Original Article

Analysis of expression of p53, p63 and proliferating cell nuclear antigen proteins in odontogenic keratocyst: An immunohistochemical study

Vidya Kadashetti[1], Nanda Patil[2], Kailas Datkhile[3], Sujata Kanetakar[2], K M Shivakumar[4]

Departments of [1]Oral Pathology and Microbiology, Forensic Odontology and [4]Public Health Dentistry, School of Dental Sciences, Krishna Institute of Medical Sciences Deemed University, [2]Department of Pathology, Krishna Institute of Medical Sciences, Krishna Institute of Medical Sciences Deemed to be University, [3]Department of Molecular Biology and Genetics, Krishna Institute of Medical Sciences Deemed University, Malkapur, Maharashtra, India

Abstract

Background: Odontogenic keratocyst (OKC) is a benign intraosseous lesions (within the jaw bone) of odontogenic origin that account for about 10% of jaw cysts. They are characterized by an aggressive behavior with a relatively high recurrence rate. Early diagnosis and follow-up of the patient with OKC is important because the possibility of such patient there is develop to other features of Nevoid basal cell carcinoma syndrome in future. Considering the roles and effects of p53, p63 and proliferating cell nuclear antigen (PCNA) in cells proliferation, this study was designed.

Objectives: To understand the behavior of epithelial cells in pathogenesis and biological aspects of OKC in diagnosis.

Materials and Methods: Immunohistochemical (IHC)technique was performed in 21 cases of OKCs.

Results: Immunological stained p53 cells were mainly located in the suprabasal layers. p63 and PCNA-positive cells were found throughout the lining epithelium including basal and suprabasal cell layers. The intensity of staining was more in p63 and PCNA than the p53 expression of the cystic epithelial lining.

Conclusions: It is possible that the biological behavior of OKCs may be related to the suprabasal proliferative compartment in the cystic epithelium as observed. These proteins may participate in the regulation of epithelial cell differentiation. Taken together, these data may favor tumerigenesis on OKCs.

Keywords: Immunohistochemistry, p53, p63, proliferating cell nuclear antigen

Address for correspondence: Dr. Vidya Kadashetti, Department of Oral Pathology and Microbiology, Forensic Odontology, School of Dental Sciences, Krishna Institute of Medical Sciences Deemed University, Malkapur, Karad - 415 110, Satara, Maharashtra, India.
E-mail: kadashettiv@gmail.com

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INTRODUCTION

The common pathology from developing tooth apparatus is an odontogenic lesion that may be cyst or tumor. These are common lesions of odontogenic origin in head-and-neck and accounts to form a major part of total biopsies received by any pathology services. These are diverse group of lesions commonly associated with younger age group (developing age) with second and third decades of life, exhibit with different behavior ranging

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from a small unnoticed lesion, which may be detected accidentally or may present as a highly aggressive and locally destructive lesion of the jaw bone that may even transform into a malignancy. Among these odontogenic lesions most notorious are odontogenic keratocyst (OKC).[1,2] It may be associated with the carious tooth, impacted tooth or tooth-bearing areas shows multiple lesions, destruction, recurrence and may convert in to malignancy associated with syndrome.

OKC was first described by Philipsen in 1956. It is the cyst arising from the cell rests of dental lamina during the development of tooth dental lamina disintegrates into small clusters of epithelium and is resorbed. In situations when the clusters are not resorbed, these remnant of the dental lamina is sometimes known as the glands of Serres, eruption cysts are formed over the developing tooth and delay its eruption into the oral cavity.[2,3] OKC is benign intraosseous lesions (within the jaw bone) of odontogenic origin that account for about 10% of jaw cysts. They are characterized by commonly occurs in second and third decades of age group with an aggressive behavior with a relatively high recurrence rate. The clinical feature and radiographic appearance of OKCs are not characteristic. That may lead to misdiagnosis, especially when the lesion is in relation to a nonvital tooth.[3,4] OKC tends to grow in an anteroposterior direction within the medullary cavity of the bone initially without causing obvious bone expansion. The radiographic appearance of OKC may range from a small unilocular radiolucency to a large multilocular radiolucency. Hence, it may resemble ameloblastoma, dentigerous cyst, lateral periodontal cyst and radicular cyst (RC).[4,5] Sometimes, multiple OKCs are also present in those cases, Nevoid basal cell carcinoma syndrome (NBCCS) should be ruled out.[6] Histologically, OKCs arise from the dental lamina and are constituted by a cystic cavity containing desquamated keratin, the cyst is lined with a uniform parakeratinized or orthokeratinized squamous epithelium of 6–10 cell layer thick, with a distinct tall columnar basal cell layer of palisaded arrangement with hyperchromatic nuclei, whose nuclei tend to be vertically oriented. The infiltrative growth with the adjacent connective tissue is normally flat with a potential for budding of the basal layer and the formation of small satellite or daughter cysts. The mitotic activity in OKC is higher than other cysts of odontogenic origin.[7-9] Pindborg and Hansen suggested the histological criteria necessary to diagnose OKC. In recent years, the World health organization recommended the term cystic neoplasm (also known as keratocystic odontogenic tumor) for this lesion, as it better reflects neoplastic characters such as aggressive clinical behavior, histologically high mitotic rate and association with genetic and chromosomal abnormalities and syndromic character one which is associated with multiple OKCs. The OKC is an enigmatic developmental cyst that deserves special attention. OKC exhibits putative high growth potential and high recurrence rate due to its nature of forming compartments within. In recent classification, it is included as OKC. The growth mechanism of OKCs has been investigated by different researchers, especially the proliferative potential of the epithelial lining including proteins involved in the cell cycle.[10-14]

P53 protein is a product of tumor protein p53 (Tp53), a tumor suppressor gene that is expressed in the G1 phase of the cell cycle. It helps repairs of DNA damage cells and prevents cells to enter synthesis phase of the damaged cell. If DNA is repaired, cell cycle arrest is ended. However, if DNA is not successfully repaired, p53induces apoptosis and leads the cell to die. In the absence or mutations of p53, DNA damage cannot be repaired and it leads to the proliferation of damaged cells and the proliferation of damaged cells or it may transform into malignancy.[14,15]

P63 TP63 (p63) is a homolog of TP53 gene and is located at the 3q27–29 locus expression of p63 protein is discovered in the skin, esophagus, oral mucosa and odontogenic epithelium of tooth germ as well as dental follicle of impacted teeth.[16]

Proliferating cell nuclear antigen (PCNA), is a nuclear protein essential for nucleic acid metabolism in DNA transcription and repair. It is expressed in high amount in growing cells during the cell cycle. The expression of PCNA can be used as a cell proliferative marker, because proliferating cells remain for a longer time in G1/S phase. Increased expression of PCNA may be stimulated by growth factors or as a result of DNA injury in the absence of cell cycle.[17] Early diagnosis and follow-up of the patient with OKC is important because the possibility of such patient there is a develop to other features of NBCCS in future. Considering the roles and effects of p53, p63 and PCNA in cells proliferation. This study was aimed to understand the behavior of epithelial cells in the pathogenesis and biological aspects of OKC would improve the success in theprevention of cyst proliferation or to prevent recurrence and better treatment procedures.

MATERIALS AND METHODS

After Institutional Ethics Committee approval paraffin-embedded tissue blocks of 21 cases of OKC cases were selected from the archive of oral pathology and Microbiology Department, School of Dental Sciences,

Krishna Institute of Medical Sciences Deemed to be University, Karad, Satara, Maharashtra, India. The samples had an adequate epithelial component to evaluate the quantity of stained cells selected on the basis of Hematoxylin and eosin-stained sections. Clinical data including lesion site, age and sex of patients were obtained from patients registered documents. Cases with uncertain diagnosis, severe inflammation, and small size lesions were excluded.

Immunohistochemistry

After reviewing and confirming the proposed diagnosis of OKC, The 4-μm thick sections of formalin-fixed and paraffin-embedded specimens were taken deparaffinized in Xylene, then rehydrated by using 100% Isopropanol and finally washed with distilled water. Most formalin-fixed tissue requires an antigen retrieval step before immunohistochemical staining can proceed. This is due to the formation of methylene bridges during fixation, which cross-link proteins and therefore mask antigenic sites. The present study was followed the heat involved epitope retrieval by using thepressure cooker method. Followed by Antigen-antibody reaction was detected by mouse monoclonal anti-p53, p63 and PCNA antibody for 1 h at room temperature. Then, diaminobenzidine was used as chromogen. Sections were counterstained with Harris' Hematoxylin, washed with tap water and covered by glass coverslips. The positive control was oral squamous cell carcinoma sections were used for all three antibodies. For analyzing the p53, p63 and PCNA expressions, mean percentage of positive cells, i.e., nuclear staining determined by 100 cells in 10 fields of ×40. The intensity of all three markers staining was also evaluated in three Groups: I (mild, light-brown), II (moderate), III (severe, dark-brown). The percentage of positive cells was calculated (in high power field) from a minimum of 1000 epithelial cells, each containing over 100 cyst-lining cells, were observed under high-power field (40 objective and 10 oculars) from the most evenly and heavily labeled areas. All positive cells were counted regardless of the intensity of staining. The percentage of p53, p63 and PCNA positivity stained cells was calculated. Statistical significance was assessed by the Student's *t*-test and a $P < 0{:}05$ was considered statistically significant.

RESULTS

Table 1 showed the mean percentages of among positive p53, p63 and proliferating cell nuclear antigen cells in basal and suprabasal layers in odontogenic keratocyst. The Comparision showed a significance difference $P<0.01$, S) between basal and Suprabasal cells in Odontogenic Keratocyst.

Table 2 showed the Intensity of p53, p63 and proliferating cell nuclear antigen expression in study groups. Mild intensity was noticed in p53 when compared to p63 and PCNA group. The moderate intensity was noticed in PCNA when compared to P53 and p63 followed by Severity intensity in p63 when compared to p53 and PCNA.

Figure 1; showed the basal and suprabasal cells using H & E Stain. Figure 2; showed the basal and suprabasal cells using p53 stain. Figure 3; showed the basal and suprabasal cells using p63 stain. Figure 4; showed the basal and suprabasal cells using Proliferating Cell Nuclear Antigen Stain. Figure 5; showed the basal and suprabasal cells with p63 stain using ×10 magnification.

DISCUSSION

P53 is a tumor suppressor gene which participates in the growth arrest, initiates repair or induces apoptosis. The expression of this protein in the cystic epithelium of OKC has not been defined, but the aggressive behavior and high recurrence rate of these lesions might be related to the immunoexpression of this protein. In the present study, 16 (76.19%) cases were positive for p53 [Figure 2] and identified in the suprabasal layers of the cystic epithelium. p63 isoforms contain the transcriptional activation domain and is involved in apoptosis and cell proliferation. p63 may act as an oncogene.[18] An increase in PCNA levels may be induced by growth factors or as a result of DNA damage in the absence of cell cycling.[17] All the cases of OKC were positive for p63 and PCNA both proteins were expressed in the basal and suprabasal layer of the cystic epithelium [Figures 3-5]. In the analysis of p53 expression in the suprabasal cell layer, standard deviation values were high [Table 1], which revealed greater dispersion around the mean. These results concordance with studies conducted

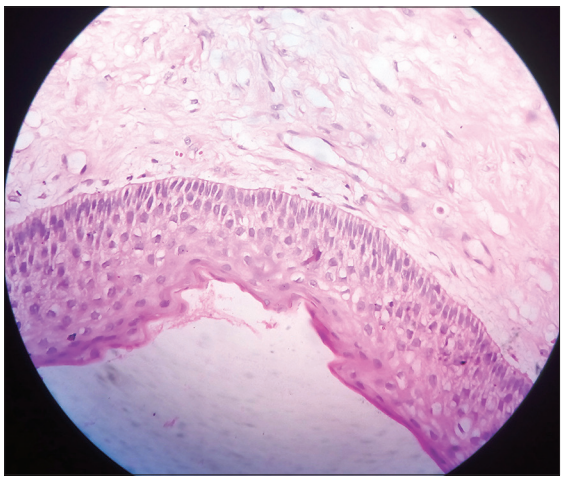

Figure 1: H&E stain

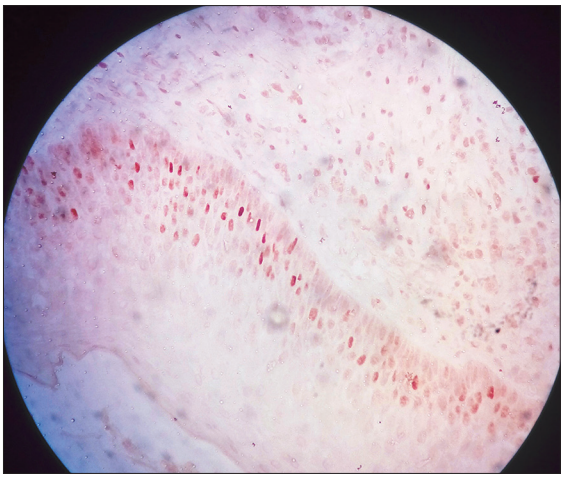

Figure 2: p53 stain

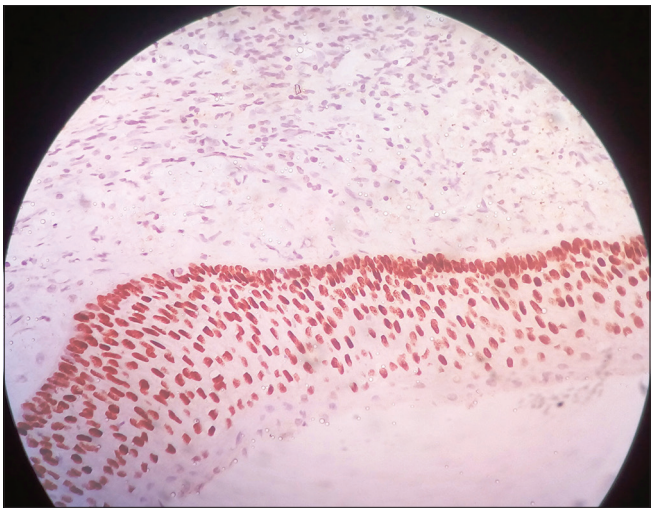

Figure 3: p63 stain

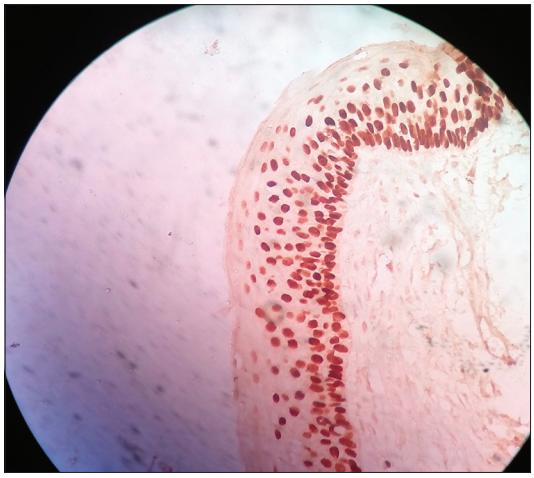

Figure 4: Proliferating cell nuclear antigen stain

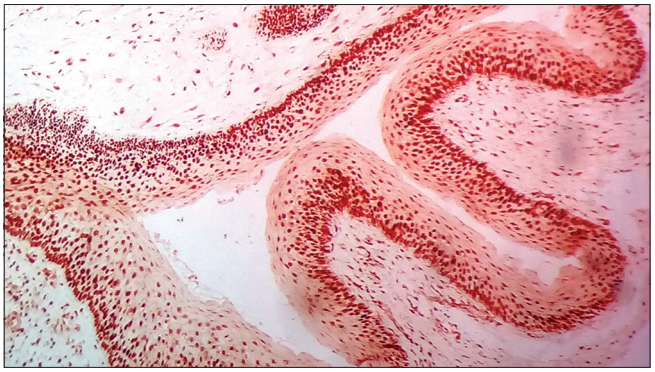

Figure 5: p63 stain in ×10

by de Oliveira *et al*.[19] There was a significant difference between values in the basal and suprabasal layers of OKC greater values were found in the basal layer. The p53 expressions of the number of positive cells in the present study were more than the study conducted by Gurgel *et al*.[20] This finding might be attributed to the use of the immunohistochemical method. The intensity of p53 stain in the epithelium of cystic lining was less or mild when compared to p63 and PCNA [Table 2]. It was previously considered that immunohistochemical detection of p53 protein is possible only when the protein is overexpressed or accumulates in cells as a result of mutation. Therefore, p53 immunostaining has been used as a marker of neoplasia, malignancy and tumor progression.[21,22] The p53 protein is expressed by proliferating cells, but its accumulation in the cell may be caused by several factors. Cell stress is one of these factors since p53 is a primary mediator of cell response to stress. Such findings may explain its clinical behavior and its tendency to recurrence. The presence of mutant p53 in OKCs should also be taken into consideration, as demonstrated by Gonzáles-Moles *et al*.[23] who used a specific antibody for mutant p53. It is possible that the accumulation of p53 in OKCs cannot be attributed to gene mutation with stabilization of this protein, but it may be due to an accumulation of normal p53 protein Gonzalez-Moles *et al*.[23] This explanation might be attributed to mdm2 defects as a response in different situations. However, it is important to state that has demonstrated lost of heterozygosity in TPp53 gene in OKCs. Further studies are needed to clarify this matter.[20] The p53 protein is expressed by proliferating cells, but its accumulation in the cell may be caused by several factors. Cell stress is one of these factors since p53 is a primary mediator of cell response to stress. The lesions under study, its etiology and its clinical behavior should be taken into consideration because all these factors contribute to the accuracy of results.

The expression of p63 antibody for the investigation of odontogenic lesions are scarce and only few reports investigating the expression of this protein in OKCs are available. Intense p63 positivity in epithelial cells of OKCs, including basal, suprabasal cell layers and also insuperficial

Table 1: Comparison of mean percentages of among positive p53, p63 and proliferating cell nuclear antigen cells in basal and suprabasal layers in odontogenic keratocyst

Lesion	Layer	*n*	P53		P63		PCNA		*P*
			Mean %	SD	Mean %	SD	Mean %	SD	
OKC	Basal	21	5.52	6.73	71.48	34.52	68.25	23.57	0.01*
	Suprabasal	21	9.12	7.63	69.53	32.03	84.34	21.31	

Significant difference (Student's *t*-test for paired samples among the expressions of the basal and suprabasal layer of cystic epithelium). SD: Standard deviation, OKC: Odontogenic keratocyst, PCNA: Proliferating cell nuclear antigen. *$P<0.01$(S), Significance level $P<0.05$(Significance), $P>0.05$ (Non-significance)

Table 2: Intensity of p53, p63 and proliferating cell nuclear antigen expression in study groups

IHC protein	Mild	Moderate	Severe	Total
p53	13	2	1	16
p63	-	5	16	21
PCNA	-	16	5	21

PCNA: Proliferating cell nuclear antigen, IHC: Immunohistochemistry

cell layers, and in 64% of cases for p63 antibody positive with high intensity of staining. In the present sample, the percentage of stained cells in each case was higher, probably because of the use of the immunohistochemical method. The results of this study suggest that p63 immunostaining might reflect the immaturity of epithelial cells in this lesion. This aspect may favour tumerigenesis and support the hypothesis of a neoplastic nature of OKCs. p63 plays a role on blocking apoptosis-inducing and growth-inhibitory actions. This may facilitate its proliferative potential on stratified epithelial as described by Yang *et al*.[24] In addition, p63 contributes to regulation of the Sonic hedgehog signaling pathway.[25] Alterations in this pathway have been described in different diseases, including OKCs, as mutations in the PTCH gene result in aberrant activation of this pathway. The current study displayed that OKCs have been found to contain the highest number of p63-positives cells and OKCs presented the most intense and diffuse immunostaining for p63. In addition, in OKC also the intermediate and superficial epithelial layers showed positives for p63 expression. Similar findings were found in a study conducted by Lo Muzio *et al*.[26] The results showed that the pattern of p63 expression varied linked to the histological type. The current study displayed that OKCs have been found to contain the highest number of p63-positives cells with the most intense and diffuse immunostaining for p63. Staining above the basal layers was typically reduced in intensity and in cell numbers, in accordance with cell maturation. Localization of p63 positive cells seems to be associated with proliferative compartments in the epithelia.

Expression of PCNA was found in all lesions analyzed in this study. The expression of PCNA positive cells within OKC linings differed significantly from that of p53, with greater than 95% of stained cells located in basal and suprabasal layers, this could probably suggest increased growth factors (epidermal growth factor receptor) in OKC, and a higher level of cytokines (interleukin 1) stimulates epithelial cell proliferation directly and/or indirectly by inducing the secretion of some factors such as keratinocyte growth factor with increased activity of keratin layers, which may contribute to the higher thickness of the epithelium in OKC. similar results found in the study conducted by Piattelli *et al*.,[27] In our study, p63 andPCNA staining was 100% in OKC and lesser intensity of expression of p53 expression.

In OKCs the expression of markers in this type of lesion may indicate a cell proliferation pattern compatible with neoplastic cells, which is independent of inflammatory stimuli. p53, p63 and PCNA were higher in the suprabasal layer, which demonstrated that this lesion has proliferation and maturation patterns that differ from those found in the other lesions studied. Such findings may explain its clinical behavior and its tendency to recurrence.[23,28] The presence of mutant p53 in OKC should also be taken into consideration. Results of this study show about p63 and PCNA expression in OKCs was higher and the number of cells expressed p53 were less. These unique findings of OKCs suggest that the proliferation compartment in these lesions is formed by the basal and suprabasal layers. In which the cells in the suprabasal layer have an increased proliferative potential in comparison with healthy epithelial tissue.[19]

OKC differs from other cyst types by a characteristic microscopic aspect and by an increased epithelial mitotic activity in basal and suprabasal layers. Clinically, OKCs are more aggressive and tend to recur with greater frequency than the other cyst types. The epithelial lining of OKCs may have some intrinsic growth potential not present in other odontogenic cyst linings. The findings of several studies appear to confirm this hypothesis. In addition, most of the PCNA cells in OKC were located in suprabasal layers. The predominant and peculiar suprabasal distribution of PCNA positive cells in OKC could mean a greater proliferative activity in OKC linings, at one with their more aggressive clinical behavior, and could represent an evidence of epithelial dysplasia. Furthermore, OKC has been found to contain the highest number of p53 cells, most of which

were located in the basal and suprabasal layers, in contrast to what happens in dendritic cells and RCs or normal oral mucosa, whereas the location is basal.

CONCLUSIONS

It is possible that the biological behavior of OKCs may be related to the suprabasal proliferative compartment in the cystic epithelium as observed by high levels of p53, p63 and PCNA. Our results indicate that these proteins contribute to the biologic profile of OKCs; In addition, p63 and PCNA immunostaining may represent the immaturity of keratinocytes in OKCs, and suggests that these proteins may participate in the regulation of epithelial cell differentiation. The more intense and diffuse expression of p53, p63 and PCNA in OKCs, especially in suprabasal cell layer, could help to explain the difference in the clinical and pathologic behavior of OKCs, pointing to an abnormal control of cell cycle leading to an intrinsic growth potential. Taken together, these data may favor tumerigenesis on OKCs.

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Conflicts of interest
There are no conflicts of interest.

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