

ORIGINAL ARTICLE

Ki-67 and p53 expression in solitary sporadic, syndrome associated and recurrent keratocystic odontogenic tumor

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ABSTRACT

Background: Ki-67 and p53 are markers expressed in actively proliferating cells, particularly in neoplasms. **Objectives:** (1) To study the proliferative potential of epithelia in Solitary Sporadic, Syndrome-associated and Recurrent Keratocystic odontogenic tumors (KCOTs) using Ki67 and p53 labeling indices (LI). (2) To derive a relationship if any between the expression of these proteins and the biologic behavior of Solitary Sporadic and Syndrome associated KCOTs. **Study Design:** Thirteen paraffin embedded blocks of KCOTs (Solitary Sporadic, $n = 03$; Recurrent, $n = 03$; Syndrome associated, $n = 07$) were stained immunohistochemically for Ki-67 and p53 and labeling indices were calculated. **Statistical Analysis:** Z test with predetermined alpha set at 0.05 was used for the comparison of Ki-67 positivity between the three groups and p53 positivity between the three groups. **Results:** Ki-67 labeling indices were: 30% in solitary sporadic; 26% in recurrent; and 32% in syndrome associated KCOTs. p53 labeling indices were: 19% in solitary sporadic; 23% in recurrent; and 21% in syndrome-associated KCOTs. There was no difference seen in the rate of proliferation in the epithelial linings between the three groups. However, in our cases where Ki-67 positivity was seen there was expression of p53. Though not statistically significant a trend was seen, reflecting the loss of balance between the proliferative potential and apoptotic activity. **Conclusion:** On the basis of proliferative index alone it is not possible to comment on biological behavior of KCOTs associated with syndrome versus those of solitary and recurrent. There is probably a mesenchymal role which needs to be researched.

Key words: Ki-67, keratocystic odontogenic tumor, nevoid basal cell carcinoma syndrome, p53

INTRODUCTION

Odontogenic keratocyst (OKC) is one of the most aggressive odontogenic cysts. In the recent World Health Organization classification of Head and Neck Tumors, keratocyst has been reclassified as a benign neoplasm, designated as "Keratocystic Odontogenic tumor" (KCOT).^[1] KCOTs can arise sporadically and about 4-5% of KCOTs arise in association with nevoid basal cell carcinoma syndrome (NBCCS).^[2] The aggressive

biological behavior of the KCOTs and its tendency to recur might be associated with cell kinetics in the lining epithelium.

Several studies have been done comparing solitary, recurrent and syndrome-associated KCOTs. The proliferative activity of the epithelial lining of KCOTs has been the focus of numerous investigations. Studies have shown conflicting results with few of them showing the expression of cell-cycle-related proteins in the lining epithelium to be different in sporadic KCOTs as compared with KCOTs associated with the NBCCS. The different biological behavior of the latter group was suggested to be due to the underlying genetic abnormalities.^[3-5] The aim of the present study was to compare the expression pattern of Ki-67 and p53 in the epithelial lining of Solitary Sporadic, Recurrent KCOTs and KCOTs-associated with NBCCS and to derive a relationship if any between the expression of these proteins and the biological behavior of Solitary Sporadic and Syndrome-associated KCOTs.

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MATERIALS AND METHODS

A total of 13 formalin fixed, paraffin embedded tissue blocks were retrieved from the archives of our department, which included three cases of Solitary Sporadic KCOTs, 3 Recurrent KCOTs and seven Syndrome-associated KCOTs.

Immunohistochemistry

Sections of 4 µm were taken from the paraffin embedded blocks and mounted on Polysilane-coated slides. The slides were incubated at 60°C overnight. The sections were deparaffinized in two changes of xylene for 5 min each and then the slides were hydrated and cleared through different grades of alcohol. The tissues were then incubated with 3% H₂O₂ in methanol for 20 min to block the endogenous peroxidase activity. Then the slides were washed in Tris Buffer solution (TBS) for 5 min and subjected to antigen retrieval. The slides were heated in microwave oven with 0.1 m Ethylene diamine tetra acetic acid (PH 8.0) at 800 w for 12 min and 200 w for 15 min for antigen retrieval. The buffer was then cooled back to room temperature and the sections were subjected to two washes of TBS. The sections were incubated for 5 min with protein block to eliminate background staining. The sections were then incubated with primary antibody: Anti Ki-67 monoclonal mouse antibody (Biogenex (BGX), Clone BGX-297) and anti p53 monoclonal mouse antibody (Dako (DO), Clone DO-7) for 45 min. Lymphoma sections were used as positive control for both Ki-67 and p53. One section from each positive control was used as negative control, by omitting the primary antibody. The slides were then washed with TBS twice for 5 min each. The Horse Radish Peroxidase (HRP) labeled polymer antimouse antibody (Biogenex) was incubated at room temperature. The slides were then washed in TBS and were incubated with fresh Diamino Benzidine (DAB) chromogen for 1-2 min. The slides were then washed with water and counter stained with Harris hematoxylin for 1 min. the slides were dehydrated through graded alcohol, cleared and mounted with Dibutyl Pthalate Xylene (DPX).

In order to evaluate the stained sections descriptively with Ki-67 and p53 antibodies, the numbers of positive cells in basal and parabasal regions in the lining epithelium of KCOTs were counted. Cell nuclei with bright brown color, regardless of the color intensity, were considered as positive cells for Ki-67 and p53. Cell counts were made at x400 magnification with conventional light microscope in ten randomly selected fields, counting minimum of 1,000 cells. Ki-67 and p53 LI were calculated.

Labeling Index (LI) = Number of IHC positive cells × 100/Total number of cells observed.

Ki-67 and p53 LI were compared between the three groups (Solitary Sporadic vs. Recurrent), (Recurrent vs. Syndrome) and (Solitary Sporadic vs. Syndrome).

Statistical analysis

The Statistical software namely Minitab v14 was used for the analysis. Z test with predetermined alpha set at 0.05 (i.e. 5% - alpha is “level of significance”) was used for the comparison of Ki-67 positivity between the three groups and p53 positivity between the three groups.

RESULTS AND OBSERVATION

Anti-Ki-67 antigen antibodies reacted with nuclei in the cells of basal and parabasal layers of the lining epithelium of KCOTs [Figures 1-3]. Ki-67 LI were 30% in solitary sporadic, 26% in recurrent and 32% in syndrome-associated KCOTs which is slightly higher than the expression seen in solitary sporadic and recurrent cases [Table 1]. There was no statistical difference in the expression of Ki-67 between the three groups. Likewise, the expression of p53 was seen in the nuclei of basal and parabasal layers of epithelium [Figures 4-6]. p53 LI were 19% in solitary sporadic KCOTs, 23% in recurrent KCOTs and 21% in KCOTs-associated with syndrome, showing statistically no difference in the expression of p53 between the three groups [Table 2]. In all the cases, Ki-67 and p53 expression was more in the parabasal layers as compared to the basal layers.

DISCUSSION

KCOT is a unique lesion in the spectrum of odontogenic cysts and tumors. Besides the clinically and radiographically aggressive features, the inherent growth potential of its epithelium, its high recurrence rate (2.5-62.5%),^[6] close relationship to the NBCCS and the alleged increased risk of neoplastic transformation have ensured that it remains extensively investigated for various aspects of its behavior. Although it is a well-known fact that behavioral differences exist between solitary, syndrome and recurrent KCOTs, the reasons for such differences are not well understood.

Some proliferation markers^[7,8] and apoptotic proteins have been investigated to assess their role in such behavioral differences but with conflicting results.^[2,9,10] In this research, we studied the expression of established markers like Ki-67 and p53 in solitary, syndrome and recurrent KCOTs.

Studies in the past have shown Ki-67 and p53 expression to be higher in the epithelium of KCOTs when compared with developmental and inflammatory cysts. Most of the Ki-67+ cells in KCOTs have been detected in the supra basal layers, suggesting high proliferative activity in these layers.^[8,11]

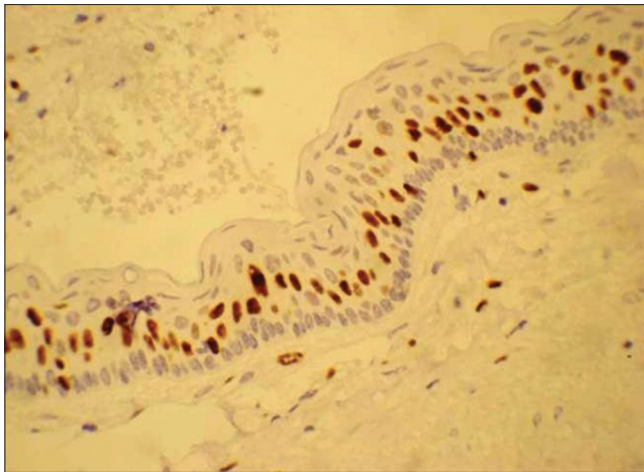


Figure 1: Ki-67 expression in basal and parabasal layer of solitary Keratocystic odontogenic tumor (IHC,×400)

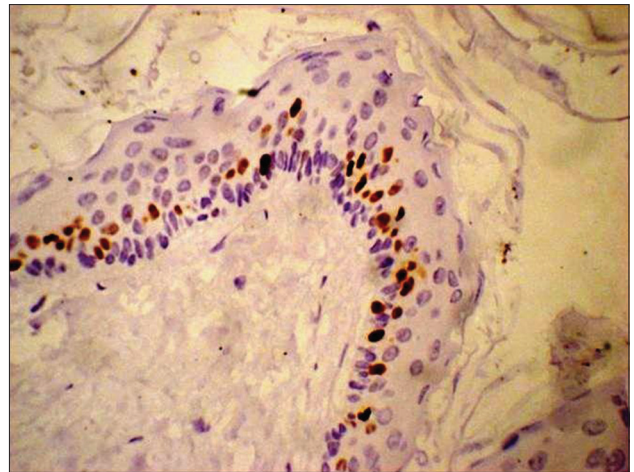


Figure 2: Ki-67 expression in basal and parabasal layer of recurrent Keratocystic odontogenic tumor (IHC,×400)

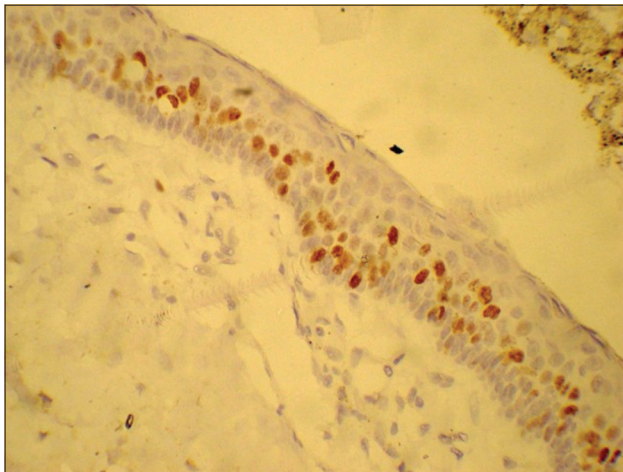


Figure 3: Ki-67 expression in basal and parabasal layer of syndrome associated Keratocystic odontogenic tumor (IHC,×400)

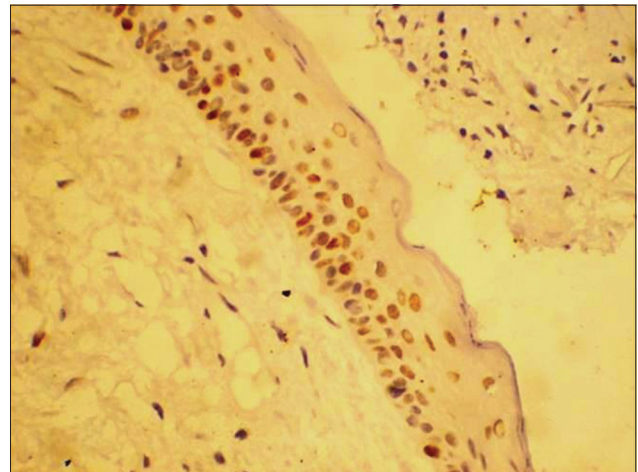


Figure 4: p53 expression in basal and parabasal layer in solitary Keratocystic odontogenic tumor (IHC,×400)

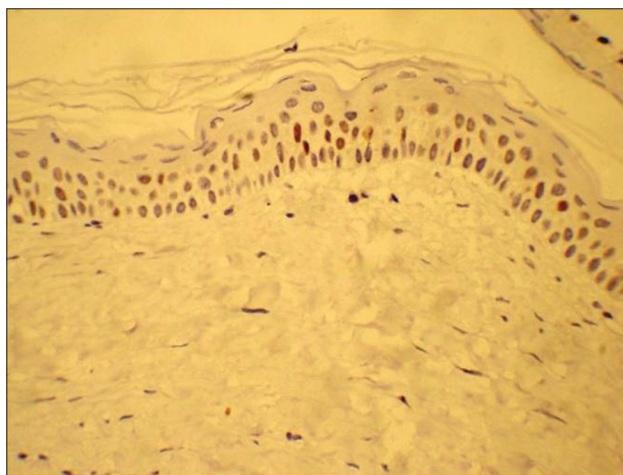


Figure 5: p53 expression in basal and parabasal layer of recurrent Keratocystic odontogenic tumor (IHC,×400)

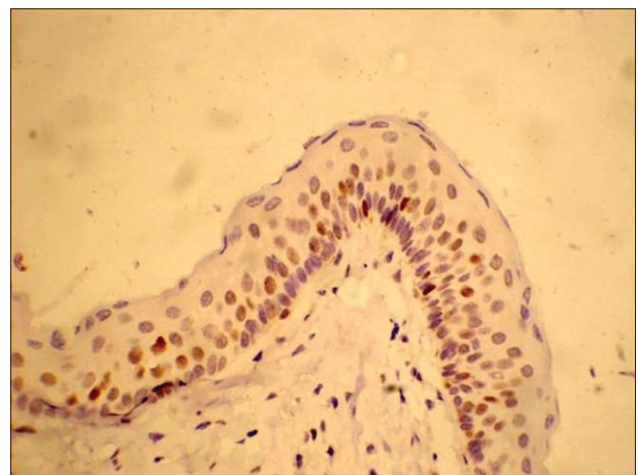


Figure 6: p53 expression in basal and parabasal layer of syndrome associated Keratocystic odontogenic tumor (IHC,×400)

Table 1: Comparison of Ki-67 and p53 expression between solitary, recurrent and syndrome-associated KCOTs

KCOT	Total no. of +ve cells	Labeling index (LI) (%)	Z test	P value
Ki67				
Recurrent (n=362)	94	25.97	-1.38	0.168
Solitary (n=397)	121	30.48		
Recurrent (n=362)	94	25.97	-1.92	0.055
Syndrome (n=783)	247	31.55		
Solitary (n=397)	121	30.48	0.37	0.709
Syndrome (n=783)	247	31.55		
P53				
Recurrent (n=337)	76	22.55	0.98	0.325
Solitary (n=339)	66	19.47		
Recurrent (n=337)	76	22.55	0.47	0.639
Syndrome (n=761)	162	21.29		
Solitary (n=339)	66	19.47	-0.69	0.492
Syndrome (n=761)	162	21.29		

Table 2: Comparison of Ki-67 and p53 expression in basal and parabasal cells in solitary, recurrent and syndrome-associated KCOT's

Types of KCOT	Ki67		P53		Z	P value
	n	+ve	n	+ve		
Basal cells						
Solitary	1,934	243	1,648	247	-2.10	0.035*
Recurrent	1,831	204	1,860	338	-6.03	<0.001*
Syndrome	4,019	530	3,817	586	-2.74	0.006*
Para basal cells						
Solitary	1,976	973	1,620	408	14.76	<0.001*
Recurrent	1,821	751	1,534	413	8.68	<0.001*
Syndrome	3,816	1,947	3,768	1,053	20.55	<0.001*

*Significant difference, KCOT: Keratocystic odontogenic tumor

Similar pattern of staining has been noted for p53 too with its expression seen in the same areas where there is increased expression of Ki-67.^[2,3,8,12] The reason for this pattern is because p53 over-expression may promote cell proliferation in odontogenic lesions.

Our results also showed higher parabasal staining of Ki-67 and p53 in all the types of KCOTs but there was no statistically significant difference seen in the three study groups [Tables 2-4]. Similar findings have been seen in other studies too.^[8,12-15] However, contradictory results have been seen in few other publications where significant difference was seen in the expression of Ki-67 and p53 in syndrome cases compared to solitary or recurrent KCOTs.^[4,9,16] Majority of them have attributed this to a genetic basis in KCOTs associated with syndrome.^[3-5] Also, the lack of difference in expression seen between solitary and recurrent KCOTs has been credited to the fact that recurrences are mainly due to the treatment modality used and not because of any inherent behavioral differences.^[9]

Table 3: Comparison of ki-67 and p53 expression between different types of KCOT in basal layer

KCOT	+ve	% +ve	Z	P value
Ki67				
Solitary (n=1,934)	243	12.56	1.35	0.177
Recurrent (n=1,831)	204	11.14		
Solitary (n=1,934)	243	12.56	-0.67	0.503
Syndrome (n=4,019)	530	13.19		
Recurrent (n=1,831)	204	11.14	-2.19	0.028*
Syndrome (n=4,019)	530	13.19		
P53				
Solitary (n=1,648)	247	14.99	-2.53	0.012*
Recurrent (n=1,860)	338	18.17		
Solitary (n=1,648)	247	14.99	-0.34	0.731
Syndrome (n=3,817)	586	15.35		
Recurrent (n=1,860)	338	18.17	2.70	0.007*
Syndrome (n=3,817)	586	15.35		

*Significant difference, KCOT: Keratocystic odontogenic tumor

Table 4: Comparison of ki-67 and p53 expression between different types of KCOT in parabasal layer

KCOT	+ve	% +ve	Z	P value
Ki67				
Solitary (n=1,976)	973	49.24	4.95	<0.001*
Recurrent (n=1,821)	751	41.24		
Solitary (n=1,976)	973	49.24	-1.29	0.199
Syndrome (n=3,816)	1947	51.02		
Recurrent (n=1,821)	751	41.24	-6.87	<0.001*
Syndrome (n=3,816)	1947	51.02		
P53				
Solitary (n=1,620)	408	25.19	-1.11	0.266
Recurrent (n=1,534)	413	26.92		
Solitary (n=1,620)	408	25.19	-2.09	0.037*
Syndrome (n=3,768)	1053	27.95		
Recurrent (n=1,534)	413	26.92	-0.76	0.450
Syndrome (n=3,768)	1053	27.95		

*Significant difference

Overall, when the labeling indices were compared across the three groups it was seen that, as the expression of Ki-67 increased the expression of p53 decreased. Though this was not statistically significant, there was a trend seen in this direction [Table 1]. This indicates that there probably was a loss of balance between proliferation and apoptosis though not translating into any behavioral differences in the three groups.

Thus, from our study, we found that the rate of proliferation and apoptosis-related proteins in the epithelium may not be the only factors responsible for the variations seen in the biologic behavior of solitary, syndrome associated and recurrent KCOTs. In addition to epithelial factors, there may be mesenchymal factors or any unknown epithelial mesenchymal interactions which possibly could explain the variations in the aggressiveness of different types of KCOTs.

CONCLUSION

In conclusion, our study showed no difference in the rate of proliferation in the epithelial linings between the three groups. In cases, where Ki-67 positivity was high, there was reduced expression of p53, reflecting the loss of balance between the proliferative potential and apoptotic activity. On the basis of proliferative index alone, it is not possible to comment on biological behavior of KCOTs associated with syndrome versus those of Solitary Sporadic and Recurrent. Rate of recurrence and rate of growth are factors not solely dependent on epithelial proliferation but is probably multifactorial with a mesenchymal role as well, which needs to be researched. This also raises the clinical question whether on the basis of proliferative index alone these commonly occurring lesions of the jaws should be categorized as a benign neoplasm.

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