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IMMUNOHISTOCHEMICAL EXPRESSION OF KI-67 IN ODONTOGENIC KERATOCYST: EVIDENCE OF AGGRESSIVE BEHAVIOR.

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ABSTRACT… The odontogenic keratocyst (OKC) well-known for its aggressiveness and high recurrence rate, comprises approximately 11% of all jaw cysts. Due to its aggressive behavior it was placed into category of tumour in 2005 by the World Health Organization (WHO). **Objectives:** The purpose of this study was to determine the Ki-67 expression in Odontogenic Keratocysts to predict its proliferative potential. **Study Design:** Descriptive study. **Setting:** Department of Morbid Anatomy and Histopathology, UHS. **Period:** June 2014- June 2018. **Material & Methods:** This is a descriptive study comprising of 39 cases of odontogenic cysts. These surgically removed samples were processed at University of Health Sciences (UHS) laboratory. Routine staining with Hematoxylin & Eosin stain along with immunohistochemistry (IHC) with Ki-67 antibody was performed. Immunohistochemical scoring was done on the basis of percentage of the nuclear staining of Ki-67. Data was entered into SPSS 22 and descriptive statistics were measured in the form of percentage and frequency. Quantitative variables such as age of patient, size of the cyst, and Ki-67 score were also measured. P value <0.05 was taken as significant. **Results:** The mean age of the patients was 25.08 $\pm$14.5 years. Significant association was observed between histological variables with odontogenic keratocyst such as parakeratinized epithelial lining ($p = 0.00$), epithelial hyperplasia both typical and atypical ($p = 0.02$) and focal spongiosis ($p = 0.04$). Foci having epithelial atypia demonstrated stronger staining intensity compared to adjacent normal epithelium. However, no significant association was observed between the histological variables and Ki-67 expression. **Conclusion:** OKC expressed low Ki-67 expression in most of the cases, however, foci of strong expression were also observed in few cases.

Key words: Immunohistochemistry, Ki-67, Odontogenic Keratocysts.

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INTRODUCTION

Odontogenic keratocysts (OKC) are common and clinically aggressive lesions which arise from dental lamina or rest of Serres.[1] OKC have mandibular predominance with a predilection for the posterior body and ramus regions followed by anterior mandible. In the maxilla, these occur most commonly in the third molar area followed by cuspid region.[2] Odontogenic keratocysts may be found in all ages ranging from infancy to old age, but about 60% of all cases are diagnosed in first four decades of life. There is a slight male predominance.[3] World Health Organization (WHO) recommended the term keratocystic odontogenic tumour (KOT) depicting its neoplastic nature in year 2005.[4]

The Ki-67 protein (also known as MKI67) is a marker for cellular proliferation.[5] It has been described that Ki-67 antigen expression rises in pre-malignant and malignant lesions of the oral mucosa, and in all conditions of high cell turnover. MIB-1 (monoclonal antibody) reacts with the epitope of the Ki-67 nuclear antigen in formalin-fixed, paraffin-embedded sections.[6] Ki-67 is commonly used to evaluate the proliferative activity in neoplastic and non-neoplastic lesions, including odontogenic cysts.[7] The purpose of this study was to determine immunohistochemical expression of Ki-67 in odontogenic keratocysts that will help in assessing the proliferative potential in these cysts.

MATERIALS & METHODS

This descriptive study was conducted in the department of Oral Pathology at University of Health Sciences, Lahore after the approval of institutional review board. A total of 39 diagnosed cases of OKC were collected after written and verbal consent of the respondents, those who did not give consent were excluded from the study. Samples were taken from de 'Montmorency College of dentistry, Lahore. Patients both genders of all ages were included in the study. Patients having history of associated debilitating chronic co-morbid conditions like uncontrolled diabetes mellitus, tuberculosis, malignancies, patients with immune disorders, with recurrence of cysts or tumor and inadequate tissue sample were also excluded from the study.

Labelled specimens after being fixed in formal-saline were brought to the department of Histopathology, UHS, where they were allocated a specific laboratory number. After gross examination, tissues were processed for preparing paraffin blocks. Paraffin embedded sections were prepared.

Histopathological diagnosis under Hematoxylin and Eosin was also confirmed. The sections were stained with monoclonal mouse anti-Human Ki-67 Antigen Clone MIB-1 Code M7240 (DAKO) and examined to assess the proliferative index. The percentage of positive nuclear staining of epithelial cells in 10 high-power field (40X) was used to classify each cystic lesion, using the following criteria; negative (<5% cells positive), low expression (5%-50% cells positive) and high expression (>50% cells positive).

The data was entered in SPSS 22. Quantitative variables such as age of patient, size of the cyst, and Ki-67 score were calculated with mean (+ S.D). Frequencies, percentages and graphs were given for qualitative variables like gender, and site of the cyst.P value <0.05 was taken as significant.

RESULTS

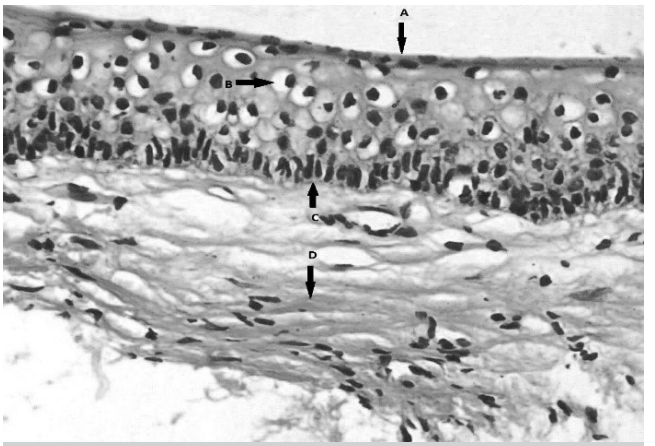
The mean age of the patients was 25.08 ±14.5 years. The age of patients was divided into two groups: ≤ 25 years of age and > 25 years with n=21 (54%) patients falling in first group and n=18 (46%) in the latter group. Painless swelling was the most frequently reported symptom (61.5%). Radio graphically, 69.2% of OKCs were < 2cm in diameter, 23.1% were 2-4cm in diameter while only 7.7% were >4cm in diameter.

Posterior mandible was the commonest site (61.5%) and 92.3% of the cysts were unilocular. On histopathological examination, following observations were made shown in Table-I.

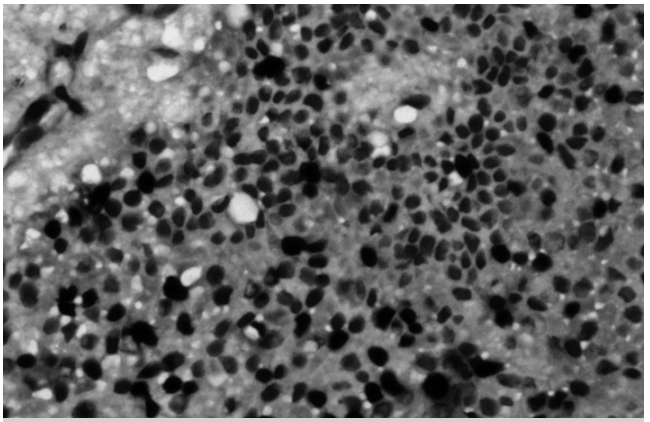
Age of the patients (p value= 0.004) and site of specimen (p value= 0.003) showed significance with the occurrence of OKC in these patients.

Photomicrograph 1-4 depicting characteristics of histopathology and ki-67 expression in OKC. When histological variables were related with odontogenic keratocyst (cross tabulation), parakeratinized epithelial lining (p = 0.00), epithelial hyperplasia both typical and atypical (p = 0.02) and focal spongiosis (p = 0.04) demonstrated significant association.

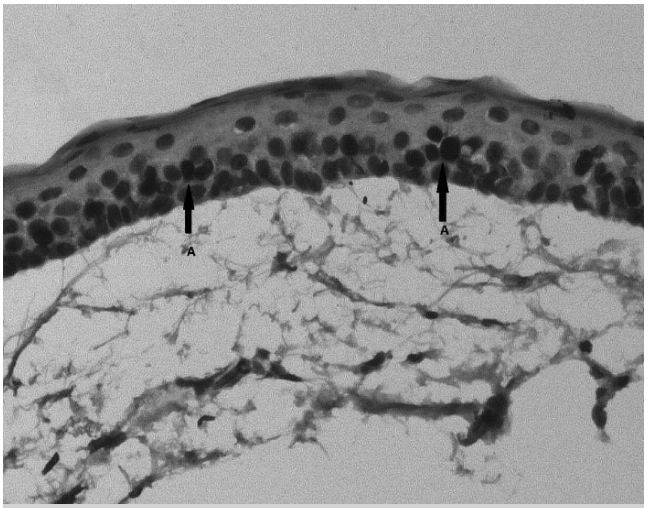
Foci having epithelial atypia demonstrated stronger staining intensity compared to adjacent normal epithelium. However, no significant association was observed between the histological variables and Ki-67 (p = 0.08).



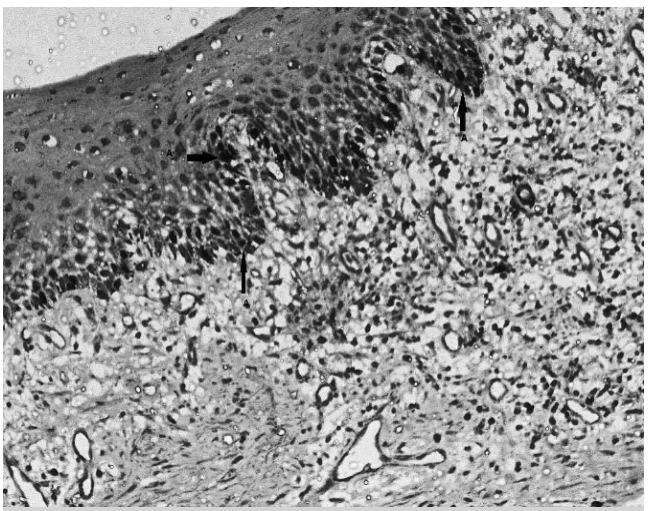
Photomicrograph-1. of OKC showing parakeratinized epithial lining (A), cytoplasmic vesiculation (B), palisaded basal layer (C), fibrous connective tissue (D)



Photomicrograph-2. of OKC showing staining intensity of Ki-67 in atypical epithelium of OKC



Photomicrograph-3. of OKC (Parakeratinized) showing staining intensity of Ki-67 in atypical epithelium of OKC



Photomicrograph-4. of OKC showing staining intensity of Ki-67 in atypical epithelium of OKC

Serial Number	Variables	Frequency
Clinical Features	Age <25 >25	 54% 46%
	Gender Male Female	 66.6% 33.4%
	Asymptomatic swelling	61.5%
	Site: Anterior, Posterior	 38.5% 61.5%
	Size < 2cm in diameter, 2-4cm in diameter >4cm in diameter	 69.2% 23.1% 7.7%
Radiographic	Unilocular Multilocular	92.3% 7.7%
Histo-pathological Chara-cteristics of OKC on H&E	Parakeratinized	61.5%
	Orthokeratinized	30.8%
	Epithelial Hyperplasia	38.5%
	Keratinocytic spongiosis	46.2%
	Acantholysis	7.7%
	Epithelial atypia	84.6%
	Nuclear pleomorphism (mild)	76.9%
	Prominent nucleoli	46.2%
	Inflammation in Connective Tissue	
	Mild	30.8%
	Moderate	53.8%
	Severe	15.4%
	Connective Tissue Changes	
	Hyalinization	15.4%
	Fibroplasia	76.9%
	Fibrosis	7.7
Ki-67 Expression in OKC	Score of Ki-67	Percentage
	Negative	15.4%
	Low Expression	76.9%
	High expression	7.7
Association of OKC with variables	OKC	P value
	Age category <25, >25	0.004
	Site anterior, posterior	0.003

Table-I. Descriptive statistics of OKC (Histological and Immunohistochemical Findings)
Mean age = 25.08 ±14.5 years

DISCUSSION

Odontogenic keratocysts in the present study were most common in third decade of life which is similar to the results of study conducted by Selvamani et al[8] (2012) but shows variation from a study conducted by Ansari et al[9] (2010) who reported OKC to be prevalent in 2nd decade of life. Although sample size is limited yet male predominance was seen in the present study (M: F ratio = 2:1) which is concordant with the results of study conducted by Ansari et al[9] (2010) (M: F=2:1) and Selvamani et al[8] (2012) (M: F =1.2:1). Posterior mandible was the most common site of occurrence for OKCs (61.5%) in the present study which is consistent with the studies conducted by Ansari et al[9] (2010) and Selvamani et al[8] (2012) as 76% and 37.5% cases of OKCs involved mandible respectively.

When size of OKCs was observed radio graphically, a total of 69% OKCs were < 2cm in diameter which is discordant with an Israelian study conducted in 2012[10] which reported >2cm as the mean diameter of Odontogenic keratocysts. A total of 95% of OKCs were unilocular in the present study which is concordant with the study conducted by Jankowski et al[11] but this finding was not supported by another study conducted in Pakistan by Khan et al (2009)[12] which showed that 83% odontogenic keratocysts were multilocular while only 17% were unilocular his is might be due to diverse biological behavior of the lesion.

Histologically most cases of OKCs (61%) were lined by parakeratinised epithelium which is similar to the findings of most of the studies conducted in different countries.[13,14] Epithelial hyperplasia was observed in 38.5% OKCs in the present study which is consistent with a study conducted on the Brazilian population.[15] Spongiosis was observed in 46% OKCs in the present study but no data is available in the literature regarding spongiosis in OKCs. Epithelial atypia comprising of increased nuclear to cytoplasmic ratio, prominent nucleoli and nuclear pleomorphism was observed in 84.6% OKCs in the present study but data is scanty in the literature in this context. Chronic inflammation was observed in connective tissue of 76.9% OKCs and it was of moderate severity (53.8%) in most of OKCs. Finding of the present study is consistent with the study of Kwon et al[16] and Nadalin et al[17] who also reported chronic inflammation in OKCs but Nadalin et al[17] reported severe inflammation in connective tissue of OKC. Fibroplasia was present in 85% OKCs and vascular congestion was observed in only 15.4% OKCs but data in literature is not available regarding these both variables.

Immunohistochemical staining with Ki-67 showed low expression in 76.9% OKCs, negative expression in 15.4% while high expression was observed in only 7.7% OKCs in the present study. The present study is discordant with the study of Nadalinet al[17] in that low Ki-67 expression was demonstrated by 94% OKCs in this study. The present study is quite comparable with the study conducted by Wahbaet al[18] who reported that 80% OKCs showed moderate staining with Ki-67.

Ki-67 was positive in 84.6% OKCs in the present study and foci showing epithelial atypia demonstrated stronger intensity of Ki-67 immunostaining which might predict some strong link with the development of preneoplastic changes as it has not yet been mentioned in the literature related to odontogenic cysts. However, Humayun and Prasad[19] reported a significant correlation between progression of oral epithelium from normal to neoplastic and increased expression of Ki-67 antigen suggesting it to be a useful biomarker of malignant transformation in oral premalignant conditions. Therefore epithelial hyper proliferation is considered to be an early indicator of disorganized growth.

MCM-2 is more sensitive marker as compared to Ki-67 in order to assess proliferation and growth rate, although all cases of OKC expressed expression of Ki-67 but intensity is lesser than MCM-2 concluded by Acharya et al (2019).[20] Recently in 4th edition of WHO classification of odontogenic tumor (2017), OKC is placed back into the category of odontogenic cysts, due to insufficient evidence regarding mutation of PTCH gene, furthermore Orthokeratinized OKC (OOKC) is declared as separate cyst as previously it was consider as variant of OKC. There are many

reasons due to which it is declared as a separate cyst such as lack of palisading, less aggressive behavior and less recurrent rate,[21] however still controversy exists.[22]

LIMITATIONS

Lastly, this study has some limitations such as limited sample size and lack of clinic pathological correlations. Further studies with large sample size and cyst with relative early diagnosis are recommended to measure proliferative behavior of this odontogenic cyst There is also need to compare proliferative potential in OKCs which have early diagnostic history (short duration of diseases) as compared to long standing history (long duration of cyst).

CONCLUSION

Ki 67 expression (labelling index) expressed its low expression in most of the cases, only few cases expressed strong expression in OKC. Even Low expression of Ki-67 indicates its proliferation potential in odontogenic keratocyst. Ki-67 believed to be a reliable marker of cell proliferation because this non-histone protein is expressed at all stages of cell cycle except G0 and therefore may be taken as a transitional point for progression of disease into malignant lesions.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Rabiya Saif	Principal investigator worked in all aspects of this research.	
2	Hafiz Majid Jehangir	Contributed in data collection and data entry.	
3	Abdul Hannan Nagi	Supervised my study.	
4	Nadia Naseem	Assisted and guided throughout the project.	
5	Zainab Rizvi	Contributed in data collection.	
6	Faiz Rasul	Data analysis and project design.	