Comparison of p53 Expression in Odontogenic Keratocyst and Dentigerous Cyst: An Immunohistochemical Study

Dr. Meenakshi, [1] Dr. Garima Sharma, [2] Dr. Reena Pabri, [3] Dr. Manju Rawat, [4] Dr. Sajan Vaish, [5] Dr. Manju Devi [6]

1. **Dr. Meenakshi**
   Senior Demonstrator, Department of Oral Pathology and Microbiology, RUHS College of Dental Sciences, Jaipur, Rajasthan, India
2. **Dr. Garima Sharma**
   Senior Demonstrator, Department of Prosthodontics, RUHS College of Dental Sciences, Jaipur, Rajasthan, India
3. **Dr. Reena Pabri**
   Senior Demonstrator, Department of Oral Pathology and Microbiology, RUHS College of Dental Sciences, Jaipur, Rajasthan, India
4. **Dr. Manju Rawat**
   Private Practitioner, MDS, Oral Pathology and Microbiology, New Delhi, India
5. **Dr. Sajan Vaish**
   Private Practitioner, MDS, Oral Pathology and Microbiology, Ahmedabad, Gujrat, India
6. **Dr. Manju Devi**
   Postgraduate Student, Department of Oral Pathology and Microbiology, RUHS College of Dental Sciences, Jaipur, Rajasthan, India

Abstract

Background: Odontogenic cysts comprise an important aspect of oral and maxillofacial pathology. These cysts arise from the same odontogenic apparatus, but with their different pathogenesis and differ considerably in their biological behavior in terms of aggressiveness. This could be due to the nature of their epithelium and alteration in their cell cycle control. The p53 protein, a product of the p53 tumor suppressor gene, and the mutations of p53 protein are closely related to the decreased differentiation of cells.

Objective: This study was carried-out to investigate the immunohistochemical expression of p53 protein in odontogenic keratocysts (OKC) and dentigerous cyst (DC).

Materials and Methods: Immunohistochemistry was performed with the p53 protein with fifteen cases of OKC and ten cases of DC.

Results: The mean scores of the marker used were found to be significantly higher in OKC in comparison to DC.

Conclusion: The higher p53 protein expression in OKC in comparison to DC suggest that p53 protein, contribute to the aggressive behaviour in OKCs.

Keywords: Immunohistochemistry, odontogenic keratocysts, dentigerous cyst, p53.

INTRODUCTION

The term cyst is derived from the Greek word "Kystis" which means a bladder or sac. Kramer has defined cyst as a pathological cavity having fluid, semi-fluid, or gaseous contents and which is not created by the accumulation of pus[1]. The term "Odontogenic Keratocyst" was introduced by Philipsen in 1956[2].

Odontogenic keratocysts (OKCs) constitutes 11.2% of all developmental odontogenic cysts. OKCs can develop from derivatives of embryologic dental lamina or it remains (Serres glands) as well as basal cell extensions from the overlaying epithelium.

Histologically, they have a consistent epithelial lining of parakeratinized-stratified squamous epithelium that is thin, ranging from six to ten cell layer thickness, and a well-defined basal layer made of columnar or cuboidal cells. OKCs are aggressive cystic lesions which have a tendency to recur if not treated properly and grow larger than other cysts with a mitotic activity observed in their epithelial lining which is more than that observed in dentigerous and radicular cysts[1]. OKCs have clinical importance due to its aggressive behavior, recurrence risk, and malignant potential[3].

Dentigerous cysts are the most frequent developmental cysts of the jaws, and they expand as osmotic pressure within their lumen increases[4]. Dentigerous cysts are benign odontogenic cysts that develop in the permanent teeth's crowns. They found more common in the mandible than the maxilla[5]. Histologically, the cyst wall of dentigerous cysts, is made up of connective tissue which lined by low cuboidal, stratified squamous epithelium of 2-3 cell layer thickness but in the presence of inflammation, the thickness of the lining epithelium may vary[1].

An increase in cell proliferation probably plays a role in the development of odontogenic cysts and tumours[6,7].

The p53 protein, which has a molecular weight of 53 kilodalton and is encoded by the p53 gene on chromosome 17, is a nuclear protein with a molecular weight of 53 kilodalton. Apoptosis, cell cycle, cell proliferation regulation, and genetic stability are all critical functions of this tumour suppressor gene[8,9,10].

Usually, the concentration of wild type p53 is low in cells due to its relatively short half-life which is approximately 20 min. Its concentration increases in cells when half-life is extended, which may found because of TP53 gene mutation, association of wild type p53 with other proteins, or disruption of p53 degradation pathway[11,12].

p53 protein is a product of mutations in the p53 gene which has an increased half-life, thus allowing this mutated protein to be expressed immuno-histochemically[13,14].

In our study p53 protein expression will be noted in OKCs and DCs to know the different biological behaviour of OKCs than DCs of oral cavity.

MATERIALS AND METHODS

This study was done on 25 archival paraffin blocks of odontogenic cysts. These cases were retrieved from the Department of Oral Pathology and Microbiology of RUHS College of Dental Sciences, Jaipur by random sampling. Ethical clearance was taken from ethical committee. The cases include 15 odontogenic keratocyst cases (OKC) and 10 dentigerous cysts (DC).

From every paraffin block, two 4 micron-thick paraffin sections were done. First section were stained with H&E and reviewed to confirm the histopathological diagnosis. The second section was processed immunohistochemically to assess P53 expression in odontogenic cysts. These sections were taken on Poly-L-Lysine adhesive coated glass slides or positive charged slides. Then the sections were deparafinized, rehydrated in graded alcohols (100%, 80%, 50%), treated with blocking reagent for 5 min and washed in phosphate buffer working solution (PBS) for 5 min. One drop of monoclonal mouse antibody to P53 (Biogenix) was then placed on each section. Then incubation was done over night in the humidity chamber with 2-3 drops of streptavidine enzyme placed on each slide. DAB (diaminobenzidine tetrahydro chloride) chromogen working solution was applied onto the slides for 1-2 minutes at room temperature. Sections were counterstained with Myer's hematoxylin, dehydrated by passing them through ascending grades of alcohol (50%, 80%, 100%) and mounted in DPX. Breast carcinoma sections for p53 protein (positive control) was run with every immunostaining to confirm the immunoactivity of the antibodies. Negative controls were used to confirm the specificity of the method and to asses nonspecific background staining by staining the test tissue in the absence of primary antibody.

The brown colored nucleus at the site of target antigen was considered immunopositive for p53. The immunoreactivity was assessed quantitatively by two investigators to overcome inter-observer variability. All positive stained cell from five randomly chosen high power fields in every case were counted while cells were scored as positive or negative. Positively stained cells were analyzed quantitatively by counting the total number of intact positively stained cells per high power field

(40x) of light microscope. Images were captured by digital camera attached with light microscope and analyzed using image analysis software (ij152-win-java8 image J).

Counting was done in five representative areas of epithelium in the OKC and DC. 200 cells were counted in per high field and the total 1000 cells immunoreactive for p53 protein was calculated. The mean of five values were calculated and expressed.

Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM).

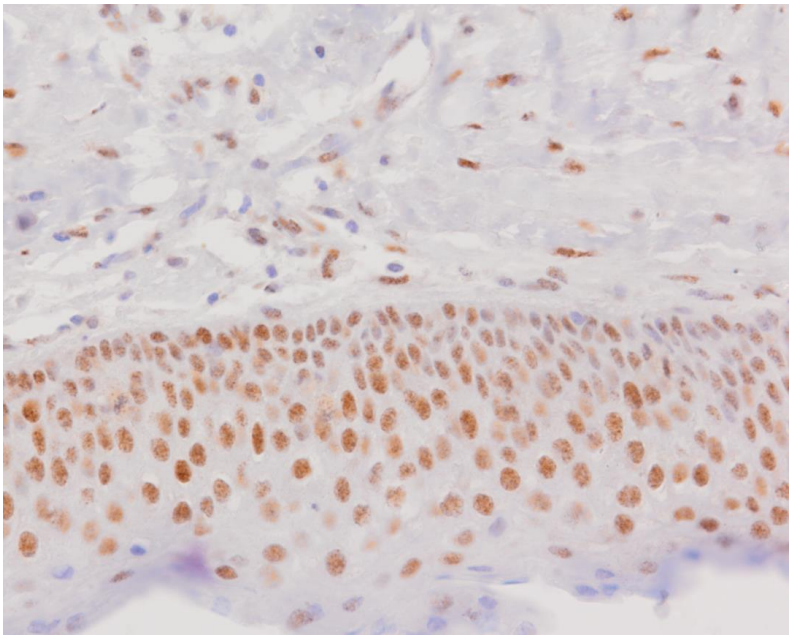


Figure 1: p53 Protein Stained Section of Odontogenic Keratocyst (40x)

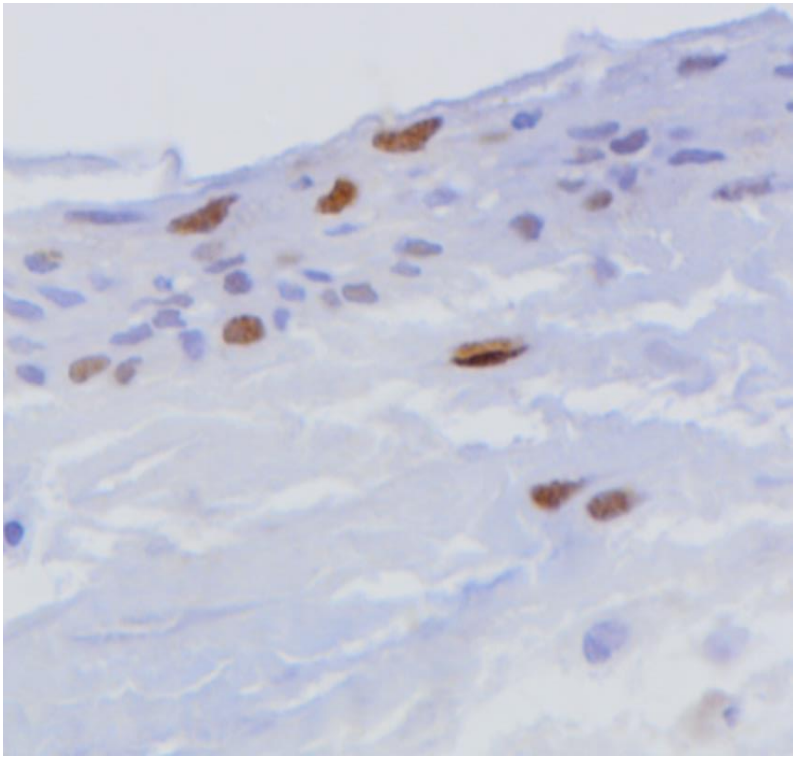
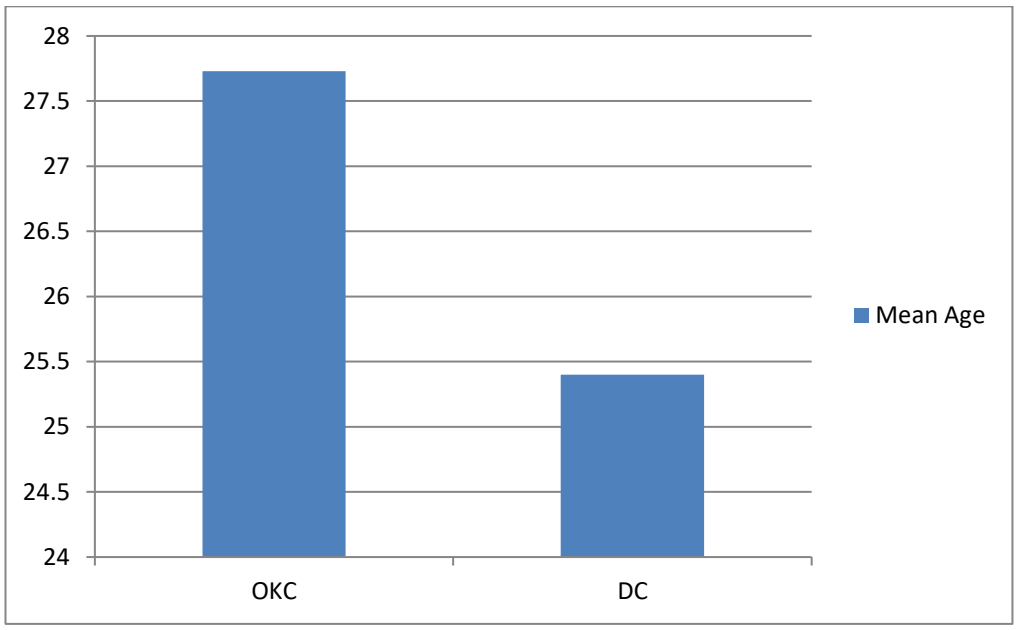


Figure 2: p53 Protein Stained Section of Dentigerous Cyst (40x)

RESULT

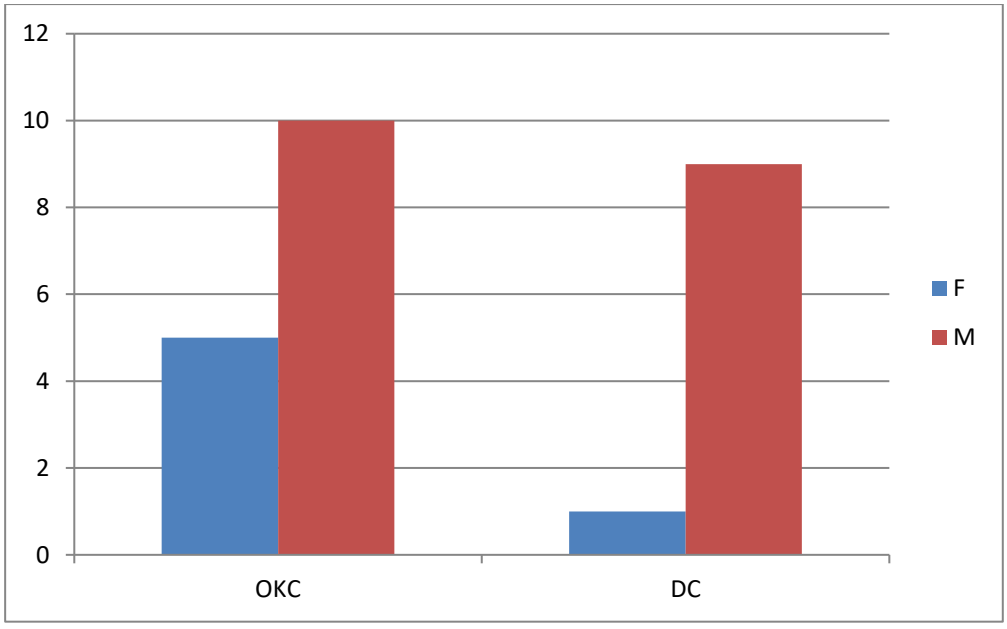
Expression of p53 in the OKC epithelium (Figure 1) is more than DC epithelium (Figure 2) was found.

Graph 1: Represents mean age of the subjects. For okc the mean age was 27.73 and for DC the mean age was 25.40.



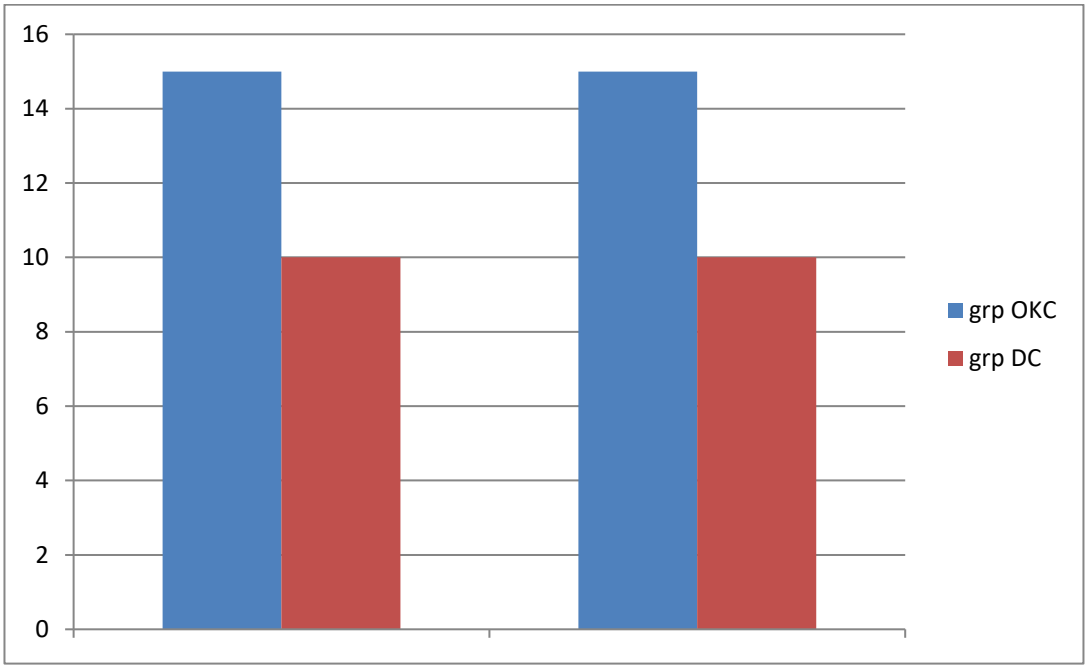
Graph 1: Comparison of Mean Age

Graph 2: Denotes distribution of two groups according to gender. In OKC & DC 66.7% & 90% were male respectively. Whereas 33.3% & 10% cases were female for OKC & DC respectively. Out of total cases 76% were male & 24% were female.



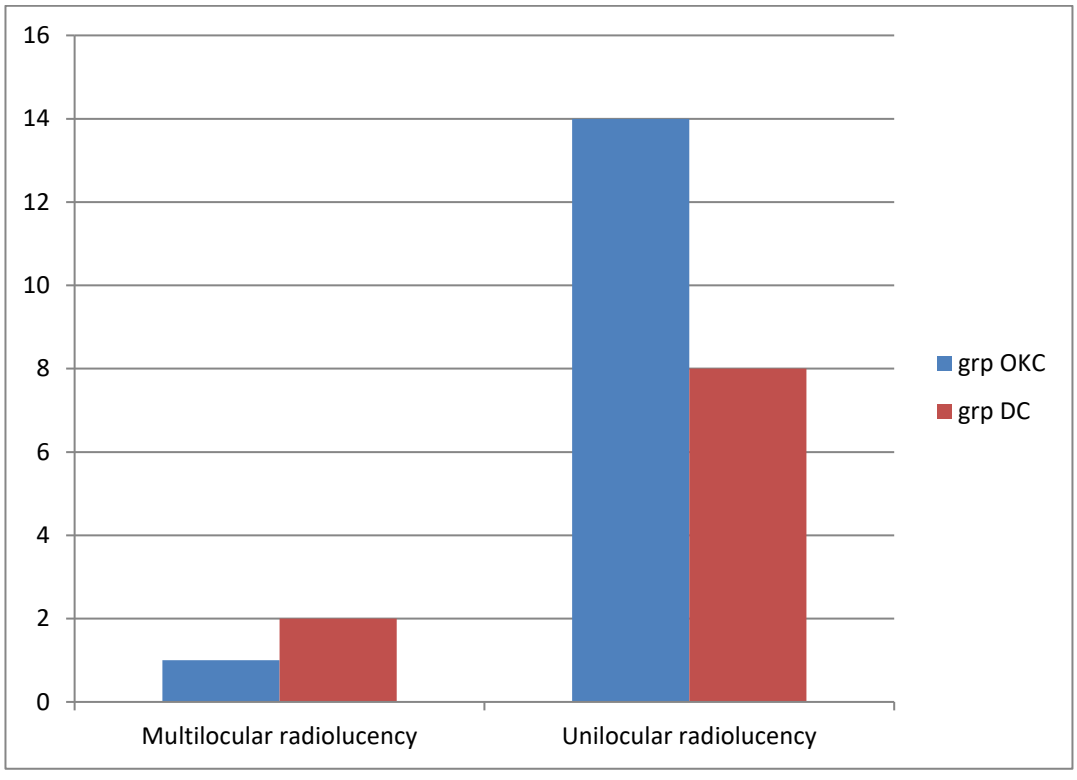
Graph 2: Distribution of different Groups according to Gender

Graph 3: Shows that in all cases the presentation was mainly swelling therefore, no statistics are computed because presentation is a constant.



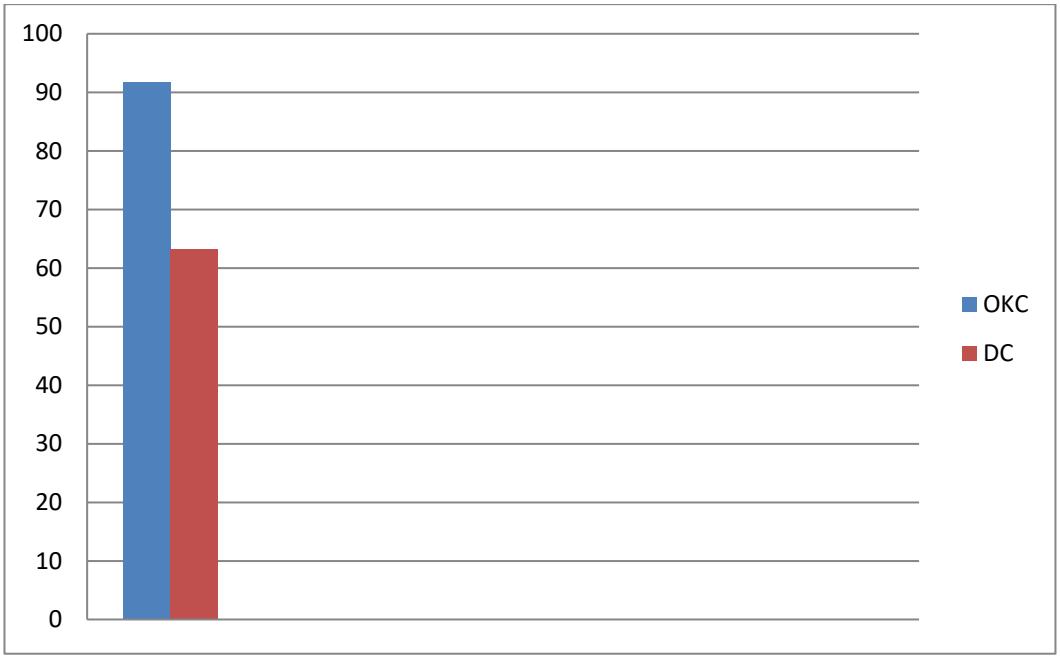
Graph 3: Clinical Presentation

Graph 4: Shows that in OKC 14 cases and in DC 8 cases presented as unilocular radiolucency while 1 and 2 cases of OKC and DC respectively showed multilocular radiolucency. There was statically non-significant difference in radiographic presentation of OKC and DC ($p>0.05$).



Graph 4: Distribution of different Groups according to Radiographic Appearance

Graph 5: Demonstrates inter group comparison of mean expression of p53 amongst OKC and DC. The mean expression for OKC was 91.72 and for DC 63.32.



Graph 5: Inter Group Comparison of Mean Expression

Table 1: Shows inter group comparison of mean expression of p53 protein quantitatively. There was a statistically no significant difference seen for the values between the OKC and DC groups (p>0.01).

Table 1: Inter Group Comparison of Mean Expression of p53 Protein Quantitatively

Group	Vs group	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
OKC	DC	54.000	-1.165	0.244#

DISCUSSION

There are lots of studies which have investigated the mutations and changes in cell-cycle regulatory, proliferative, and apoptotic proteins in OKC and dentigerous cyst[15-21].

In normal cells, p53 is a negative regulator of cell division and inactivation of this gene is one of the most common genetic changes in human cancer[22], Changes of the p53 gene result in a gene product (p53 protein: p53) which has an increased half-life in comparison with the wild-type protein. Therefore, if one is able to demonstrate p53 immunohistochemically in an individual cell. It is often assumed that in that particular cell the p53 gene is abnormal. However, there is evidence that non-mutational stabilization of p53 can occur and that heightened or even normal levels of wildtype p53 may be detected depending on the methods employed in detecting the antigen[23,24,25].

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

It was found that in this study the higher mean age in OKC (27.73 yrs) and lower in DC was (25.40 yrs) where as in Gaballah E T *et al*[26]. (2010) study the mean age was higher in OKC (37±16.1 yrs) and lower in DC (31±20.2 yrs).

The predominance of males in cases of the present study was reported in OKC and DC cases and similar finding was reported in Ochsenius *et al*[27]. (2007), Tortorici *et al*[28]. (2008) studies.

In this study the clinical presentation of both groups is swelling similarity with previously reported study conducted by Doll C *et al* [29]. (2018). Most Common radiographic appearance of both groups in this study is unilocular in accordance with Robert J *et al*[30]. (1999).

In our study the inter group comparison of mean expression of p53 amongst OKC and DC was done. The mean for OKC was 91.72 and for DC 63.32. The higher values in group OKC and lower in group DC which was in accordance with Piattelli A *et al*[31]. (2001), Slootweg PJ[7] (1995) and Li TJ *et al*[32]. (1996) where as De Oliveira MG *et al*[12]. (2008) concluded higher expression of p53 in DC followed by OKC.

CONCLUSION

In our study we found high expression of p53 in OKC compared to DC because the greater proliferation activities of the epithelial lining in OKC. A p53 gene mutation may be one of the causes of cell proliferation. The results suggest that p53, contribute to the aggressive behaviour in OKCs. In odontogenic cysts, p53 play an important role in the pathology of both inflammatory and developmental lesions.

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