

p73 Expression in Odontogenic Lesions - An Immunohistochemical Study

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ABSTRACT

Introduction: p73 may have a role in oncogenesis of odontogenic lesions. The aim of the study was to carry out the immunohistochemical study of p73 in a total of 80 cases of dentigerous cyst, Keratocystic odontogenic tumor (KCOT), Unicystic Ameloblastoma and Ameloblastoma.

Material and methods: Immunohistochemical method was carried out using p73 antibody. The percentage of immunopositive cells was calculated in the cyst lined by epithelium and ameloblastoma islands.

Result: p73 expression was marked in all types of odontogenic lesions. The immunopositivity of p73 was marked throughout the lining epithelium of KCOT except parakeratinized surface layer. In dentigerous cysts, very few basal and suprabasal cells expressed immunopositivity. Peripheral cells of ameloblastoma showed p73 expression. Immunopositivity of p73 expression in lesions of odontogenic origin suggests that p73 is important in differentiation and proliferation of odontogenic epithelial cells.

Conclusion: p73 protein represents a basal cell marker, and it is not expressed in mature differentiated cells. Clinical significance is to establish p73 as an independent prognostic biomarker in Odontogenic lesions.

Keywords: p73, Dentigerous Cyst, Keratocysticodontogenic Tumor, Unicystic Ameloblastoma, Ameloblastoma

INTRODUCTION

The lesions of odontogenic origin encompass a number of lesions with diverse behaviour, from innocuous hamartomatous proliferations to cysts with significant development and malignant capacity. p53 gene is a tumor suppressor gene that is often transmutated in tumors.^{1,2} It plays a role in cell cycle arrest or apoptosis in response to genomic damage or oxidative stress.³ Two p53 corresponding genes, p63 and p73, have been determined at loci 3q27-29 and 1p36, respectively.^{4,5} These genes encode variable proteins that have a remarkable degree of sequence homology, particularly in the activation of a gene by the presence of another at a different locus, DNA-binding and oligomerization domains.^{4,6}

p53 corresponding genes though have a property of tumor suppressor, alteration of genes, loss of heterozygosity (LOH), they are less frequent than those associated with p53.^{7,6,8,9} Recent studies indicate up-regulated expression and activity of p63 and p73 in few malignancies.¹⁰⁻¹⁶

The main purpose of this study was to review the current observation of P73 expression in Dentigerous cyst, KCOT, Unicystic Ameloblastoma and Ameloblastoma by IHC to better understand their part in oral Oncogenesis.

MATERIAL AND METHODS

This study is a retrospective study carried out in the Department of Oral Pathology and Microbiology after obtaining approval from the Institutional Ethical Committee. The study included histopathologically diagnosed cases of Dentigerous cyst, KCOT, Ameloblastoma, Unicystic Ameloblastoma retrieved from archives of the Department. The study was performed on paraffin embedded tissue, which was stained for the expression of p73 antibody by immunohistochemistry.

Sample size

A total of 80 cases were selected. They were divided into four groups.

Inclusion criteria

- Histopathologically diagnosed 20 cases of Dentigerous cyst.
- Histopathologically diagnosed 20 cases of KCOT
- Histologically diagnosed 20 cases of Unicystic Ameloblastoma.
- Histologically diagnosed 20 cases of Ameloblastoma

Exclusion criteria

- Patients who is suffering from OSCC.

Primary Antibody

P73 (H-79) sc-7957 IgG rabbit polyclonal antihuman antibody. Santa Cruz Biotechnology, INC

Secondary Antibody

HRP labeled Polymer Antimouse (Dako Envision+System, Product Code: K4000, Dako North America Inc.) Labelling Index was calculated.

Methodology - Immunohistochemistry

Sections stained with p73 antibody were examined under Leica DMLB2 (Leica microscope) at 40X magnification. The sections were examined for the presence of a coloured end product at the site of target antigen (DAB chromogen brown

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end product).

Cells were considered positive for p73 which showed nuclear staining (brown colour). The stained nucleus was scored positive regardless of intensity of staining. Cells that lacked a clear staining were excluded. Minimum of 100 cells were counted in each section. Tissue sections positive for p73 were examined for the presence of brown stained nucleus and evaluated by locating the most heavily labelled by scanning the sections at 10 X magnification. Cell count were made at 40X magnification with Leica DMLB2 (Leica microscope) in 5 randomly selected fields. P73 labelled cells counting was done among all the groups. The number of positively stained nuclei were expressed as a percentage of the total number counted.

P73 Labelling index = Number of IHC positive cells (p73) X 100 / Total number of cells observed.

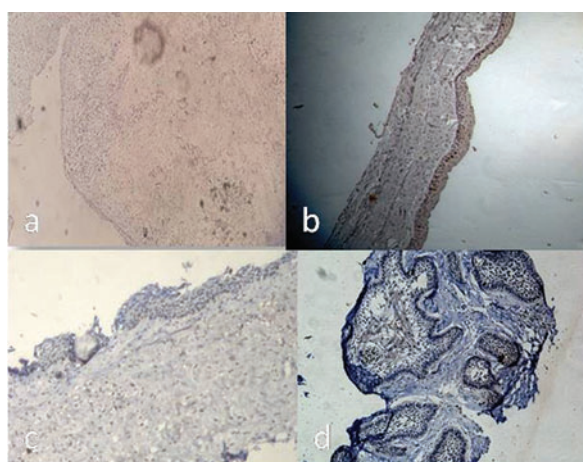


Figure-1: Shows p73 expression in Dentigerous cyst (a), KCOT (b), Unicystic Ameloblastoma (c) and Ameloblastoma (d)

STATISTICAL ANALYSIS

Microsoft office 2007 was used for the statistical analysis. Statistical analysis was done by using descriptive and inferential statistics using Kruskal Wallis Chisquare test (Table 1) and multiple comparison Turkey test (Table 2). Kruskal Wallis Chisquare value=42.91,p-value=0.0001,S. Turkey test and software used in the analysis was SPSS 17.0 version and p<0.05 is considered as level of significance(p<0.05).

RESULT

The immunohistochemically stained sections were evaluated by counting approximately 100 cells in high power field wherever possible and the labelling index was obtained. Staining was observed as nuclear staining for p73. The results were presented with detailed analysis of p73 expression in individual groups as descriptive statistics and their significant differences in the groups.

DISCUSSION

Odontogenic cysts encompasses a varied group of lesions. It comprises of a group of intraosseous lesions within the jaw and are one of the main causes for bone destruction. The most common odontogenic cysts are Radicular cyst (52.3%) followed by Dentigerous cyst(16.6%) and Odontogenic Keratocyst (11.2%).^{17,18} The Dentigerous cyst can be defined as cyst of odontogenic origin that envelops the crown of an impacted tooth; caused by collection of fluid between the reduced enamel epithelium and the enamel surface. The pathogenesis of odontogenic keratocyst is dental lamina or its remnants. Different from radicular and Dentigerous cyst, the odontogenic keratocyst acquire locally aggressive and destructive course.¹⁹ If the cyst is not managed properly, it

Group	N	Mean	Std. Deviation	Std. Error	95% confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Lower Bound		
Dentigerous cyst	20	1.50%	3.28	0.73	-0.037	3.03	0.00	10.00
KCOT	20	36.00%	15.00	3.35	28.97	43.02	20.00	60.00
Unicystic ameloblastoma	20	9.00%	10.71	2.39	3.98	14.01	0.00	30.00
Ameloblastoma	20	32.00%	13.61	3.04	25.62	38.37	10.00	60.00

Kruskal Wallis Chisquare Value = 42.91, p-value=0.0001, S

Table-1: Comparison of p73 expression in Dentigerous cyst, KCOT, Unicystic Ameloblastoma and Ameloblastoma -Kruskal Wallis Chisquare test

Group	N	Mean Difference	Std. Error	p-value	95% confidence Interval for Mean	
					Lower Bound	Lower Bound
Dentigerous cyst	KCOT	-34.50	3.66	0.0001,S	-44.11	-24.883
	Unicystic ameloblastoma	-7.50	3.66	0.180,NS	-17.11	2.11
	Ameloblastoma	-30.50	3.66	0.0001,S	-40.11	-20.88
KCOT	Unicystic ameloblastoma	27.00	3.66	0.0001,S	17.38	36.61
	Ameloblastoma	4.00	3.66	0.695,NS	-5.61	13.61
Unicystic ameloblastoma	Ameloblastoma	-23.00	3.66	0.0001,S	-32.61	-13.383

Table-2: Comparison of p73 expression in Dentigerous cyst, KCOT, Unicystic Ameloblastoma and Ameloblastoma- Multiple Comparison:Turkey test

leads to a significant damage.

Tumors originating from the odontogenic apparatus or its derivatives exhibit variable histological patterns and are grouped into benign and malignant categories. Ameloblastoma is the most common odontogenic tumor we come across which is characterized by a benign but locally invasive behaviour with a high risk of recurrence. Studies have identified gene alterations in these lesions but the detailed mechanisms still remain unknown.²⁰

The nature and behaviour of any lesion is generally known by its growth potential. The proliferation of cells indicate the potentiality of growth and its aggressive behaviour. The growth potential thus acts as a marker in the prognosis and the treatment plan.

To evaluate and to predict the behaviour and prognosis of the odontogenic lesions, we have decided to carry out a study on Dentigerous cyst, KCOT, Ameloblastoma and Unicystic ameloblastoma.

In the present study, immunohistochemistry for p73 was carried out to evaluate the cell proliferation in Dentigerous cyst and proliferative potential and aggressive behaviour of KCOT, Unicystic Ameloblastoma and Ameloblastoma.

In Dentigerous cyst, very mild to no immunopositivity for p73 was found. This is in accordance with the study Seyedmajidi M et al in 2011.²⁰ Thus, it can be concluded that the pathogenesis of expansion of Dentigerous cyst could be some distinct factors other than the proliferating epithelium which is the main reason for the expansion of other cysts. This is established by lack of potential for increased cell proliferation in the cystic lining of Dentigerous cyst.

In Ameloblastoma, p73 immunopositivity was seen predominantly in the peripheral cells than in the central cells. The proliferative potential is known to be higher in peripheral neoplastic cells than in central cells indicating that p73 plays a role in differentiation and or proliferation of cells.

In Unicystic ameloblastoma, p73 immunopositivity was seen in the lining of cyst. The basal cells showed positivity for p73 indicating the proliferative behaviour of the lesion.

In, KCOT the expression of p73 was seen in the basal and suprabasal layers. This is in accordance with the study by Lo Muzio et al., Foschini et al. and Gurgel et al., where in a greater proliferative potential in the suprabasal layers of KCOT was shown. The Labeling Index for p73 was seen to be marked in the peripheral columnar cells in case of Ameloblastoma, while it was higher in the suprabasal layers of KCOT. Similar studies done using other proliferative markers PCNA, IPO-38, Ki-67 by Takahashi H et al.,²¹ Thosaporn et al.,²² concluded that proliferation indices are useful in predicting the different biological behavior of odontogenic lesions and also that KCOT showed a higher proliferative rate than Ameloblastoma.

CONCLUSION

The results of the study suggests that KCOT and Ameloblastoma have similar proliferative potential in the basal layer whereas in the suprabasal layer, KCOT has a higher proliferative potential when compared to Unicystic

ameloblastoma and Ameloblastoma. This indicates that the nature of OKC is at least on par if not much higher than that of Ameloblastoma thus supporting the new nomenclature for this lesion as 'Keratocystic odontogenic tumor. The study concludes that p73 plays an important role in the cytodifferentiation and oncogenesis of the odontogenic epithelium.'

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