



## Quantitative analysis of Cytokeratin 19 expression in Odontogenic Keratocyst, Dentigerous cyst and Radicular cyst

### KEYWORDS

Odontogenic keratocyst, Dentigerous cyst, Radicular cyst, Cytokeratin-19, Immunohistochemistry.

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### ABSTRACT

*Introduction: Odontogenic cysts and tumors arise from the enamel organ or remnants of dental epithelium after odontogenic progression completes. Odontogenic keratocyst will have high recurrence rate when compared with other odontogenic cysts. To evaluate proliferation in odontogenic cysts with Cytokeratin 19 marker. Aim&Objectives: The aim of this present study is to analyze the cytokeratin 19 expression in the epithelium of the odontogenic keratocyst (OKC) and to compare it with dentigerous cyst (DC) and radicular cyst (RC). Materials and methods: The present study was carried out using the histological sections of odontogenic lesions which were selected from the archives of the Department of Oral and Maxillofacial Pathology, SIBAR Institute of Dental Sciences, Guntur. Each group has 10 archival blocks been taken and routine hematoxyline and eosin staining was performed for confirmatory diagnosis. Later immunohistochemistry was performed using CK-19 antibody. The expression of CK-19 is assessed in basal, suprabasal and superficial cells of OKC, DC and RC. CK-19 positive cells were counted under oil immersion lenses and number of cells showing positivity was counted among a total of 100 cells counted all together in five different fields. Data analysis was done using Kruskal Wallis ANNOVA test. Results: It has been observed that the highest positivity of 64.9% is shown in DC and the positivity is seen in basal, suprabasal and superficial layers. RC showed a positivity of 58.4% and this positivity is greater in suprabasal layers compared to basal and superficial layers. OKC show only 13.5% positivity and this positivity is seen in superficial layers. Conclusion: CK 19 expression correlates with the degree of differentiation of the epithelium. So the cysts with a well-differentiated epithelium (RC and DC) express CK19, while the cysts with a less well-differentiated epithelium (OKC) show slight positivity. Thus it can be useful to differentiate OKC from DC and RC.*

### Introduction:

Odontogenic cyst is arising from the enamel organ or remainants of dental epithelium. After or before odontogenesis process occurs these remainants will proliferate with some stimulation with in the jaw bones and lead to odontogenic cysts or tumors.<sup>1</sup> Those cysts which are arising from the dental remainants will have aggressive behavior and high recur rate. When compared all the odontogenic cysts and tumors, odontogenic keratocyst will have high recurrence rate.<sup>2</sup> Odontogenic keratocyst will proliferate anterioposterior direction before clinical manifestation<sup>3</sup> WHO in 2005 they changes the term Odontogenic keratocyst with Keratocystic Odontogenic tumor (KCOT) based on its neoplastic nature arising from dental remainants<sup>3</sup> Radicular cyst is one of the most common inflammatory cysts in jaw bones. Even those it is inflammatory cyst they categorized into odontogenic cysts because of its epithelial nature i.e. non keratinized stratified squamous epithelium.<sup>4</sup> Radicular cyst usually associated with apices of non vital tooth.<sup>5</sup> Den-

tigerous cyst is one of the commonly associated with neck of impacted teeth. It will be either unilocular or multilocular by radiographically and diagnosis cannot be made by radiographically because there are some odontogenic tumors and cysts are associated with unilocular or multilocular radiolucency such as Ameloblastoma and Odontogenic keratocyst.<sup>6</sup> Ochsenius G et al., in 2007 they evaluated frequency of odontogenic cysts among these Radicular cysts accounts 50.7%, Dentigerous cysts 18.5% and Odontogenic keratocysts accounts only 14.3%.<sup>7</sup> Cytokeratins (CK) are the majority complex members of the intermediate filament protein family. Cytokeratins are grouped as acidic and basic subfamily. Most of the cytokeratins are high molecular weight.<sup>8</sup> Cytokeratins are completely expressed in epithelial cells and cancer cells. Cytokeratins can be identified in altered epithelial cells and this cytokeratins can be identified by immunohistochemical markers. These markers will help to determining the epithelial origin of undifferentiated malignant neoplasm.<sup>9</sup> Cytokeratin-19 (CK-

19) is a protein that is encoded by KRT 19 gene.<sup>10</sup> CK19 expressed in supra basal layer this will indicate that over-excited propagation of cells in potentially malignant disorders.<sup>11</sup> Due to its high sensitivity, CK19 is the most commonly used marker for the RT-PCR-mediated detection of tumor cells disseminated in lymph nodes, peripheral blood, and bone marrow of breast cancer patients.<sup>12</sup> Odontogenic keratocyst will have high repetition rate when contrast with other odontogenic cysts. The aim of this study to scrutinize the pattern of expression of CK-19 in Odontogenic keratocyst, Dentigerous cyst and Radicular cyst and to find out whether it can be used to discriminate in all these cysts.

#### Materials and Methods:

The present study was carried out using archival of paraffin embedded blocks from Department of Oral and Maxillofacial Pathology & Microbiology. In each category ten blocks were selected those histopathologically diagnosed as Odontogenic keratocyst, Radicular cyst and Dentigerous cyst (DC) from the year 2009 – 2012.

#### The study consists of 30 samples of which

- Group 1: odontogenic keratocyst
- Group 2: Dentigerous cyst
- Group 3: Radicular cyst

All the archival blocks were done serial sections of 4 µm thicknesses, first section subjected for routine Hematoxylin and eosin stain observed under binocular microscope to confirm the histopathological diagnosis. After conformation of diagnosis with histopathologically all the three groups sectioning was carefully fixed on the prepared micro slides coated with poly-L-Lysine. These slides were subjected for immune histochemical analysis using Monoclonal mouse anti human cytokeratin- 19 (Dako product Ltd, Agilent Technologies, Bostan, USA). The standard technique used for immune histochemistry was based on the labeled streptavidin – biotin methods. After labeled with streptavidin-biotin method the endogenous peroxidase activity of the tissue was blocked by using 3% hydrogen peroxide for 5 mints, then all these sections were kept in woven with primary antibody. The non specific bindings sites were blocked using horse peroxidase serum. Biotinylated secondary antibody and strepta – viridian peroxidase conjugated were added and the sections was incubated for 30 mints in each of the them. The substrate chromogen solution prepared by mixing 1 ml of substrate buffer and 1 drop DAB (Diaminobenzidine) was added to the specimen and incubated for 5 minutes at room temperature and later gently rinsed twice with phosphate buffer saline. The slides were then immersed in a bath of haematoxylin for 2-5 min and washed under tap water for 5 min. Cytokeratin 19 expression positive was observed as light brown color, the cells taken up stain considered as positive and those cells are not taken up stain considered as negative. All brown stained cells demonstrated positive for cytokeratin 19 were counted in 40 X magnification (MAGNUS) and total number of cells counted were 100 in ten high power fields in each slide.

Data were analyzed using SPSS (Stastical package for Social Sciences) software. Data analysis was performed using Kruskal Walli's ANOVA for comparison of all three groups and Mann –Whitney U test with  $p < 0.05$  level of significant.

#### Results:

The present study was undertaken to assess the immune histochemical expression of Cytokeratin-19 in basal, supra

basal and superficial cells of in all three groups. The expression of cytokeratin 19 in basal cells in all three groups, the mean number of cells positive in Dentigerous cyst ( $20.80 \pm 5.65$ ) followed by Radicular cyst ( $11.30 \pm 8.41$ ). Expression of cytokeratin 19 in all three groups shown stastically significant with  $p < 0.05$ . (Table 1 and Graph 1) the expression of cytokeratin 19 observed in supra basal and superficial layer in all three groups, radicular cyst shown more expression ( $30.60 \pm 10.9$ ) followed by Dentigerous cyst ( $23.50 \pm 3.8$ ). In both supra basal and superficial cells expression was more in radicular cyst and statistically significant with  $p < 0.05$  (Table 2 & Graph 2). Comparison of basal, supra basal and superficial cells in all three groups Dentigerous cyst shown more expression than Odontogenic keratocyst and Radicular cyst, the expression was stastically significant with  $p < 0.05$  (Table 3 & Graph 3)

#### Discussion:

The epithelium of Odontogenic keratocyst, Radicular cyst and Dentigerous cyst are similar histological appearance i.e. stratified squamous cystic epithelium. In inflammatory odontogenic keratocyst lining will be non – keratinized stratified squamous epithelium which may appear similar to Radicular cyst and Dentigerous cyst.<sup>13</sup> Such misdiagnoses should be avoided since the biological behavior of odontogenic keratocyst epithelium differs from those of Radicular cyst or Dentigerous cyst. Odontogenic keratocyst shows a more aggressive biological behavior with a high recurrence rate as compared to Radicular cyst and Dentigerous cyst.<sup>14</sup> To rule out its role in differentiation of Odontogenic keratocyst from Dentigerous cyst and Radicular cyst with a help of Cytokeratin 19. Morgan P et al., in 1988 shown that Cytokeratin 19 was expressed in all the layers of Dentigerous cyst. This observation was consistent with our study. In our study also we observed that Dentigerous cyst showed more expression in all the layers<sup>15</sup> Wagner Y et al in 1999 shown that 71% of the cells are shown positivity for Cytokeratin – 19 expressions in Dentigerous cyst. In our study we have observed that 64.9% of the cells shown positivity in Dentigerous cyst , this findings are close to Wagner Y et al., study<sup>16</sup>

Hormia M et al., in 1987 done a study to see the expression of cytokeratin 19 in basal, supra basal and superficial cells of radicular cyst, they observed that all the layers of the cells are shown positive expression, in our study also we have observed that expression of Cytokeratin 19 shown that all the layers of epithelial cells are expressed the stain<sup>17</sup> Stoll C et al in 2005 compared the expression of Cytokeratin 19 between Dentigerous cyst, radicular and odontogenic keratocyst and showed that 52% of dentigerous cysts, 49% of radicular cysts and no odontogenic keratocysts showed positivity for cytokeratin 19. In our study the highest positivity of 64.9% is shown in Dentigerous cyst, Radicular cysts showed a positivity of 58.4% and odontogenic keratocysts showed only 13.5% positivity.<sup>18</sup>

#### Conclusion:

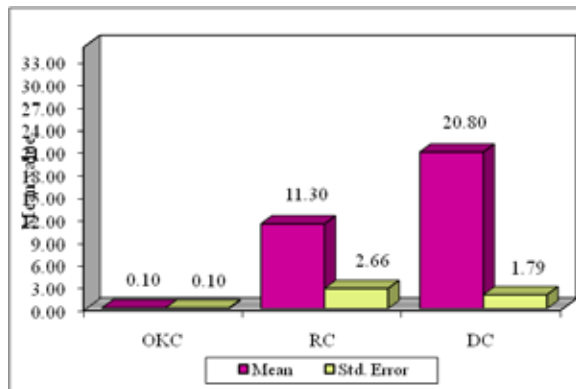
Expression of Cytokeratin 19 correlates with the degree of differentiation of the epithelium. So the cysts with a well-differentiated epithelium such as Radicular cyst and Dentigerous cysts will express the Cytokeratin 19, while the cysts with a less well-differentiated epithelium such as Odontogenic keratocyst show slight positivity. Thus it can be useful to differentiate Odontogenic Keratocyst from Dentigerous cyst and Radicular cyst. There is a clinical correlation between the degree of differentiation and its clinical behavior with well-differentiated tumors tends to be less aggressive than poorly-differentiated ones. Thus the reduced expression of Cytokera-

tin-19 in Odontogenic keratocyst in relation to Radicular Cyst and Dentigerous cyst shows the aggressive nature of Odontogenic keratocyst. In Odontogenic keratocyst the positivity is greater in superficial layers in comparison to basal and supra-basal layers. So basal cells have a more role in the aggressive nature of the OKC or recurrence of the cyst.

**Table 1: Comparison of three groups (OKC, RC and DC) with respect to basal cells by Kruskal Wallis ANOVA**

Group	Minimum	Maximum	Mean ± SD	Std Error	P value
OKC	0	1	0.10 ± 0.3	0.10	
RC	0	22	11.3 ± 8.4	2.66	0.00001*
DC	12	31	20.8 ± 5.6	1.79	

**Graph 1: Comparison of three groups (OKC, RC and DC) with respect to basal cells**

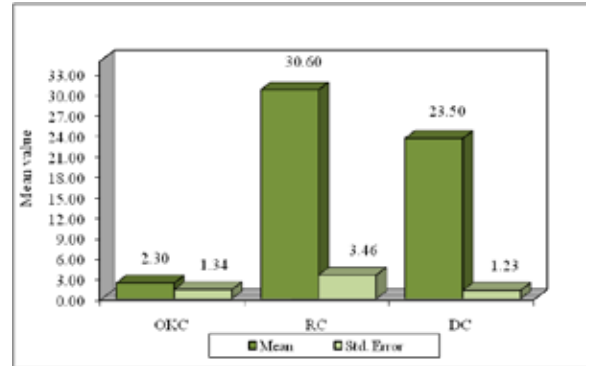


**Table 2: Comparison of three groups (OKC, RC and DC) with respect to Supra basal cells by Kruskal Wallis ANOVA**

Group	Minimum	Maximum	Mean ± SD	Std Error	P value
OKC	0	13	2.30 ± 4.2	1.34	
RC	22	51	30.6 ± 10.9	3.46	0.00001*
DC	16	28	23.5 ± 3.8	1.23	

\* p<0.05

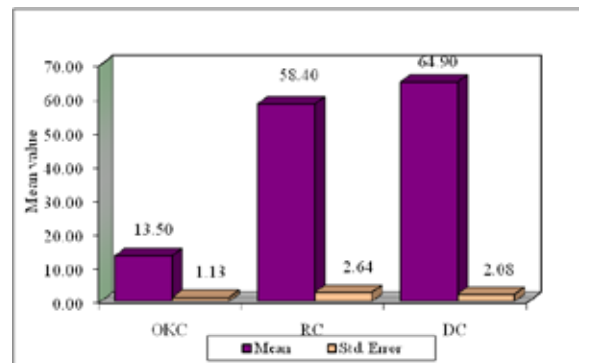
**Graph 2: Comparison of three groups (OKC, RC and DC) with respect to supra basal cells**



**Table 3: Comparison of three groups (OKC, RC and DC) with respect to basal, supra basal and superficial cells by Kruskal Wallis ANOVA**

Group	Minimum	Maximum	Mean ± SD	Std Error	P value
OKC	6	20	13.5 ± 3.5	1.13	
RC	43	69	58.4 ± 8.3	2.64	0.00001*
DC	56	73	64.9 ± 6.5	2.08	

**Graph 3: Comparison of three groups (OKC, RC and DC) with respect to basal, supra basal and superficial cells**



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