

# p63 expression in randomized odontogenic cysts

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## ABSTRACT

**الأهداف:** التقييم النسيجي الكيميائي المناعي لانتشار ظهور بروتينات بي 63 في الأكياس السننية وذلك اعتماداً على اختلاف الأعراض السريرية فيما بينها.

**الطريقة:** أُجريت هذه الدراسة في قسم أمراض الفم التابع لجامعة بابل للعلوم الطبية، بابل، إيران وذلك خلال الفترة من مارس 2003م إلى فبراير 2008م. شملت الدراسة 34 عينة من الأكياس السننية التي تم وضعها في البرافين، وقد تم تقسيمها إلى 3 مجموعات وهي كالتالي، الأكياس التاجية وكان عددها 12 عينة، والأكياس الجذرية وعددها 9 عينات، والأكياس المتقرنة الورمية السننية وعددها 13 عينة. لقد قمنا بالتقييم الكيميائي المناعي لعينات الأنسجة بواسطة نظام إنفيجن وذلك من أجل تحليل نسبة ظهور بروتينات بي 63.

**النتائج:** أشارت نتائج الدراسة إلى ارتفاع نسبة ظهور بروتينات بي 63 في مجموعة الأكياس المتقرنة الورمية السننية مقارنة بالمجموعات الأخرى، ولقد كان الاختلاف بين المجموعات الثلاثة كبيراً من الناحية الإحصائية.

**خاتمة:** أثبتت الدراسة فعالية الاستدلال بظهور بروتينات بي 63 في تشخيص الأكياس المتقرنة الورمية السننية والتي يظهر عليها بعض الأعراض السريرية المرتبطة بالأمراض الخطيرة.

**Objectives:** To find out the immunohistochemical assessment of p63 expression in odontogenic cysts based on the differences among their clinical behaviors.

**Methods:** This study was carried out on 34 archival paraffin-embedded specimens of odontogenic cysts. We obtained the specimens from the Pathology Department of Babol University of Medical Sciences, Babol, Iran from March 2003 to February 2008. The specimens comprised 12 dentigerous cysts, 9 radicular cysts, and 13 keratocystic odontogenic tumors (KCOTs). The immunohistochemical technique was performed using the Envision system for evaluation of p63 expression.

**Results:** The KCOT revealed the highest p63 expression and the differences between the 3 groups was statistically significant.

**Conclusion:** P63 expression might be helpful when identifying cyst types with more aggressive and invasive phenotype.

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**P**63, a new member of the Tp53 gene family, shows similarities in exon/intron organization to p53.<sup>1,2</sup> The p63 is placed at the 3q27-29 area containing 15 exons.<sup>1,2</sup> The p63 gene has a significant role in epithelial development, and the proliferation of limb and craniofacial structures.<sup>3,4</sup> The epithelial lining of odontogenic cysts have an ectodermal origin. Although there is a possibility that the odontogenic epithelial lining causes odontogenic and non-odontogenic tumors, malignant transformation of odontogenic cysts seems to be extremely rare.<sup>5</sup> There has been little focus on the expression of p63 in odontogenic cysts so far in the current literature.<sup>6</sup> A more aggressive biological behavior is observed in odontogenic keratocysts (OKCs), with a noticeable tendency of recurrence, when compared to the other types of cysts such as dentigerous, or radicular cysts (RCs).<sup>6</sup> Due to the clinical features of OKCs, they are usually described as benign cystic tumors named as keratocystic odontogenic tumors (KCOT) by some authors.<sup>6-8</sup> Likewise, the presence of epithelial dysplasia has been shown by some KCOTs. Also, KCOTs have an apparent tendency to malignant transformation.<sup>9</sup> Some authors believe that in rare instances, development of squamous cell carcinoma has been reported within RCs, and they have mentioned the possibility of neoplastic transformation to an ameloblastoma, squamous cell

carcinoma, and mucoepidermoid carcinoma in a dentigerous cyst (DCs).<sup>7</sup> Due to the rather restricted expression of p63 in epithelial cells, its infrequent mutation, its overexpression in different types of solid tumors indicating an oncogenic role in the regulation of proliferation and distinction in premalignant and malignant lesions of epithelial origin, a noticeable interest has recently been focused on p63. So far, only a few papers have dealt with the expression of p63 in odontogenic cysts.<sup>6</sup> The objective of this study is to assess, by immunohistochemistry, the expression of p63 in the epithelial lining of different odontogenic cysts. We also analyzed the capability of p63 for identifying cases with more aggressive phenotype, which provides novel diagnostic information.

**Methods.** After Institutional Ethics Committee approval, 34 paraffin-embedded archival specimens of odontogenic cysts were obtained from the Oral Pathology Department of Babol University of Medical Sciences, Babol, Iran from March 2003 to February 2008, comprising 12 DCs, 9 RCs, and 13 KCOTs. None of the patients had been treated in the past. Excisional biopsy was performed in all cases. The KCOTs were not associated with nevoid basal cell carcinoma syndrome. Only para-keratinized KCOTs were included in this study.

Sections from formalin-fixed, paraffin-embedded blocks were stained by hematoxylin & eosin, and histological slides were reviewed. Only sections containing adequate epithelium were considered. The Envision System (DakoCytomation, Glostrup, Denmark) and the monoclonal mouse anti-human p63 protein, clone 4A4 (DakoCytomation, Glostrup, Denmark) were used. Four micrometer sections were deparaffinized in xylene and rehydrated through graded concentrations of alcohol. Then the sections were heated in a microwave oven for 2 cycles of 15 minutes (min) each in citrate buffer (DakoCytomation, Glostrup, Denmark). Endogenous peroxidase activity was blocked with H<sub>2</sub>O<sub>2</sub> solution in methanol (0.01 M) for 30 min. Nonspecific binding was attenuated by incubating the sections for 30 min with blocking solution (DakoCytomation, Glostrup, Denmark). After washing with TRIS-buffered saline (TBS) for 5 min, the antibodies were incubated for 30 min in room temperature, and that the slides were washed for 10 min with TBS. Diaminobezidine tetrahydrochloride (DAB) was used as a chromogen. All sections were counterstained with hematoxylin. Then they were mounted with a permanent mounting medium, and examined by light microscopy (Olympus BX41 [Shibuya-ku, Tokyo, Japan]). Positive controls comprised tissue specimen sections of skin squamous cell carcinoma with known antigenic reactivity. For negative controls, nonimmunized mouse sera were used, and the

primary antibody was omitted. Negative controls in all samples led to a negative immunoreactivity for p63. We only observed nuclear staining of epithelial cells. For analyzing the p63 expression, a mean percentage of positive cells was determined from the percentage of positive nuclei obtained from the analysis of 100 cells in 10 random areas at 40x magnifications, and intensity of the stain was also determined. A pathologist who was blinded to the clinicopathological data assessed the positivity for p63. An evaluation of p63 expression was performed. Approximate percent of cells: (a) score 1, when the stained cells were included <1% of the total; (b) score 2, when the stained cells were included from 1-10% of the total cell population; (c) score 3, when the stained cells were included from 11-33% of the total cell population; (d) score 4, when the stained cells were included from 34-66% of the total cell population; (e) score 5, when the stained cells were accounted >66%. Approximate intensity of staining; (a) score 0, when no cell stained; (a) score 1, when the cells were stained mildly (light brown); (b) score 2, when the cells were stained moderately (brown); (c) score 3, when the cells were stained severely (dark brown). Then the sum total of this 2 were announced as the final score.

Data were assessed using Statistical Package for the Social Sciences, version 17 (SPSS Inc. Chicago, IL, USA), Kruskal Wallis and Mann Whitney test, and significant differences ( $p < 0.05$ ) among the groups were determined.

**Results.** According to the data analysis, statistically significant positivity of p63 expression was observed in KCOTs in comparison with RCs and DCs using the Kruskal Wallis test ( $p = 0.000$ ). In other words, the percentage of positive cells and the intensity of staining by p63 was greater in the epithelial lining of KCOTs in comparison with RCs and DCs (Tables 1, 2, & 3). These findings were statistically significant ( $p = 0.000$ ). The p63 expression in KCOTs was more than RCs ( $p = 0.000$ ), and DCs ( $p = 0.000$ ), and more than DCs in RCs ( $p = 0.001$ ). In nearly all the DCs, immunostaining for the p63 was restricted to the basal and parabasal layer of the epithelium. There was only nuclear staining in all cases. In the majority of DCs (83.3%), the stained cells were included <1% of the total, and for the intensity of staining, the score was 0 in most cases (75%). For the final score, the score was one in most cases. (Figure 1a). Almost all the RCs indicated positivity not only in the basal and parabasal layer, but also in the intermediate layer. Approximately 44% of RCs showed a percentage of stained cells between 11-33%. For the intensity of staining, the score was one in most cases, and the final score was 3, and 4 in most cases (Figure 1b). A more intense and diffuse p63 expression was shown by KCOTs. Most of the KCOTs displayed not only

**Table 1** - The p63 expression in odontogenic cysts (regarding percentage of stained cells).

Levels	Dentigerous cysts n=12	Radicular cysts n=9	Keratocystic odontogenic tumor n=13
Score 1	10(83.3)	1(11.1)	0 (0.0)
Score 2	2(16.7)	3(33.3)	0 (0.0)
Score 3	0 (0.0)	4(44.4)	3(23.1)
Score 4	0 (0.0)	1(11.1)	1 (7.7)
Score 5	0 (0.0)	0 (0.0)	9(69.2)
<i>p</i> =0.000			

**Table 2** - The p63 expression in odontogenic cysts (regarding intensity of staining).

Levels	Dentigerous cysts n=12	Radicular cysts n=9	Keratocystic odontogenic tumor n=13
Score 0	9(75)	1(11.1)	0(0)
Score 1	3(25)	9(66.7)	2(15.4)
Score 2	0(0)	2(22.2)	2(15.4)
Score 3	0(0)	0 (0)	9(69.2)
<i>p</i> =0.000			

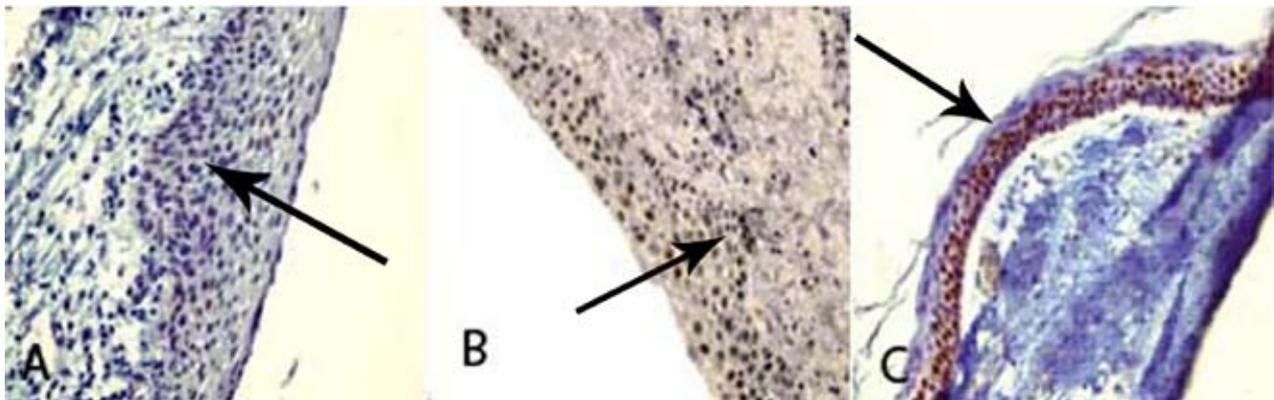
**Table 3** - The p63 expression in odontogenic cysts (regarding final score).

Levels	Dentigerous cysts n=12	Radicular cysts n=9	Keratocystic odontogenic tumor n=13
Score 1	9(75.0)	1(11.1)	0
Score 2	1 (8.3)	0	0
Score 3	2(16.7)	3(33.3)	0
Score 4	0	3(33.3)	2(15.4)
Score 5	0	1(11.1)	11(7.7)
Score 6	0	1(11.1)	0
Score 7	0	0	2(15.4)
Score 8	0	0	8(61.5)
<i>p</i> =0.000			

a positivity in basal and suprabasal layers, but also in the intermediate and superficial cells of the epithelium. In KCOTs, 69.2% of cases displayed a percentage of stained cells between 66-100%. For the intensity of staining, in most cases the score was 3, and the final score was 8 in most cases. (Figure 1c).

**Discussion.** In this study, p63 expression in KCOTs was more than other cysts. In another study,<sup>7</sup> the researchers believed that KCOTs show a different growth mechanism and biologic behavior from the more common DCs and RCs. Clinically, KCOTs are more aggressive and usually recur more frequently than the other types of cyst.<sup>9</sup> Epithelial dysplasia and squamous cell carcinoma may seldom develop into KCOTs, and there is contradictory data on the tendency to undergo ameloblastomatous transformation.<sup>10</sup> There might be some intrinsic growth potential in the epithelial lining of KCOTs that does not exist in other odontogenic cysts.<sup>9</sup>

Many researchers believed that apparently there is a suprabasal proliferation compartment in KCOT epithelium.<sup>9</sup> Clone 4A4 used here only detects ΔNp63 in squamous epithelium. This hypothesis was suggested by Nylander et al.<sup>11</sup> We found 3 studies on the use of anti-p63 antibody for investigation of odontogenic cysts that also were in agreement with the present study. Lo Muzio et al<sup>9</sup> offered that p63 expression might be helpful to identify odontogenic cysts with more aggressive behavior. They found a high and statistically significant positivity of p63 expression in KCOTs in comparison with RCs and DCs, and no differences in p63 expression in the basal-parabasal layer, but they observed a statistically significant difference when they compared the intermediate and superficial layers of KCOTs with RCs and DCs.<sup>9</sup> Foschini et al<sup>12</sup> found that p63 in recurrent KCOT had more homogenous



**Figure 1** - Expression of p63 protein in a) dentigerous cyst with low expression of p63 protein in the epithelial cells of the basal and parabasal layers (×400), b) radicular cyst with medium expression of p63 protein in the epithelial cells of the basal, parabasal, and intermediate layers (×400) and c) keratocystic odontogenic tumor with high positivity for p63 protein in the epithelial cells of the basal, intermediate, and superficial layers (×400).

and superficial distribution than non-recurrent KCOT. Gurgel et al<sup>13</sup> believed that p63 immunostaining in KCOT might due to immaturity of epithelial cells and supported the neoplastic nature of this lesion. They found that p63 may play a role like an oncogene.<sup>13</sup>

The results of the present assessment indicate that the pattern of p63 expression differed dependent to the type of cyst. It is likewise indicated that KCOTs contained the highest number of p63-positives cells, and they presented the most intense and diffuse immunostaining for p63. Moreover, in KCOT, intermediate and superficial epithelial layers were positive for p63 expression. Conversely, p63-positive cells were localized in the basal and parabasal layers of DCs and RCs. In fact, research on human normal skin has proved that in the epidermis p63 staining was restricted to almost all the basal and suprabasal cells, and gradually reduced in the middle layer of the epidermis, without any expression in the spinous layer where terminal differentiation occurred.<sup>4</sup> In the epidermis, staining above the basal layers normally decreased in intensity and in cell numbers according to cell maturation. Localization of p63-positive cells seems to be linked to proliferative compartments in the epithelia.<sup>4</sup>

The current findings in the linings of KCOTs supports an alteration in cell cycle control. Imbalanced expression of p63 can influence the prognosis of many tumors like squamous cell carcinoma of lung, larynx, and oral cavity,<sup>14-17</sup> and it may be correct for KCOT that showed high expression of p63. Many researchers believed that  $\Delta$ Np63 isoforms can inhibit the function of the wild-type of p53.<sup>13</sup> Thus, p63 may block the induction of apoptosis and inhibition of growth.<sup>13</sup> It may also cause proliferation in squamous epithelium.<sup>18,19</sup>

In conclusion, high expression of p63 in KCOTs, could be useful to explain the difference in the clinical and pathological behavior of KCOTs. Also it seems to point to an alteration in cell cycle control. In addition, the p63 overexpression found in KCOTs can be a diagnostic marker of odontogenic cysts with more aggressive clinical behavior. "Previous researchers showed that 4A4 antibody does not recognize the TAp63 isoforms in tissue sections".<sup>11</sup> On the other hand, staining was limited to  $\Delta$ Np63 isoforms. Therefore, studies related to evaluation of TAp63 isoforms with appropriate antibody is needed.

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