Classifying odontogenic keratocysts as benign cystic neoplasms: a molecular insight into its aggressiveness

Devi Charan Shetty¹✉ · Aadithya B Urs² · Suhas Godhi³ · Sandeep Gupta⁴

¹Professor and HOD

²Professor

Dept. of Oral and Maxillofacial Pathology and Microbiology, I.T.S.-CDSR, Ghaziabad

⁴ Dept. of Oral and Maxillofacial Pathology

³ Professor, Dept. of Oral and Maxillofacial Surgery

I.T.S.-CDSR, Ghaziabad

Received: 20 December 2009 / Accepted: 19 February 2010
© Association of Oral and Maxillofacial Surgeons of India 2009

Abstract

Objective The present study was conducted with the aim to evaluate the expression of p53 in Odontogenic Keratocyst (OKC) and Ameloblastoma to correlate with the aggressiveness of these lesions.

Material and methods Immunohistochemical (IHC) staining was performed using anti-p53 antibody in eighteen cases each of OKC and ameloblastoma.

Results p53 positivity was seen in all the cases of OKCs and ameloblastomas. p53 positive cells were seen predominantly in the suprabasal cell layer of OKC and in the peripheral pre-ameloblast like cells in ameloblastoma. Total p53 count was significantly higher in ameloblastoma as compared to OKC. But the intensely stained p53 cell count showed no statistically significant difference between the two lesions.

Conclusion The high p53 expression in OKC when compared with ameloblastoma, acknowledges OKC as an aggressive lesion and hence a more aggressive treatment modality is recommended.

Keywords Odontogenic keratocyst · Ameloblastoma · p53 · Intensity · Aggressiveness

Address for correspondence:

Devi Charan Shetty

I.T.S Center for Dental Studies & Research
Delhi-Meerut Road, Murad Nagar
Ghaziabad, Uttar Pradesh, India
Ph: +919873525076
Email: shettydivya76@gmail.com

Introduction

There has been a great amount of interest in the Odontogenic Keratocyst (OKC) since it became apparent that they may grow to a large size before manifesting clinically, and also because of their tendency to recur following surgical treatment [1]. The term 'Odontogenic Keratocyst' was introduced by Pindborg (1956) to designate any jaw cyst in which keratin was formed to a large extent [2]. Although the aggressive potential of ameloblastomas has been documented, the aggressive nature of OKC with its biologic potential needs to be researched.

The intrinsic growth potential of OKC epithelium can be clearly appreciated by the infolding of the epithelial lining into the capsule which suggests that this is a result of active epithelial proliferation [3]. The reported literature reviews behavior of some OKCs in every way as a benign neoplasm, similar to that of ameloblastoma.

Main, in his study showed that the mitotic value of keratocyst ranged from 0 to 19, with a mean of 8.0 [1]. WHO classification of head and neck pathology 2005 have classified OKC as Keratocystic Odontogenic Tumour and placed it in the subgroup of tumours consisting of odontogenic epithelium with or without odontogenic ectomesenchyme along with ameloblastoma [4].

Mutations and alterations in tumour suppressor pathways of p53 gene have been implicated in almost all human cancers [5]. Normal p53 exerts antiproliferative effect on the cell showing reversible DNA damage, and apoptosis in cells with irreversible cell damage [6]. The mutant p53 protein lack these activities and has a much longer half-life, resulting in large amount of mutated proteins in transformed cells, thus detected by IHC.

The present study was carried out with the aim of quantifying the expression of p53 in OKC and ameloblastoma. Also the

intensity of p53 expression between them was correlated to highlight the aggressiveness of the respective lesions.

Materials and methods

The present study was designed to evaluate the expression of p53 in odontogenic keratocyst and ameloblastoma, using immunohistochemical technique in paraffin embedded tissues. The study was conducted in the Department of Oral and Maxillofacial Pathology and Microbiology, ITS Center for Dental Studies and Research, Murad Nagar, Ghaziabad, Uttar Pradesh.

Tissue specimens were retrieved from the archives, of 50 histopathologically confirmed cases. The selected specimens consisted of 25 Odontogenic keratocyst and 25 ameloblastomas. Tissue specimens were fixed with 10% neutral buffered formalin. A standard IHC method involving

fixation in 10% neutral buffered formalin, followed by tissue processing, sectioning of tissue in 4 micron thick sections, dewaxing, antigen retrieval followed by staining with primary, secondary antibodies and counterstaining with haematoxylin was done.

Immunohistochemistry procedure was carried out using anti-p53 mouse monoclonal primary antibody; Clone: D07; Isotype: Ig_{2a} (Biogenex Ind. Pvt. Ltd) along with Poly HRP secondary antibody detection system (Biogenex Ind. Pvt. Ltd). Breast carcinoma was taken as positive control for the expression of p53. Negative control was obtained by omitting the use of primary antibody and carrying out the subsequent steps of immunohistochemistry.

All the slides were viewed under the light microscope, and cells which took up the brown stain were taken as p53 positive. A set of three examiners evaluated the slides for the intensity of p53 staining. Pre-standardized p53 stained breast carcinoma slides were used as controls. In OKC, a total of 100 from the basal cells and suprabasal cells were counted. Likewise, in Ameloblastoma 100 cells comprising of preameloblast like cell and stellate reticulum like cells were counted.

The total number of p53 positive cells as well as intensity of the immunohistochemical staining graded on a two point scale: (+) mild staining and (++) dense staining was evaluated. A standardized protocol for evaluating the expression was followed, and care was taken to minimize intra and inter observer variability. The absence of expression in those cases can be attributed to technical errors such as prolonged fixation etc.

Out of 25 cases of ameloblastoma, 2 cases lacked representative areas, 3 cases did not show p53 expression, and 2 cases showed only focal p53 expression. Hence these were excluded from the study. Similarly, 5 cases of OKC showed focal p53 expression and 2 cases did not show the representative area.

Results

The mean p53 count in basal and suprabasal cells in OKC was 21.33 ± 8.30 and 27.56 ± 8.35 respectively (Table 1 and Fig. 1). p53 expression was significantly higher in suprabasal cells than the basal layer ($p < 0.05$). In cases of ameloblastoma, the mean p53 count expression in preameloblast like cells and stellate reticulum like cells were 29.06 ± 4.62 and

Fig. 1 p53 immunostaining in OKC (X10)

Fig. 2 p53 immunostaining in ameloblastoma (X10)

Fig. 3 Densely stained p53 positive cells in OKC (X40)

Fig. 4 Densely stained p53 positive cells in ameloblastoma (X40)

Graph 1 Quantitative assessment of total p53 expression in ameloblastoma and OKC

Graph 2 Qualitative assessment of total dense p53 expression in ameloblastoma and OKC

Table 1 Quantitative assessment of p53 expression in OKC

Layer	Mean	Standard deviation	Mean rank	Sum of ranks	Mann-Whitney U	Asymp. sig (2-tailed)
Total	48.89	15.095	14.36	258.50	90.500	p= 0.023
Basal	21.33	8.30	14.53	261.50		
Suprabasal	27.56	8.35	22.47	404.50		

Table 2 Quantitative assessment of p53 expression in ameloblastoma

Layer	Mean	Standard deviation	Std. error mean	T	p value of sig. (2-tailed)	Mean difference
Total	57.28	7.828	22.64	0.512	0.612	0.833
Preameloblast like cells	29.06	4.62	1.089			
Stellate reticulum like cells	28.22	5.14	1.211			

Table 3 Total mild p53 expression in ameloblastoma and OKC

Lesion	Mean	Standard deviation	Mean rank	Sum of ranks	Mann-Whitney U	Asymp. sig (2-tailed)
Ameloblastoma	32.44	4.841	25.17	453.00	42.000	0.000
OKC	24.11	5.540	11.83	213.00		

Table 4 Total dense p53 expression in ameloblastoma and OKC

Lesion	Mean	Standard deviation	Mean rank	Sum of ranks	Mann-Whitney U	Asymp. sig (2-tailed)
Ameloblastoma	24.83	4.878	19.75	355.50	139.500	p=0.476
OKC	24.78	11.123	17.25	310.50		

28.22 + 5.14 respectively (Table 2 and Fig. 2). Difference between the means was found to be insignificant using independent T test ($p > 0.05$).

Total mean p53 expression in OKC was $48 + 15.095$ which was found to be significantly lower than that of ameloblastoma i.e. $57.28 + 7.828$ ($p < 0.05$) (Graph 1).

With respect to qualitative assessment, the total mild p53 expression in OKC was $24.11 + 5.540$ that was found to be significantly lower than that of ameloblastoma i.e. $32.44 + 4.841$ ($P < 0.01$) (Table 3). The total dense p53 expression in OKC (Fig. 3) and ameloblastoma (Fig. 4) was $24.78 + 11.123$ and $24.83 + 4.878$. No significant difference could be found using nonparametric test ($p > 0.05$). Thus dense p53 expression in both OKC and ameloblastoma were similar (Table 4 and Graph 2).

Discussion

The ameloblastoma is probably one of the most conventional neoplasms of the facial skeleton. In 1868, Broca first described this tumour and in 1934, Churchill coined the

term 'Ameloblastoma' [7]. As defined by Robinson 'Ameloblastoma is unicentric, non-functional, intermittent in growth, anatomically benign and clinically persistent' [8]. Since then, ameloblastoma is known as a tumour with complex biological behavior and presents a high local recurrence rate even in patients treated with radical therapy. The local aggressiveness of this tumour is justified by the higher proliferative activity proven by the positive Ki67 and PCNA staining [9–11].

First described by Philipsen in 1956 [12], the odontogenic keratocyst is now designated by World Health Organization (WHO) as 'Keratocystic Odontogenic Tumour' (KCOT) and is defined as a benign unicystic or multicystic, intraosseous tumour of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and potential for aggressive, infiltrative behavior [13] WHO recommends the term Keratocystic Odontogenic Tumour as it reflects its neoplastic nature.

The propensity for OKC to recur ranges from 3 to 60%. OKC may penetrate cortical bone and involve the surrounding soft tissue. Cases have been reported where

OKC extended from the mandible and eventually involved the base of skull, behaving rather like a low grade squamous cell carcinoma [3].

The clinically aggressive behavior of OKC is a result of the properties of the lining epithelial cells and the connective tissue capsule. Raised osmolality of cystic fluid also plays an important role in expansile growth [15], while an alternative view to this is that, the mural proliferations contributes to the enlargement of these lesions. This latter view is supported by authors who believed that the multilocular and loculated outline exhibited by OKC suggested a multicentric pattern of cyst growth brought about by the proliferation of local group of epithelial cells against a semisolid cystic content [3].

The aggressive behavior of OKC was further attributed to the infolding of the epithelial lining into the capsule which suggested that this was the result of the active epithelial proliferation [3]. Few authors have reported that the behavior of some OKCs is in every way as aggressive as a benign neoplasm such as ameloblastoma. Toller P [16] cited that the inherent growth potential of the epithelial lining was found to have a labeling index 7 times more than that of

apical cyst. These findings are confirmed by Partridge M [17] who showed that mitotic index of OKC epithelium is similar to that of ameloblastoma [8].

The fundamentals of aggressive behavior of a neoplasm are its proliferative activity and alteration in cell cycle tumour suppressor genes. In the present study, the role of tumour suppressor gene p53 has been used to corroborate its levels of expression and intensity in OKC and ameloblastoma to establish the hypothetical assumption as prevalent in the current literature that changes in OKC were reminiscent of ameloblastoma.

The proliferative activity of the OKC lining have been studied using various proliferative markers like PCNA, Ki67, IPO, which are also expressed in normal physiologically proliferating cell, and p53 which is detected only in neoplastic but not in normal odontogenic epithelium, because of very short half life of wild type p53 [18].

The mutated p53 that does not bind to DNA produces a defective protein which blocks the activity of normal p53 protein (wild type). With homozygous loss of p53, DNA damage goes unrepaired [19].

p53 mutation is a fundamental mechanism involved in disordered growth control, and not a secondary consequence of neoplastic phenotype. In addition to abolishing the tumour suppressor activity of the protein, there is also stabilization of the protein, resulting in its accumulation to levels detectable by immunohistochemistry [20].

In the present study comparative immunohistochemical analysis of p53 expression in OKC and ameloblastoma was carried out with respect to total number of cells showing the expression and the difference in intensity of staining between the two groups.

Suprabasal cells of OKC showed higher p53 expression than the basal cells. This observation was similar to studies of Piattelli et al. [21] and Oliveira et al. [22] in which p53 expression studied in radicular, dentigerous cyst and OKC showed increased expression of p53 predominantly in suprabasal cells of OKC [3,7]. According to Browne (1996) the epithelial lining of odontogenic keratocysts contains more cells in the cell cycle predominantly in suprabasal cells, than that in other types of odontogenic cysts which is reflected by increased number of mitotic figures in suprabasal cell layer [23].

Difference between the mean p53 expression in preameloblast like cells and

stellate reticulum like cells was found to be insignificant using independent 't' test. This result is contradictory to the studies carried out by Barboza et al. and El Sissy, in which they found p53 to be predominantly expressed in preameloblast like cells and occasionally in the stellate reticulum like cells [11].

In qualitative assessment of p53 expression, the total mean mild p53 expression in OKC was 24.11 ± 5.540 . This was found to be significantly lower than that of ameloblastoma which showed 32.44 ± 4.841 . The difference was highly significant ($p=0.00$). As studied by Kumamoto, there is increased number of apoptotic cells in ameloblastoma, thus reflecting cells with normally acting p53 gene probably taking mild p53 staining as the wild p53 is not stable to be accumulated to a concentration to provide intense p53 staining as they undergo destruction by p53-MDM2 pathway [24].

The total intense p53 expression in OKC and ameloblastoma was 24.78 ± 11.123 and 24.83 ± 4.878 respectively. No significant difference could be found using nonparametric Mann-Whitney U test ($p>0.05$). According to Slootweg, intense p53 expression correlates with higher proliferative activity of the cells [25]. It also explains the molecular basis of the proliferative advantage gained by the cell due to p53 dysfunction, p53 mutation and accumulation of this stable mutated p53 protein leading to intense p53 expression immunohistochemically.

Slootweg also proposed that there is a relationship between intensity of staining and the lesions that were characterized clinically by proliferation i.e. ameloblastoma, odontogenic carcinoma and OKC. He postulated that it was the overexpression of p53 rather than the increased number of p53 positive cells that were related to the proliferative capacity of OKC [25].

Conclusion

The present study, showed an intense p53 expression in OKC when compared with ameloblastoma, which lends support to the new nomenclature for this lesion as Keratocystic odontogenic tumour; warranting the need for more aggressive treatment modality such as enucleation with application of carnoy's solution [26].

Although the literature contains many reports regarding management of OKC, debate still exists as to the most effective

treatment for this lesion. Further studies need to be done where p53 expression would be correlated with clinical parameters such as size, extent, location, perforation of cortical plates and recurrence in order to formulate a more appropriate treatment plan.

References

1. Shear M, Speight PM (2007) Cysts of the oral and maxillofacial regions. 4th edition. Singapore: Blackwell Munksgaard 6–58
2. Payne TF (1972) An analysis of the clinical and histopathologic parameters of the odontogenic keratocyst. *Oral Surg Oral Med Oral Pathol* 33(4): 538–546
3. Shear M (2002) The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 1. Clinical and early experimental evidence of aggressive behaviour. *Oral Oncol* 38(3): 219–226
4. Branes L, Everson JW, Reichart P, Sidransky D (2005) Pathology and genetics of head and neck tumours. Lyon: IARC Press, WHO classification of tumours series.
5. Jayade BV, Bhat K, Patil BR, Nayak R, Sant A (2009) Histological significance of p53 gene expression in squamous cell carcinoma of buccal mucosa. *J Maxillofac Oral Surg* 8(3): 205–210
6. Robbins (2006) Pathological basis of diseases. 7th edition. New Delhi: Thomson press. 288–318
7. Reichart PA, Philipsen HP, Sonner S (1995) Ameloblastoma: Biological profile of 3677 cases. *Eur J Cancer B Oral Oncol* 31B(2): 86–99
8. Shafer, Hine, Levy (2001) Sahafer's text book of oral pathology. 4th edition. Noida: Elsevier Publication. Gopsons paper Ltd. 258–285
9. Piattelli A, Fioroni M, Santinelli A, Rubini C (1998) Expression of proliferating cell nuclear antigen in ameloblastoma and odontogenic cysts. *Oral Oncol* 34(5): 408–412
10. Jaaskelainen K, Jee KJ, Leivo I, Saloniemi I, Knuutila S, Heikinheimo K (2002) Cell proliferation and chromosomal changes in human ameloblastoma. *Cancer Genet Cytogenet* 136(1): 31–37
11. Barboza CA, Pereira Pinto L, Freitas Rde A, Costa Ade L, Souza LB (2005) Proliferating cell nuclear antigen (PCNA) and p53 protein expression in ameloblastoma and adenomatoid

- odontogenic tumour. *Braz Dent J* 16(1): 56–61
12. Brannon RB (1977) The odontogenic keratocyst. A clinicopathologic study of 312 cases. Part II. Histologic features. *Oral Surg Oral Med Oral Pathol* 43(2): 233–255
 13. Madras J, Lapointe H (2008) Keratocystic odontogenic tumour: reclassification of the odontogenic keratocyst from cyst to tumour. *J Can Dent Assoc* 74(2): 165–165h
 14. Toller PA (1970) The osmolality of fluids from cysts of the jaws. *Br Dent J* 129(6): 275–278
 15. Toller PA (1970) Protein substances in odontogenic cyst fluids. *Br Dent J* 128(7): 317–322
 16. Toller P (1967) Origin and growth of cysts of the jaws. *Ann R Coll Surg Engl* 40(5): 306–336
 17. Partridge M, Towers JF (1987) The primordial cyst (odontogenic keratocyst): Its tumour like characteristic and behavior. *Br J Oral Maxillofac Surg* 25(4): 271–279
 18. Shear M (2002) the aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 2. Proliferation and genetic studies. *Oral Oncol* 38(4): 323–331
 19. Hollstein M, Sidransky D, Vogelstein B, Harris CC (1991) p53 mutations in human cancers. *Science* 253(5015): 49–53
 20. Li TJ, Browne RM, Prime SS, Paterson IC, Matthews JB (1996) p53 expression in odontogenic keratocyst epithelium. *J Oral Pathol Med* 25(5): 249–255
 21. Piattelli A, Fioroni M, Santinelli A, Rubini C (2001) p53 protein expression in odontogenic cysts. *J Endod* 27(7): 459–461
 22. Oliveira MG, Lauxen IS, Chaves AC, Rados PV, Filho SA (2008) Immunohistochemical analysis of the patterns of p53 and PCNA expression in odontogenic cystic lesions. *Med Oral Patol Oral Cir Bucal* 13(5): E275–280
 23. Browne RM (1996) Per[cyst]ent growth: the odontogenic keratocyst 40 years on. *Ann R Coll Surg Engl* 78(5): 426–433
 24. Kumamoto H (1997) Detection of apoptosis related factors and apoptotic cells in ameloblastoma: analysis by immunohistochemistry and an in situ DNA nick end-labeling method. *J Oral Pathol Med* 26(9): 419–425
 25. Slootweg PJ (1995) p53 protein and Ki 67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. *J Oral Pathol Med* 24(9): 393–397
 26. Godhi SS, Kukreja P (2009) Keratocystic odontogenic tumour: a review. *J Maxillofac Oral Surg* 8(2): 127–131

Source of Support: Nil, **Conflict of interest:** None declared.