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Clinicopathological Features and Expression of Ki-67 in Odontogenic Keratocyst, Dentigerous Cyst and Radicular Cyst

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ABSTRACT

Background: Biological behaviour of Odontogenic Keratocyst (OKC) is aggressiveness than others Odontogenic Cysts (OCs) like Dentigerous Cyst (DCs) and Periapical Cyst/Residual Cysts (RCs). The aim of the study was to determine clinicopathological features and expression of Ki-67 in Odontogenic Cysts of the oral cavity.

Methodology: This cross-sectional study was conducted at de'Montmorency College of Dentistry (DCD from Feb, 2020 to Feb, 2022 after approval from Institutional Review Board (IRB) of DCD. A total 78 cases of OCs were collected from hospitals which are affiliated with the DCD. Routine lab process for Hematoxylin & Eosin and Immunohistochemistry was performed. Data entry and statistical analysis was carried out in SPSS 21. A Chi- square test was applied to observe the association between cyst and Ki-67. P value < 0.05 was taken as statistically significant.

Results: Among total 78 cases the mean age was 25.08 ±14.5 years with an age range of 6-70 years. Most OCs (64.1%) were reported in males than females (35.89%). Most OCs were reported in mandible (56.4%. Ki-67 expression in OKC was high 7.7%, low in 76.9% and negative in 15.4%). Most of the DCs and RCs expressed low expression of Ki-67 (84.6% and 76.9% respectively).

Conclusion: Most of the odontogenic cysts expressed low expression of Ki-67 while few cases of OKC and DC expressed high expression.

Key words: Dentigerous Cyst, Odontogenic Keratocyst, Immunohistochemistry, Ki-67, Odontogenic Cysts, Periapical Cyst, Radicular Cyst.

Authors' Contribution:
[1]Conception; Literature research; [2,3]manuscript design and drafting; [4,6]Critical analysis and manuscript review; [6]Data analysis; Manuscript Editing.

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Introduction

Odontogenic cysts are classified on the basis of etiology, development, and histology. World Health Organization (WHO) has also classified cysts in 2017.

OCs (derived from remnants of tooth forming epithelium), include Dentigerous cyst (DC), Odontogenic Keratocyst (OKC), Orthokeratinized odontogenic cyst, Lateral periodontal and botryoid odontogenic cyst, Gingival cyst, Glandular odontogenic cyst, Calcifying odontogenic cyst and Radicular cyst. Radicular cysts are inflammatory OC and are most common cyst of the jaw.[1] Epithelial lining of RC is derived from the proliferation of rests of Malassez within the periodontal ligament. DC is derived from proliferation of enamel organ remnants or reduced enamel epithelium (REE) and OKC from cell rests of the dental lamina.[2] OKC and DC have highest risk of malignant transformation.[3]

WHO reclassified OKC as Odontogenic Keratocyst instead of Odontogenic tumor due to insufficient evidences to be placed it in tumors.[4] However the exact mechanism of the development and malignant transformation of these cysts is unknown.[3] Studies show that Ki-67 and p53 reveal intense positivity in OKC as compared to other OCs.[5] Clinicopathological features (age, gender, site, ragiographic presentation and histology) are important parameters of OCs yet Ki-67 depict potential transformation of OCs into neoplasia.[5,6] Expression of Ki-67 was variable in OKC as reported by many authors, however few foci expressed higher expression of Ki-67.[7] Orthokeratinized odontogenic cyst (OOC) revealed low expression of Ki-67 as compared to OKC.[8] Expression of Ki-67 among these cyst is different in literature, however it is still a challenge to determine that which cyst has the highest expression, and highest potential of malignant ,transformation. Literature shows OKC has highest expression of Ki-67, followed by DC, and then RC.[9-11]

Literature about the Odontogenic Cysts in local setting does not reveal the behaviour, clinicopathological features and proliferative activities of OCs. The objectives of current study are to measure demographic characteristics, size, site, radiographic presentation, locularity, histopathological characteristics and Ki-67 expression of common OCs (OKC, DCs, and RCs) in local setting in Punjab Dental Hospital/DCD.

Methodology

This cross-sectional study was conducted at de'Montmorency College of Dentistry (DCD from Feb, 2020 to Feb, 2022. Ethical approval was taken from Institutional Review Board (IRB) of de'Montmorency College of Dentistry, Lahore (DCD). A total 78 cases of OCs were collected from hospitals which are affiliated with the DCD. Equal cases of OKCs, DCs and RCs were collected from DCD after getting written consent from all 78 cases and each group comprised of 26 cysts. Males and females of all ages were included in the study, however patients having any systemic diseases were excluded from the study. Recurrent cases of OCs and inadequate tissue samples were also excluded from the study. Clinical data was noted on individual Proforma. Routine H&E staining and Immunohistochemistry for Ki-67 staining were performed on collected specimens.

Histological features of OCs were recorded on H&E staining. Expression of Ki-67 was recorded on 10 high-power fields (40X). Positive nuclear staining of epithelial cells were counted according to the following criteria; negative (<5% cells positive), low expression (5%-50% cells positive) and high expression (>50% cells positive).[7] Clinicopathological features and Immunohistochemical scores were analysed using SPSS 21. Mean $\pm$ S.D were given for quantitative variables like age of patient, and size of the OCs. Percentages and frequencies were given for variables like gender, site of the cyst, type of cyst, locularity, microscopic features and score of Ki-67. A Chi- square test was applied to observe association between cyst and Ki-67, P value < 0.05 was taken as significant.

Results

Table I: Clinical o radiological features of Odontogenic cysts (n=78)			
Sr. No.	**Variables**	**Frequency (n)**	**Percentage (%)**
Age	< 25	44	56.4
	> 25	34	43.6
Gender	Males	50	64.1
	Females	28	35.89
Type cyst	Radicular cyst	26	33.33
	Dentigerous cyst	26	33.33
	Odontogenic keratocyst	26	33.33
Site of OCs	Lower Jaw (Mandible)	44	56.4
	Upper Jaw (Maxilla)	34	43.6
Size of OCs	< 2 cm	60	76.9
	2-4cm	16	20.5
	>4 cm	02	2.6
Locularity	Unilocular	74	94.9
	Multilocular	04	5.1

The mean age of the patients was 25.08 ±14.5 years with an age range of 6-70 years. Male were more common (64.1%) as compared to female (35.89%). In 56.0% mandible was involved while maxilla was involved in 41.1% cases. About 95% cysts were unilocular while only 5 % were multilocular. Most of the Odontogenic cysts were small in size (< 2 cm) 76.9%, 20.5% were of 2 -4cm, and 2.6% had size greater than 4cm (Table 1).

All cases of OKC were lined by keratinized (parakeratinized) epithelium while all DC and all RC cases were lined by non-keratinized epithelium. OKC showed epithelial Hyperplasia in 20 cysts out of 26 (76.9%) while all DC and RC showed hyperplastic epithelium (table 2).

Table II: Histopathological Features of Odontogenic cysts (Radicular cyst, Dentigerous cyst and Odontogenic keratocyst) in n=78			
Histopathological Features	**Cyst type**		**Frequency (%)**
Epithelial Lining	OKC	Keratinized	26 (100)
		Non-Keratinized	00
	DC	Keratinized	00
		Non Keratinized	26 (100)
	RC	Keratinized	00
		Non Keratinized	26 (100)
Epithelial Hyperplasia	OKC	Present	20 (76.9)
		Absent	6 (23.1)
	DC	Present	26 (100)
		Absent	00
	RC	Present	26 (100)
		Absent	00
Spongiosis	OKC	Present	12 (46.2)
		Absent	14 (53.8)
	DC	Present	24 (92.3)
		Absent	2 (7.7)
	RC	Present	20 (76.9)
		Absent	6 (23.1)
Acantholysis	OKC	Present	2 (7.7)
		Absent	24 (92.3)
	DC	Present	2 (7.7)
		Absent	24 (92.3)
	RC	Present	22 (84.6)
		Absent	4 (15.4)
Inflammation in connective tissue	Present		26 (100)
	Absent		00

Table III: Expression of Ki-67 in Odontogenic Cysts in n=78					
Odontogenic Cyst	**Ki 67 Expression**				**P value**
	Negative expression (<5% cells positive)	Weak positive (5-50% cells positive)	Strong Positive (>50% cells positive)	Total	
Odontogenic keratocyst	4 (15.4%)	20 (76.9%)	2 (7.7%)	26 (100%)	**0.9**
Dentigerous cyst	2 (7.7%)	22	2 (7.7%)	26 (100%)	
Radicular cyst	6 (23.1%)	20 (76.9%)	0 (0%)	26 (100%)	
Total	12 (15.4%)	62 (79.5%)	4 (5.1%)	78 (100%)	

Expression of Ki-67 in Odontogenic keratocysts was low in 76.9%, high expression in 7.7 % and negative in 15.4%. Expression of Ki-67 in DCs was low in 84.6%, high in 7.7 % and negative in 7.7%. Expression of Ki-67 in Periapical Cyst was low in 76.9% and negative in 23.1%. Four cases 5.1% showed high expression, however insignificant association was observed (p= 0.90) between Ki-67 and type of cysts (Table 3).

Discussion

World Health Organization classified OCs in 1992, 2005 and 2017. OKC is re classified as Odontogenic cyst rather than Odontogenic tumor. Biological behaviour of odontogenic cysts is variable according to type of cyst, among them OKC shows aggressive behaviour and high recurrence rate.[1, 2, 7]

In the present study, 54% of the odontogenic cysts were seen in patients less than 25 years of age which means odontogenic cysts were more common in younger age group. This finding is similar to the study conducted by Bhat et al in 2019, which reported that 43% cases in the age ranging from 21-40 years. Male gender was affected more in the present study as compared to females. Posterior mandible followed by anterior maxilla was the most common site of Odontogenic cysts in the present study. These findings are concordant with the study conducted by Bhat et al in 2019.[13]

In the current study, the size was measured on radiograph, and it was found that most of the cysts (76.9%) were of small size i.e. < 2cm in diameter and 94.9% were unilocular. Another local study reported a mean size of 2.24cm of OCs. Radicular cysts on the whole were smaller lesions (mean=1.58 cm) as compared to dentigerous cysts (mean=3.22 cm) and odontogenic keratocysts (mean=3.67 cm). However OCs were more common in mandible than maxilla in the current study while OCs were more common in maxilla in another study conducted by Ali et al.[14]

Dentigerous cysts were the most common in the first and second decades of life (85% cases). In Dentigerous cyst, posterior mandible followed by anterior maxilla was the most common site. Microscopically Dentigerous cyst was lined by non-keratinized epithelium. Epithelial hyperplasia (92.3%) and spongiosis (92.3%) were prominent features. No dysplastic changes were observed. All cases of dentigerous cysts showed chronic inflammation with different degrees such as mild (15.4%) and severe (15.4%) however moderate inflammation was (69.2%). In another study of DCs with a large sample size (n= 338), the mean age was 33 years and was predominantly found in males. The most prevalent site was mandible as compared to maxilla. Microscopically, 317 cysts were lined by stratified squamous epithelium, 9 by mucoepidermoid-type epithelium, and 12 by ciliated pseudostratified columnar epithelium.[15]

In the current study RC was most common in second and third decades of life (69% cases) and the most common site was anterior maxilla. All cases of radicular cysts were lined by nonkeratinized epithelium and showed hyperplasia. However, 76.9% RC showed spongiosis and 84.6% showed acantholysis. Moderate (38.5%) to severe inflammation (61.5%) was seen in all the radicular cysts. Another Pakistani study reported that the mean age of patients with radicular cyst was 24.81±14.8 years and was mostly seen in males (58.3%). Predominant site of radicular cyst was anterior maxilla. Histopathological review revealed that 65.7% cysts were lined by nonkeratinized epithelium which is different from the current study. However, 2 radicular cysts were lined by keratinized epithelium.[16]

In another study with large sample of RCs (n=232) mean age of patients was 40.5 years, further it was observed that 98.2 % cysts were lined by nonkeratinized stratified squamous epithelium and only 0.9% lining of RCs were of mucoepidermoid type and 0.9% of respiratory epithelium.[17] In the present study most cases of OKCs were lined by keratinized epithelium with 38.5% hyperplasia, 46 % spongiosis and 7.7% acantholysis. Chronic inflammation was also observed in connective tissue of OKCs. In 2017 a study in Pakistani population also revealed that majority of OKC were lined by parakeratinized epithelium which is similar to current study.[18]

As far as Immunohistochemical expression of Ki-67 is concerned, in 76.9% OKC it was low (5-50% cells stained positive) in 7.7 % OKC it was high expression ((>50% cells stained positive) and negative (<5% cells stained positive) in 15.4% OKC. Expression of Ki-67 in DCs was low in 84.6%, high in 7.7 % and negative in 7.7%. Expression of Ki-67 in RC was low in 76.9% cysts and negative in 23.1% periapical cysts. Four cases 5.1% showed high expression however insignificant association was observed (p= 0.90) between Ki-67 and type of cysts (Table 3). A study reported that expression of ki-67 is observed highest in OKC than DC and RC (12.76 ± 4.78, 5.87 ± 4.24 and 5.08 ± 3.11) respectively. Expression was more prominent in suprabasal cell layers in OKC whereas it was more common in basal layer cells of DC and RC. As far as current study is concerned strong positive expression was observed same in DC and OKC however in Modi's study it was more in OKC than DC.[19]

In another study mean expression of Ki-67 LI was 7.7%, 1.7% and 15.3% for OKC, DC and RC respectively. Out of 4 cases 66.7 % weak positive expression was observed which was focal rather than diffuse, out of 8 cases of DC only one case 12.5% expressed Ki-67 in mild intensity in focal pattern, and only 1/10 cases of RC expressed moderate expression of Ki-67 which was diffuse in nature.[9] RC when compared with Residual Cyst in terms of Ki-67 expression (mean proliferative index) on lining epithelial cells was 1.25% for Radicular Cysts (ranging from 0% to 5.31%) in comparison with 3.51% in Residual Cyst (ranging from 0% to 16.3%) (p=0.017). This shows that duration of Radicular cyst may be lesser than Residual cyst.[11] A recent review (meta-analysis) of OTs and OCs reported that among OCs, the highest Ki-67 LI expression was seen in OKC (3.58±0.51%), and the lowest was observed in RCs (0.98±0.47%). The order of Ki-67 LI expression in OCs was as follows: OKC (3.58±0.51%), COC (2.43±0.65%), DCs (1.29±0.62%), and RCs (0.98±0.47%) in descending order. These findings might be useful for diagnostic purposes. It was concluded that Ki-67 is expressed in DOCs (developmental odontogenic cysts) to a greater extent than IOCs (inflammatory odontogenic cysts), such as Radicular cysts (0.98±0.47%). Among DOCs, the highest expression of Ki-67 was seen in OKC (3.58±0.51%) with the lowest expression in DCs (1.29±0.62%).[12]

Expression of Ki- 67 was greater in OKC than DCs as mentioned in studies.[21, 22] While in the current study both cysts have same expression of Ki-67 i.e. strong positive in 7.7 %. In another study Ki-67 expressed in all cases of DCs (n=15) with moderate positive

(n=14) and strong positive (n=1) which is different from the current study.[23] A case report of Orthokeratinized odontogenic keratocyst revealed expression of Ki-67 in the basal layer and also in suprabasal layer in focal area which is similar to literature and WHO new classification of OKC.[24] OKCs expressed high expression of Ki-67 as compared to OTs.[25]

Conclusion

Most of the odontogenic cysts expressed low expression of Ki-67 while few cases of OKC and DC expressed high expression. Majority of OCs were having small size which may depict early diagnosis and less transformation of epithelium and less aggressiveness.

Recommendation

In this study the sample size of odontogenic cysts was small. New studies may be carried out in future to observe patient health seeking behavior (early diagnosis, small size versus large size lesions) and biological behavior (strong positivity versus weak positivity) in odontogenic cysts particularly in OKC.

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