ORIGINAL ARTICLE

c-Myc oncogene expression in selected odontogenic cysts and tumors: An immunohistochemical study

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ABSTRACT

Aim: To investigate the role of c-Myc oncogene in selected odontogenic cysts and tumors. **Materials and Methods:** Ten cases each of ameloblastoma, adenomatoid odontogenic tumor (AOT), odontogenic keratocyst (OKC), dentigerous cyst, and radicular cyst were selected and primary monoclonal mouse anti-human c-Myc antibody was used in a dilution of 1: 50. Statistical Analysis was performed using Mann Whitney U test. **Results:** 80% positivity was observed in ameloblastoma, AOT and OKC; 50% positivity in radicular cyst and 20% positivity in dentigerous cyst. Comparison of c-Myc expression between ameloblastoma and AOT did not reveal significant results. Similarly, no statistical significance was observed when results of OKC were compared with ameloblastoma and AOT. In contrast, significant differences were seen on comparison of dentigerous cyst with ameloblastoma and AOT and radicular cyst with AOT. **Conclusion:** From the above data we conclude that (1) Ameloblastoma and AOT have similar proliferative potential and their biologic behavior cannot possibly be attributed to it. (2) OKC has an intrinsic growth potential which is absent in other cysts and reinforces its classification as keratocystic odontogenic tumor.

Key words: **Adenomatoid odontogenic tumor, ameloblastoma, c-Myc oncogene, dentigerous cyst, immunohistochemistry, keratocystic odontogenic tumor, odontogenic keratocyst, radicular cyst**

INTRODUCTION

Odontogenic cysts and tumors, although originate from the same primitive odontogenic epithelium, differ widely in their pathogenesis and biologic behavior. Various concepts have been proposed which explain the pathogenesis of different odontogenic cysts and tumors. Studies using proliferative markers suggest that increased cell proliferation plays a role in the development of odontogenic cysts and tumors. This increased cell proliferation in odontogenic epithelium can result from perturbations in cell cycle regulators (Cyclin D1, $p16^{INK4a}$, $p21^{WAF/Cip1}$, $p27^{Kip1}$), mutations in oncogenes (Ras, Myc, Fos), or tumor suppressor genes (p53, adenomatous polyposis coli (APC).[1] Genetic mutations have also been proposed as a possible cause in the development of odontogenic cysts like odontogenic keratocyst (OKC)[2] and dentigerous cyst.[3] Studies investigating the cell kinetics of epithelial linings of odontogenic cysts indicate that the lining of OKC may have some intrinsic growth potential which is not present in other cysts[4,5] and thus OKC is now regarded as a benign cystic neoplasm rather than a cyst.[6,7]

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The c-Myc protein is a nuclear transcription factor which centrally regulates cell proliferation, arrest, differentiation and death. By accelerating cells through G1 and S phases of the cell cycle, abrogating cell cycle checkpoints and increasing cell metabolism, c-Myc eventually renders genomic instability to the cells.[8-12] c-Myc also plays a role in the early stages of embryonic tooth development[13,14] and its expression here is related to the proliferation of odontogenic epithelial cells and its degree of differentiation.[15] Currently few data exist on the occurrence of c-Myc in odontogenic lesions. The aim of the present immunohistochemical study is to investigate the role of c-Myc oncoprotein in selected odontogenic cysts and tumors.

MATERIALS AND METHODS

Fifty cases of buffered formalin fixed, routinely processed, paraffin embedded tissues, histologically diagnosed as

odontogenic cysts and tumors were selected. These included ten cases of ameloblastoma, (five follicular and five plexiform), ten cases of adenomatoid odontogenic tumor (AOT), ten cases of OKC (only sporadic cases), ten cases of dentigerous cyst and ten cases of radicular cyst. Well differentiated squamous cell carcinoma was taken as positive control.

Immunohistochemistry

Sections of 4 µm thickness were taken on silanized slides (SIGMA diagnostics, USA), deparaffinized in xylene, hydrated through graded alcohol and washed in distilled water. Antigen retrieval was performed by heating slides immersed in 10 mM citrate buffer (pH 6.0) in a pressure cooker. Sections were brought to room temperature and the immunohistochemical procedure was performed. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 min. The sections were washed in tris buffered saline (TBS– wash solution) and incubated with primary monoclonal mouse anti-human c-Myc antibody (clone 9E10; Dako Cytomation, USA) at 4°C overnight, diluted to a ratio of 1:50 in TBS containing bovine serum albumin (TBS– antibody diluent solution) to block non-specific binding. The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibody with the use of a Labelled streptavidin biotin (LSAB) + Visualization system – Horseradish peroxidase (HRP) (Dako Cytomation, USA) [Figure 1]. The slides were visualized by DAB solution and weakly counterstained with hematoxylin. Negative controls were prepared by replacing primary antibody with TBS and were confirmed to be unstained.

Evaluation and statistical analysis

Immunohistochemical expression of c-Myc oncoprotein was evaluated independently by three investigators. Presence of brown colored product at the site of target antigen was indicative of positive immunoreactivity for c-Myc expression. Staining reactions were evaluated and graded as positive or negative (0). The positive results were assessed further for intensity of staining as mild (1), moderate (2) and intense (3) staining and localization as either nuclear or cytoplasmic or nuclear and cytoplasmic staining. In cases with staining heterogeneity, the expression was grouped according to the predominant staining intensity.

The statistical significance of differences in the staining intensities between various study groups were analyzed by Mann Whitney U test and significance determined at 5% level of significance.

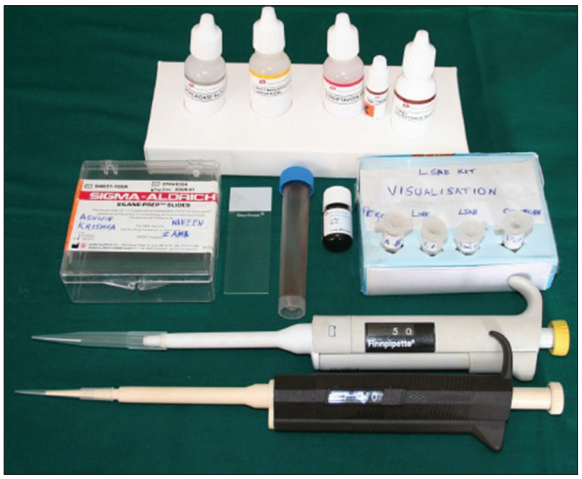

Figure 1: Photograph of immunohistochemical reagents and kit

RESULTS

The results of immunohistochemical staining of c-Myc in various study groups is summarized in Table 1. Among the odontogenic tumors studied, both follicular and plexiform ameloblastomas and adenomatoid odontogenic tumor expressed 80% positivity. In odontogenic cysts, OKC showed maximum number of cases with positive immunoreactivity (80%), followed by radicular cyst (50%) and dentigerous cyst (20%). Table 2 shows mean and standard deviation values of intensity of c-Myc expression in the various study groups. Statistical comparison of staining intensity of c-Myc between different types of ameloblastoma and between ameloblastoma and AOT did not reveal any significance. Among the cysts, OKC showed staining intensity significantly higher than dentigerous cyst or radicular cyst while no significance was seen between dentigerous cyst and radicular cyst. When the staining results of the three cysts

Table 1: c-Myc expression in different study groups

Study group	Intensity of staining				% of positivity
	Negative	Mild	Moderate	Intense	
AMB (F)[b] *n*[a]=5	1	1	2	1	80
AMB (P)[c] *n*=5	1	0	2	2	80
AOT[d] *n*=10	2	1	3	4	80
OKC[e] *n*=10	2	1	3	4	80
DC[f] *n*=10	8	0	1	1	20
RC[g] *n*=10	5	2	3	0	50

[a]*n*: Number of cases studied, [b]AMB (F): Follicular ameloblastoma, [c]AMB (P): Plexiform ameloblastoma, [d]AOT: Adenomatoid odontogenic tumor, [e]OKC: Odontogenic keratocyst, [f]DC: Dentigerous cyst, [g]RC: Radicular cyst

Table 2: Mean±SD of study groups

Study groups	Mean	SD[a]
AMB (F)	1.60	1.14
AMB (P)	2.00	1.22
AOT	1.90	1.20
OKC	1.90	1.20
DC	0.50	1.08
RC	0.80	0.92

[a]SD: Standard deviation, AMB (F): Follicular ameloblastoma, AMB (P): Plexiform ameloblastoma, AOT: Adenomatoid odontogenic tumor, OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

were compared individually with the tumors, the staining result of OKC was similar to that of ameloblastoma and AOT. Significant difference was observed in the staining intensities between dentigerous cyst and the tumours. Radicular cyst showed variable result with no significant difference when compared with ameloblastoma and a significant difference observed when compared with AOT [Table 3].

Reactivity for c-Myc protein in follicular and plexiform ameloblastomas was observed both in peripheral columnar ameloblast like cells and central polyhedral stellate reticulum like cells. Stellate reticulum like cells showed mainly nuclear staining while peripheral columnar ameloblast like cells showed both nuclear and cytoplasmic expression [Figures 2 and 3]. In AOT, predominantly nuclear staining was seen in whorls, ducts and sheets of epithelial cells [Figure 4]. Uniform staining was observed in all the layers of cystic lining in OKC, basal layer of cystic lining in dentigerous cyst and arcading epithelial lining in radicular cyst. Both nuclear and cytoplasmic staining was observed in the lining cells of all the cysts [Figures 5 and 6].

DISCUSSION

The etiology and pathogenesis of odontogenic tumors and cysts is a subject which is debated for decades. Various molecular alterations are now proposed to be responsible for the development of these lesions, one of which is the role of oncogenes. The role of c-Myc proto-oncogene in the regulation of normal cellular proliferation suggests that its deregulation contributes to deregulated DNA synthesis and genomic instability.[8] Overexpression of the c-Myc protein and its gene amplification has been identified in various neoplastic and non-neoplastic lesions. The fact that oncogene products can be detected by immunohistochemistry[16] and the advantage of maintaining the cellular and tissue architecture in this method[4] has prompted us to study the role of c-Myc oncogene product in selected odontogenic cysts and tumors.

Table 3: Comparison of intensity of c-Myc expression between different study groups using Mann Whitney U test

Lesions	Study groups compared	*P*	Significance
Tumors	AMB (F) versus AMB (P)	0.5308	NS[a]
	Total AMB versus AOT	0.7913	NS
Cysts	OKC versus DC	0.0257	S[b]
	OKC versus RC	0.0452	S
	DC versus RC	0.3847	NS
AMB versus cysts	AMB versus OKC	0.7913	NS
	AMB versus DC	0.0312	S
	AMB versus RC	0.0588	NS
AOT versus cysts	AOT versus OKC	1.0000	NS
	AOT versus DC	0.0257	S
	AOT versus RC	0.0452	S

[a]NS: Not significant, [b]S: Significant, AMB (F): Follicular ameloblastoma, AMB (P): Plexiform ameloblastoma, AOT: Adenomatoid odontogenic tumor, OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

The results of our study demonstrate a similar expression of c-Myc oncoprotein between ameloblastoma and AOT. Our findings are consistent with the argyrophilic nucleolar organizer regions counts studied in these two lesions. Since c-Myc expression is related to the proliferative status of the cells, our results in accordance with the findings of Carmo and Silva[17] suggest that the differences in the biologic behavior of ameloblastomas and AOT's are probably not related to their cellular proliferation ratio. In the ameloblastomas studied, there was no statistically significant difference observed in the expression of c-Myc between follicular and plexiform ameloblastomas which is in accordance with the findings of Kumamoto *et al*.[18] Reactivity for c-Myc protein in the follicular and plexiform ameloblastomas in our study was observed both in peripheral columnar cells and central polyhedral cells. The staining reaction of central polyhedral cells was mainly nuclear and that of peripheral columnar cells varied from being only nuclear to both nuclear and cytoplasmic. Our results are in compliance with those of Barawy *et al*.,[15] who also observed both nuclear and cytoplasmic staining. Considerable debate exists regarding the subcellular distribution of c-Myc oncogene product. Persson and Leder[9] have shown that c-Myc oncoprotein is predominantly found in the nucleus while Loke *et al*.,[19] have shown that though the c-Myc protein is predominantly localized in the nucleus, certain fixation procedures may allow these proteins to diffuse into the cytoplasm. They also observed that despite a variety of fixation methods, tissues either rich in hydrolytic enzymes or metabolically very active, exhibit cytoplasmic staining for c-Myc protein. The appearance of c-Myc protein in the nucleus and cytoplasm of the peripheral columnar ameloblast like cells in our study could be because these cells may be similar to the ameloblasts of the enamel organ which are metabolically very active whereas the predominant nuclear staining in the stellate reticulum like cells denotes its less activity when compared with the peripheral cells.

To date, data on the immunohistochemical expression of c-Myc oncoprotein in odontogenic cysts do not exist with only 1 study which determines the expression of c-Myc mRNA in OKC using *in situ* hybridization.[20] Our results showed 80% positivity for c-Myc expression in OKC which was significantly higher than dentigerous cyst (20%) and radicular cyst (50%). Previous studies assessing cell proliferation in the three major odontogenic cysts using various markers for proliferation like Proliferating cell nuclear antigen PCNA,[4] Ki67,[5,21] p53[5,22] and Ipomeanol IPO-38,[23] have shown similar results wherein increased proliferative cells were observed in the lining of OKCs which was significantly higher than the dentigerous cyst and radicular cyst. While the expression of p53, Ki67, PCNA and IPO-38 was predominantly seen in the suprabasal layers, our results showed a uniform distribution of c-Myc expression in both basal and parabasal layers of the lining epithelium.

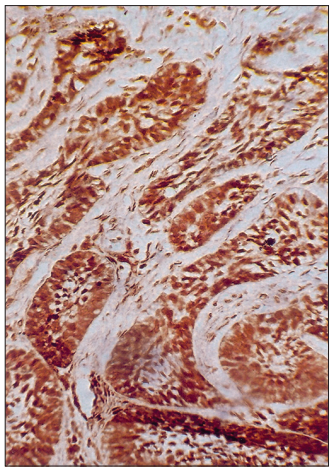

Figure 2: Positive c-Myc expression showing intense staining in follicular ameloblastoma. Predominant nuclear staining of central polyhedral cells and cytoplasmic staining of peripheral columnar cells (×400).

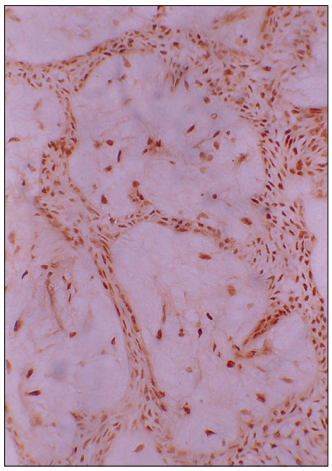

Figure 3: Positive c-Myc expression showing moderate staining in plexiform ameloblastoma (×250)

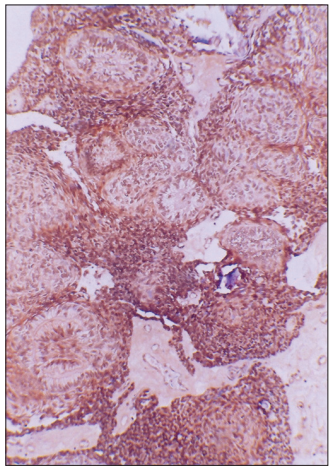

Figure 4: Positive c-Myc expression showing moderate staining in adenomatoid odontogenic tumor (×250)

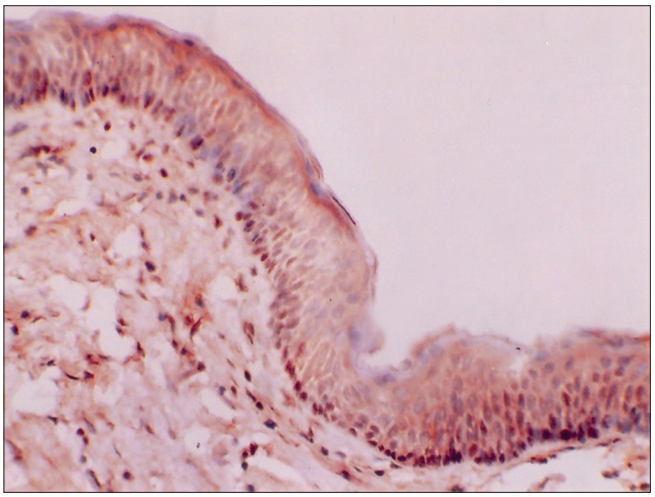

Figure 5: Positive c-Myc expression showing intense staining in odontogenic keratocyst (×250)

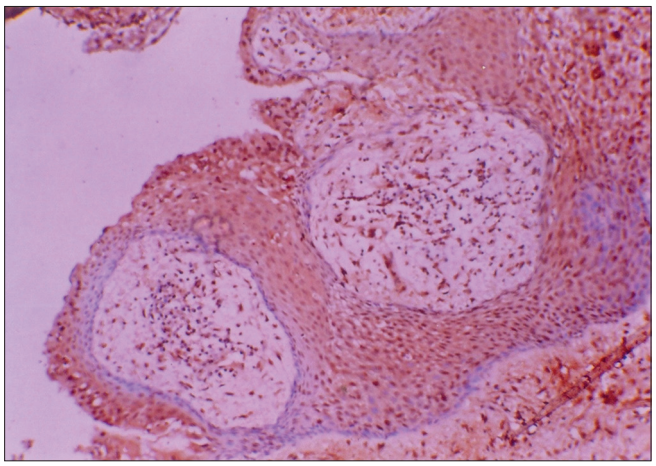

Figure 6: Positive c-Myc expression in radicular cyst (×250)

Comparison of c-Myc expression between OKC (80% +ve) and ameloblastoma (80% +ve) and OKC and AOT (80% +ve) in our study was not significant. Li *et al*.,[20] studied the expression of c-Myc mRNA and showed 75.0% positivity in OKC and 81.5% positivity in ameloblastoma. When OKC and ameloblastoma were compared for expression of PCNA,[24] IPO-38[23] and p53[5,22] the percentage of positivity in the lining of OKC was not significantly different from those of ameloblastoma. Our findings are in accordance with other studies comparing epithelial cell proliferation between OKC and ameloblastoma. Collectively, these data explain that OKC has a proliferation index which is similar to those of ameloblastoma and AOT and significantly higher than in other kinds of odontogenic cysts. Hence its addition as 'keratocystic odontogenic tumor' seems to be justified.

Comparison of c-Myc expression between dentigerous cyst and ameloblastoma and dentigerous cyst and AOT in our study showed statistically significant differences. Previous comparative studies between dentigerous cyst and ameloblastoma by Thosaporn *et al*., on IPO-38,[23] Slootweg on p53[5] and Piattelli[25] using Ki67 have shown similar results. Together these data suggest that factors other than epithelial proliferation, like fluid

accumulation in the follicle and contents of cystic fluid that increase the osmolality and hydrostatic pressure are of greater importance in the development and enlargement of dentigerous cyst.

Bando *et al*.,[26] showed that epithelial proliferation in the radicular cyst is stimulated by inflammatory cytokines (Interleukin-1) released by the inflammatory cells. Treatment with mitogens, including cytokines or growth factors such as interleukins results in a rapid increase in c-Myc mRNA and protein levels[27] and the expression of c-Myc in inflammatory gastric and colonic mucosa increased with greater degrees of inflammation.[16] Our results showed no statistically significant difference in the expression of c-Myc between ameloblastoma and radicular cyst, while a significant difference was observed when compared with AOT. This variable expression of c-Myc in radicular cyst could be attributed to inflammatory cytokines released by the inflammatory cells, which is a strong component in the development of radicular cysts.

CONCLUSION

The results of our study show a similar proliferative potential between ameloblastoma and AOT and thereby question the role of proliferation in determining the biologic behavior of these lesions. Among the cysts studied highest intensity of c-Myc expression was observed in OKC which was similar to the staining results of the tumors. This finding highlights the intrinsic growth potential present in the lining of OKC, and supports the hypothesis of OKC to be a benign cystic neoplasm rather than a cyst and attests for its addition under the benign odontogenic tumors as an entity termed 'keratinizing cystic odontogenic tumor'. However, further studies are needed using DNA assays to determine the role of c-Myc oncogene and any mutations if any which may play a role in the pathogenesis of these odontogenic lesions.

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