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Original Article

Analysis of P53 Protein Expression in Odontogenic Cyst – An Immunohistochemical Study

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Abstract

Introduction: p53 is a tumor suppressor gene which arrests the cell cycle at G1 phase. At this phase, it allows the repair of possible DNA damage and prevents the cell cycle progression to S phase. In normal cells, it is found at low level whereas its expression is elevated in many tumor. Hence, p53 is used as a marker of neoplasia, malignancy, and tumor progression.

Purpose: The aim of the study was to evaluate the expression of P53 protein in biopsy specimen of Odontogenic Keratocyst (OKCs), Dentigerous cyst (DCs), and Radicular cyst (RCs) by immunohistochemistry (IHC) to know the biological behavior of these cases.

Materials and Methods: This study is carried out in histopathologically diagnosed 15 cases of OKCs, 10 cases of DCs and 10 cases of RCs that were formalin fixed, processed, and paraffin embedded. Sections of 4-micron thickness were cut from paraffin embedded tissue blocks and stained with IHC marker p53 following standardized protocol. The presence of brown colored nuclear reaction at the site of target antigen was considered immune positive for p53.

Results: In our study, we found in OKCs, the intensity of p53 expression was more for supra basal layers (46% in the supra basal layers showed intense positivity) than basal and superficial layers. In DCs and RCs, the more intensity of p53 expression in basal layer than supra basal and superficial layers was found.

Conclusions: p53 was over expressed in OKCs compared with other lesions. Mutations in the p53 gene yield a p53 protein which has an increased half-life, thus allowing this mutated protein to be detected immunohistochemically. p53 protein is related to cell proliferation activities in OKCs.

Key words: Dentigerous cyst, Immunohistochemistry, Odontogenic cysts, Odontogenic Keratocyst, p53 protein, Radicular cyst

INTRODUCTION

Odontogenic cysts are the cysts which arise from the enamel organ or their remnants.

Odontogenic cysts are because of second most common lesions in the oral and maxillofacial specimens, it has an important role in oral and maxillofacial pathology.[1] The three common odontogenic cysts include odontogenic keratocysts (OKCs), dentigerous cysts (DCs), and radicular cysts (RCs).[2] OKCs and DCs are developmental origin whereas RCs are the result of inflammation.[3]

The p53 protein is a nuclear protein with a molecular weight of 53 kilodalton, which is coded by the p53 gene located on chromosome 17. This tumor suppressor gene has a valuable role in apoptosis, cell cycle, regulation of cell proliferation, and genetic stability.[4-6] The p53 protein is a regulative factor of many processes necessary for the proper functioning of cells, and it corresponds to a number of processes associated with its life and death. The p53 protein regulates the repair of cellular DNA and induces apoptosis when the damage of the gene is too serious and it is impossible to repair. This protein is also

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responsible for the regulation of the senescence of cells and the cell entering and leaving the subsequent stages of the cellular cycle.

Under normal conditions, p53 protein is continuously formed. It binds to the MDM2 protein and forms MDM2/p53 complexes in the nucleus and then this complex exported to the cytoplasm followed by degradation by proteosomes. The cell concentration of p53 maintained low through this process. Thus, the increase in p53 protein concentration does not depend on gene activation, transcription, and translation, but rather on inhibition of its degradation. Under stress, certain types of protein are released from the nucleus to the nucleoplasm, where it binds to MDM2 or to the MDM/p53 complex, blocking p53 export to the cytoplasm and later preventing its degradation, which results in the accumulation of the p53 protein in the nucleus.[7]

Mutations in the p53 gene yield a p53 protein which has an increased half-life, thus allowing this mutated protein to be detected immunohistochemically.[8,9]

Over expression of p53 protein in the lining epithelium of OKCs compared to DCs and RCs has been documented in a few articles.[10-13]

In this study, p53 protein expression will be noted in OKCs, DCs, and RCs to know the different behavior of OKCs than other cysts of oral cavity.

MATERIALS AND METHODS

An immunohistochemical study was done on total 35 histopathologically diagnosed cases (15 cases of OKC, 10 cases of DC, and 10 cases of RC) that were formalin fixed (10% formalin for 24–48 h), processed and paraffin embedded. These cases were retrieved from the Department of Oral Pathology and Microbiology by random sampling. Ethical clearance was taken from ethical committee.

Inclusion Criteria

The following criteria were included in the study:

1. Non-inflammatory OKCs and DCs cases
2. Only primary cases are included in the study.

Exclusion Criteria

The following criteria were excluded from the study:

1. Recurrent cases and syndromic cases
2. Patients with multiple OKCs
3. OKCs and DCs with neoplastic changes are excluded from the study
4. Cases of orthokeratinized odontogenic cyst were also excluded from the study.

Immunohistochemical Staining with p53 Protein Using Universal Immunohistochemistry Staining Kit (Biogenex)

Sections of 4 μm thickness were taken on Poly-L-Lysine adhesive coated glass slides and kept in universal hot air oven at 60°C for 60 min for proper attachment of tissue to the slides. Sections were treated with three changes of xylene 5 min each, rehydrated with descending grades of alcohol (100%, 80%, and 50%) 3–5 min each, washed twice with Tris-buffer solution 2 min, kept in 0.3% peroxidase solution for 7–10 min to block the endogenous peroxidase activity, washed again with Tris-buffer solution for 2–3 min.

Antigen retrival (using EZ Antigen Retrival Solution 2) was done in pressure cooker at 95°C temperature for 60 min. Slides were arranged on racks of immunostain humidity chamber, washed with Tris-buffer 2 times, and rounded the pap pen in surrounding tissue.

Protein block (Background sniper) was applied for 5 min to minimize the nonspecific binding of antibodies (blot only step, no washing), primary antibody p53 protein applied and incubated for 1 h and 30 min in immunostain humidity chamber. The slides were washed with Tris-buffer twice, power block mouse probe was applied for 15 min and washed again with Tris-buffer twice. SS Label Universal HRP Polymer was applied for 30 min, washed with Tris-buffer twice and diaminobenzidine tetrahydro chloride chromogen working solution was applied for 1–2 min at room temperature. Slides were washed with deionized water, counterstained with Harris hematoxylin for 1 min (5–7 dips), dehydrated by passing them through ascending grades of alcohol (50%, 80%, 100%), cleared in three changes of xylene 2 min each, and mounted in DPX using coverslips.

The intensity, pattern of distribution, and localization of the immune reactive cells were determined using trinocular light microscope with digital camera (Motic). Images were captured by digital camera attached with light microscope and analyzed using image analysis software (ij152-win-java8 imageJ). Examination was carried on trinocular light microscope (Motic) with provision for photomicrograph with SC 600, 6MP digital microscope camera. Five different areas of the epithelium were selected under low power magnification (×10) of light microscope. These representative areas should have sufficient number of cells. Positively stained cells were assessed per high power field (×40) of light microscope qualitatively by grading the immune reactivity in four grades scoring system. The intensity of p53 expression was scored varying from 0, 1, 2, 3. Here, 0 was being defined by negative and 1, 2, and 3 as being weak, moderate, and intense, respectively.

RESULTS

Table 1 represents mean age of the subjects. For OKC, the minimum age was 15 years and maximum was 47 years, for DC, the minimum age was 12 years and maximum was 45 years and for RC, the minimum age was 8 years and maximum was 45 years. The result was statistically non-significant for the values between the groups ($P > 0.05$).

Table 2 denotes distribution of different groups according to gender. In OKC, DC, and RC 66.7%, 90%, and 40% were male, respectively, whereas 33.3%, 10%, and 60% cases were female for OKC, DC, and RC, respectively.

Table 3 shows that in OKC maximum cases involved posterior mandible and DC, the most common site involved was anterior maxilla and posterior mandible. Most of the RC cases were found in the posterior mandible. There was a statistically non-significant difference seen for the frequencies between the groups ($P > 0.05$).

Table 4 shows comparison of qualitative analysis of p53 expression in basal layer of OKC, DC, and RC. Intensity of p53 expression in basal layer was weak in 11 cases of OKC, moderate expression in four cases of DC and weak to moderate expression in RC. There was a statistically non-significant difference seen for the values between the groups ($P > 0.05$).

Table 5 shows comparison of qualitative analysis of p53 expression in supra basal layer of OKC, DC and RC. The intensity of p53 expression in supra basal layer was intense in seven cases and moderate expression in five cases of OKC. In DC, the intensity was weak in five cases and weak to negative expression in RC. There was a statistically significant difference seen for the values between the groups ($P < 0.05$).

Table 6 shows comparison of qualitative analysis of p53 expression in superficial layer of OKC, DC, and RC. The intensity of p53 expression in superficial layer was weak to negative in OKC. In DC and RC, the intensity was negative. There was a statistically non-significant difference seen for the values between the groups ($P > 0.05$).

Table 7 shows qualitative analysis of p53 expression in different layers of OKC, DC, & RC. In OKC, the intensity was more for supra basal layers than basal and superficial layers [Figure 1]. In DC, the more intensity in basal layer than supra basal and superficial layers [Figure 2]. In RC, the more intensity in basal layer than supra basal and superficial layers [Figure 3]. The result was statistically highly significant for the values in each the groups ($P > 0.05$).

DISCUSSION

Odontogenic cyst is a cyst of jaws originated from odontogenic apparatus or its remnants. Being the second most common lesions in the oral and maxillofacial specimens, it has an important role in oral and maxillofacial pathology. There are three common odontogenic cysts including OKCs, DCs, and RCs.[1]

The p53 is one of the most common tumor suppressor genes located on the short arm of chromosome 17.[14,15] The disturbed function of p53 results in uncontrolled proliferation of the cell. Mutations of p53 are present in more than 50% of malignant tumors and are commonly related to the decreased differentiation of cells and early recurrence.[16]

In this study, comparison of p53 expression between OKC, DC, and RC was done to assess the aggressiveness of cysts.

It was found that in this study, the highest mean age in OKC 27 ± 9 years and lowest in RC 25.1 ± 14.8 years and in DC was 25.4 ± 10.3 years where as in Gaballah and Tawfik[17] study, the mean age was 37 ± 16.1 years, 31 ± 20.2 years, and 33 ± 15.8 years in OKC, DC, and RC cases, respectively. In this study, we take this age group because most common age of occurrence for cysts is this age.

The predominance of males in cases of the present study was reported in OKC and DC cases and female predominance was reported in RC cases where as the predominance of males in all the odontogenic cyst cases was reported in Ochsenius *et al.*,[18] Tortorici *et al.*[19] studies.

We saw that the most common site for OKC was posterior mandible, for DC anterior maxilla and posterior mandible

Table 1: Intergroup comparison of mean age

	n	Mean	SD	SE	95% CI for mean		Minimum	Maximum	*F*	*P*
					Lower bound	Upper bound				
OKC	15	27.73	9.043	2.335	22.73	32.74	15	47		
DC	10	25.40	10.352	3.273	17.99	32.81	12	45	0.208	0.813#
RC	10	25.10	14.881	4.706	14.46	35.74	8	45		

CI: Confidence interval, SD: Standard deviation, SE: Standard error, OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 2: Intergroup percentage distribution and comparison according to gender (male and female)

	Group			Total
	OKC	DC	RC	
Sex				
Female				
Count	5	1	6	12
Percentage within sex	41.7	8.3	50.0	100.0
Percentage within group	33.3	10.0	60.0	34.3
Male				
Count	10	9	4	23
Percentage within sex	43.5	39.1	17.4	100.0
Percentage within group	66.7	90.0	40.0	65.7
Total				
Count	15	10	10	35
Percentage within sex	42.9	28.6	28.6	100.0
Percentage within group	100.0	100.0	100.0	100.0

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 3: Distribution of different groups according to clinical site involved

Site	Group			Total
	OKC	DC	RC	
Ant. maxilla	2	4	3	9
Ant. mandible	1	1	0	2
Ant. and post-maxilla	2	1	1	4
Post-mandible	9	4	5	18
Post-maxilla	1	0	1	2
Total	15	10	10	35
Chi-square tests	**Value**	**df**		***P***
Pearson Chi-square	4.148[a]	8		0.844

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 4: Comparison of qualitative analysis of p53 expression in basal layer of OKC, DC, and RC

Intensity of p53 expression	Group			Total
	OKC	DC	RC	
0	2	1	0	3
1	11	3	5	19
2	2	4	5	11
3	0	2	0	2
Total	15	10	10	35
Chi-square tests	**Value**	**df**		***P***
Pearson Chi-square	11.295[a]	6		0.080

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

and for RC was posterior mandible. Similar finding was seen by Carvalhais *et al.*[20] in OKC and RC cases.

In the OKCs, the p53-positive ratios of cells in the lining epithelium revealed remarkably high values; about 77.33% in the basal layer showed weak positivity, 46% in the supra basal layers showed intense positivity, 53% in the superficial layers showed weak positivity. Although these results cannot be compared directly with previously reported

Table 5: Comparison of qualitative analysis of p53 expression in supra basal layer of OKC, DC, and RC

Intensity of p53 expression	Group			Total
	OKC	DC	RC	
0	1	3	2	6
1	2	5	8	15
2	5	1	0	6
3	7	1	0	8
Total	15	10	10	35
Chi-square tests	**Value**	**df**		***P***
Pearson Chi-square	19.396[a]	6		0.004

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 6: Comparison of qualitative analysis of p53 expression in superficial layer of OKC, DC, and RC

Intensity of p53 expression	Group			Total
	OKC	DC	RC	
0	8	9	8	25
1	7	1	2	10
Total	15	10	10	35
Chi-square tests	**Value**	**df**		***P***
Pearson Chi-square	4.457[a]	2		0.108

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 7: Qualitative analysis of p53 expression in different layers of OKC, DC, and RC

Layers	*n*	Mean	SD	SE	*F*	*P*
OKC						
Basal layer	15	1.00	0.535	0.138	24.662	0.000**
Suprabasal layer	15	2.20	0.941	0.243		
Superficial layer	15	0.47	0.516	0.133		
DC						
Basal layer	10	1.70	0.949	0.300	10.218	0.000**
Suprabasal layer	10	1.00	0.943	0.298		
Superficial layer	10	0.10	0.316	0.100		
RC						
Basal layer	10	1.50	0.527	0.167	20.053	0.000**
Suprabasal layer	10	0.80	0.422	0.133		
Superficial layer	10	0.20	0.422	0.133		

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

data because the methods used were different as used by Ogden *et al.*,[11] Muzio *et al.*[21] and Kimi *et al.*,[22] they revealed that in OKCs, the p53-positive ratios of cells in the lining epithelium revealed remarkably high values; about 66% in the basal, 72% in the intermediate, and 45% in the surface. We found that the highest p53 positive ratio was seen in the supra basal layer was in accord with previous reports by Ogden *et al.*[11] and Kimi *et al.*[22] This was seen because the cells constituting the intermediate or supra basal layers possess the highest proliferative activity in the OKCs. Low p53 expression in surface layers is because of apoptosis in surface layers.

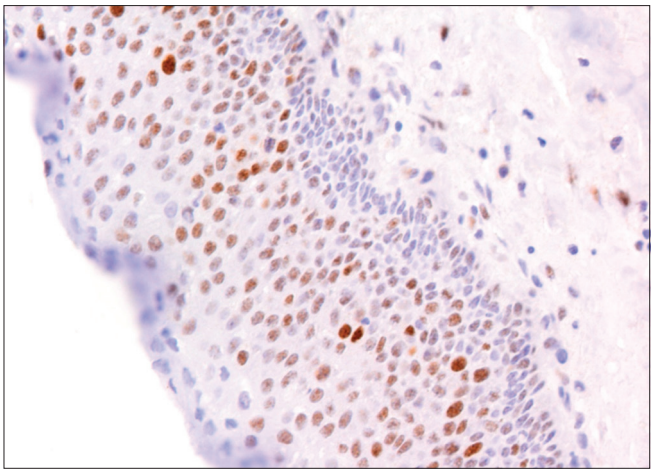

Figure 1: p53 protein stained section of odontogenic keratocyst (×40)

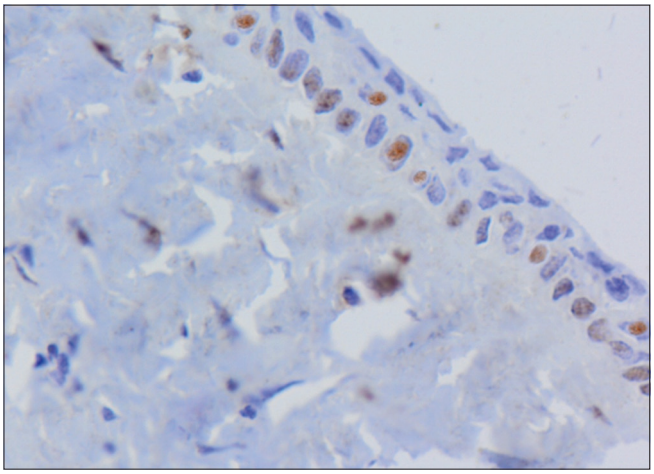

Figure 2: p53protein stained section of dentigerous cyst (×40)

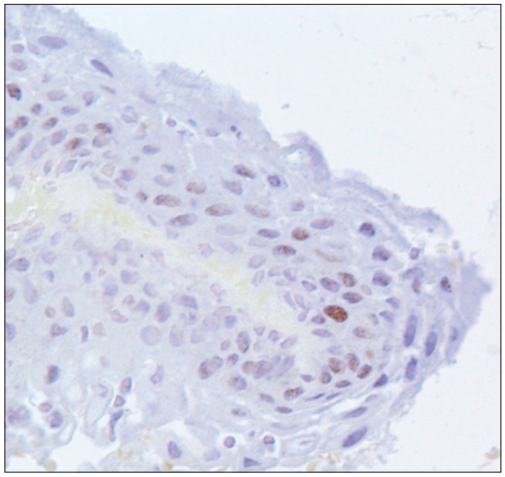

Figure 3: p53protein stained section of radicular cyst (×40)

In the DCs, the p53-positive ratios of cells in the lining epithelium revealed remarkably high values; about 40% in the basal layer showed moderate positivity, 50% in the supra basal layers showed weak positivity, 20% in the superficial layers showed weak positivity. The results found out by Kichi *et al.*[23] (2005), Piattelli *et al.*[13] (2001), and Li *et al.*[12] (1996) were in accordance with our result. It was seen because of high mitotic activity in basal layer.

In the RCs, the p53-positive ratios of cells in the lining epithelium revealed remarkably high values; about 50% in the basal layer showed moderate positivity, 80% in the supra basal layers showed weak positivity, 20% in the superficial layers showed weak positivity. The results found out by Kichi *et al.*[23] (2005), Piattelli *et al.*[13] (2001), and Li *et al.*[12] (1996) were in accordance with our result. It was seen because of high mitotic activity in basal layer.

There was a statistically significant difference between in each layer of three types of cyst ($P < 0.001$) [Table 7].

In our study, we found high expression of p53 in OKC compared to DC and RC because the greater proliferation activities of the epithelial lining in OKC. A p53 gene mutation may be one of the causes of cell proliferation.

CONCLUSION

Our results show a statistically higher occurrence of p53 in OKCs, compared with DCs and RCs. Thus, it can be stipulated that p53 protein expression can be used as a prognostic marker in odontogenic cyst suggest that p53 overexpression may be involved in the pathogenesis of some odontogenic cysts and tumors.

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