported more recurrence rate in younger patients. The reason for these results may be related to more conservative treatment methods and retaining the involved teeth in younger patients.

According to this result, there was a male predominance, similar to most other studies. Some researchers have stated that gender has an important role in the recurrence rates. But, in this study, similar to results of other studies, gender did not interfere with the recurrence rates ($P > 0.05$).

Like most other studies, in this study the posterior area of the mandible, which includes the molar region, angle and ramus, was the commonly affected site. Although, in this study there was no significant relationship between anatomic locations of KCOTs and recurrence rates ($P > 0.05$), the lesions which were involved in the mandibular posterior area had higher recurrence rates than other locations. These results are in line with the results of the studies carried out by Selvi and Habibi. Generally, in the time of diagnosis in most of the patients, especially those who had involvement of posterior mandibular area and were asymptomatic, the lesion induces more extension and destruction of the jaw bone. As a result, the complete removal of the lesion might become more difficult, with retention of the epithelial lining of the KCOT followed by recurrence.

Although the histopathological features of KCOTs are characteristic, variability is observed in most cases. The results of this study did not show a statistically significant difference between recurrent and non-recurrent KCOTs for some histopathological criteria, including the thickness of the keratin layer, corrugated surface, epithelial folding, intercellular oedema, and reversed polarity of the basal cell nuclei ($P > 0.05$). These results are in agreement with the findings of Cottom’s study. On the other hand, statistically significant differences were observed between the two groups for epithelial budding of the basal layer, odontogenic rests, and daughter cysts ($P < 0.05$). Some studies have reported that these characteristics are associated with recurrence rates.

Overall, daughter cysts in the connective tissue wall are seen in 7-44% of KCOTs. In this study 12.8% of all cases had this histopathological feature. Similar to this study, previous studies conducted by Myoung et al and Lam & Chan also revealed that observation of daughter cysts was associated with greater recurrence rates. In this study, the epithelial budding of basal cell layer had the most correlation with the recurrence rates. According to other studies, the epithelial lining of KCOTs do not often have rete pegs, but in some samples, especially in cysts associated with NBCCS, irregularity and basal budding can

<table>
<thead>
<tr>
<th>Groups</th>
<th>SID score (%)</th>
<th>( %)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0(%)</td>
<td>1(%)</td>
</tr>
<tr>
<td>Recurrent</td>
<td>3 (11.5)</td>
<td>11 (42.4)</td>
</tr>
<tr>
<td>Primary</td>
<td>16 (30.8)</td>
<td>21 (40.4)</td>
</tr>
</tbody>
</table>

*SID: Distribution and staining intensity score

![FIG. 3: Immunohistochemical staining showing p53-positive cells in epithelial lining of KCOT(×100 (A), ×400 (B))](image)
be seen.\textsuperscript{39} Although, in this study the syndromic patients of KCOTs were excluded, the epithelial budding of basal cell layer was observed in recurrent cases. So, this objective came true with regard to the recurrence rates. p53 is a tumour suppressor gene that exerts its effect on the cell cycle.\textsuperscript{22,23} Detection of this protein by immunohistochemical staining seems to be related to the stabilization of the p53 production that plays a role in cell cycle regulation. An intrinsic growth potential and aggressive, locally invasive behavior of KCOTs may explain the presence of mutant or otherwise inactive p53 protein.\textsuperscript{24,40} p53 detection in the epithelial layer of KCOT is strongly correlated with the abnormal cell cycle and uncontrolled cell proliferation.\textsuperscript{24} Li et al. argued that the overexpression of p53 in KCOTs in comparison with other odontogenic cysts might be related to cell proliferation due to overproduction and/or stabilization of normal p53 production. This result, however, was not related to p53 gene mutation.\textsuperscript{41} In Gonzalez-Moles’s study, negative p53 expression was reported in 85.4\% of all cases,\textsuperscript{42} but in this study 25.6\% of all samples were negative.

Moreover, in this study, there was significant difference between primary and recurrent cases of KCOTs in terms of p53 expression ($P < 0.05$). Some studies have showed the expression of p53 is associated with the histological features and aggressive behavior of KCOTs.\textsuperscript{17,26} Furthermore, some other studies have reported higher expression of p53 in NBCCS keratocyst than sporadic cases.\textsuperscript{43}

However, few studies have been conducted on the relationship of p53 expression and recurrence rate of KCOT. In these studies overexpression of p53 was associated with recurrent lesions.\textsuperscript{44} While, in the studies carried out by Lombardi et al. and Gurgel et al. about p53 expression, there were no statistically significant differences between primary and recurrent KCOTs, and no correlation between expression and histopathological criteria.\textsuperscript{45,46} findings that were contradictory to results of our study. These findings indicate that KCOTs can show different behaviours, the differences in the results being because of the benign nature of some lesions. Other reasons may be technical problems in the adopted immunohistochemical method. However, our result is similar to the findings of Kuroyanagi’s study which found high expression of p53 protein in recurrent cases.\textsuperscript{44}

It should be noted that radiological features and treatment method can be used to investigate the prediction factors in recurrence rates of KCOTs, but in this study due to incomplete information, we could not examine them.

**Conclusion**

We can conclude that younger patients and lesions with involvement of the mandibular posterior area have a slightly high recurrence rate. The presence of epithelial budding, odontogenic rests, and daughter cysts in the connective tissue wall of KCOTs is predictive of a greater tendency for recurrence.

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1. Philipsen HP. Om keratocyster (kolesteatom) i kaerberne. Tandlaege bladet. 1956; 60: 963-81.


