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Research Article

Association of Twist expression with multilocular and unilocular variant of odontogenic keratocyst

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ABSTRACT

Objective: To evaluate the association of twist expression with multilocular and unilocular variant of odontogenic keratocyst. Material &

Methods: This study was performed at the department of Pathology, Khyber Medical University Peshawar from January 2022 to June 2022. A total of 83 subjects meeting the inclusion criteria were included. According to WHO criteria, diagnosis of OKC was done. Twist immunoexpression of unilocular and multilocular OKC blocks were performed according to IHC protocol. The data were analysed using statistical software.

Results: Amongst the 43 cases of multilocular variant of odontogenic keratocyst, 41 (95.3%) were associated with the twist immuno-reactivity and 2 (4.75%) cases were negative, while amongst the 40 cases of unilocular variant of odontogenic keratocyst, 10 (25%) cases were associated with the twist immuno-reactivity and 30 (75%) cases were negative.

Conclusion: It is concluded that twist immunohistochemical expression was more common in the multilocular variant than unilocular variant. In multilocular variant of OKC, whether solitary or syndromic, its increased rate of recurrence and pathogenesis is greatly influenced by Twist expression and suggest its place in the neoplastic category.

Keywords: Impacted tooth, Multilocular, Odontogenic keratocyst, Twist gene.

INTRODUCTION

Odontogenic cysts represent the most frequently occurring odontogenic intrabony pathology affecting the maxilla and mandible. Histologically it is composed of a fibrous wall and a lining derived from developmental odontogenic epithelial residue.[1] Odontogenic Keratocyst (OKC) was initially described by Philipsen in 1956. As the origin of the lesion was believed to be the tooth primordium, the term "primordial cyst" was first used to describe an odontogenic keratocyst. It is found both in the maxilla and mandible, but most common location is posterior mandible. Mandibular ramus and angle are particularly prone to large lesions. The most typical

locations of origin in the maxilla are the anterior part. OKC can be found in three different locations: periapical, pericoronal, and lateral root.[2]

Males are slightly more affected by odontogenic keratocyst as compared to females. A ratio of 1.4 to 1 exists between men and women. Highest prevalence occurs in twenty to thirty years of life [3]. Radiographically, odontogenic keratocyst appear as multilocular or unilocular radiolucency, possessing distinct borders. In 2005, the World Health Organization chang ed the classification of OKC from an odontogenic cyst to an odontogenic tu mour.[4]

It was renamed as odontogenic keratocyst in 2017 by WHO.[5]Still it is not confirmed whether to place OKC in the neoplastic or cystic category.

Odontogenic keratocyst are classified into two types, solitary or non-syndromic odontogenic keratocyst and multiple or syndromic odontogenic keratocyst. The syndrome which is most commonly associated with OKC is Gorlin Goltz syndrome or nevoid basal cell carcinoma syndrome.[6]Other less common associated syndrome is Ehler Danlos syndrome.[7]

Because of its local aggression and high recurr ence rates, odontogenic keratocyst is notorious among the odontogenic lesions.[8,9] Rate of recurrence of OKC is 62.5%.[10] Odontogenic keratocyst occurs with a frequency of 3-11%.[11]

The most typical symptoms are pain and swelli ng, however they are virtually absent in the early stages.[12]Lininig of odontogenic keratocyst can be transformed into Squamous cell carcinoma.[13]Twist is a transcription protein and its structure is a basic –helix- loop- helix {bHLH}. It is a mesoderm determining factor. It is crucial for the embryo's shaping and is found in a subset of adult mesenchymal cells. In (EMT) "epithelial-mesenchymal transition" Twist is a key component. The epithelial mesenchymal transition (EMT) is a chemical process comprising of connection of polarized epithelial cells with the basement membrane through its bottom layer. It is involved in invasion and tumour spread.[14] It conforms that for mesenchymal morphology of the cells Twist is essential.[15] In EMT process lack of E-cadherin expression is the salient feature. In the process of EMT, polarity and adhesion is lost in the epithelial cells. It assumes a mesenchymal appearance and gain increased motility. Reduction of E-cadherin is linked with Twist up regulation. Other than EMT process, OKC had positive cytoplasmic and nuclear immunostaining for mouse double minute 2 (MDM2) in all layers of epithelial lining. It indicates a neoplastic origin for OKC and distinct biological behaviour from other benign odontogenic cysts in terms of its growth potential, programmed cell death i.e apoptosis, and in the process of organization and differentiation.[16] The EMT is characterised by decreased adhesive capacity, cytoskeletal rearrangement, and increased mobility.[17] Multilocular variant of OKC are more aggressive, causes local infiltration and possess more recurrent potential as compared to unilocular variant. On the basis of these features we can assume that overexpression of twist may be involved in the pathogenesis of multilocular variant of odontogenic keratocyst. This study will help the clinicians to better understand the multilocular and unilocular variant of OKC and thus will help in planning for accurate treatment modalities of these radiological variant. Odontogenic keratocyst may be treated medically by targeting the Twist. The Twist expression can be used as an adjuvant to conventional H&E stain for diagnosis of odontogenic keratocyst especially in the more aggressive recurrent cases.

MATERIAL & METHODS

This study was carried on a total of 83 diagnosed cases of odontogenic keratocyst between the period from January 2022 to June 2022 at "Department of Pathology Khyber Medical University Peshawar". Ethical approval was received from the institute of pathology, Khyber Medical University, Peshawar. Sampling was done through convenience nonprobability technique. This study was conducted according to the declaration of Helsinki**.** Patient selection was done in the following manner.

Inclusion criteria

1. Patients of all ages and both genders diagnosed clinically and histologically as odontogenic keratocyst were included.
2. Both the newly diagnosed and recurrent cases were included.
3. Syndromic and non-syndromic odontogenic keratocyst cases were included.

Exclusion criteria

1. Patients of odontogenic keratocyst having carcinomatous transformation were excluded.

2. Cases with incomplete patient medical history and clinical record were excluded.

After taking consent from all the patients, a detailed history and clinical examination of the patient was done. An OPG (Orthopantomogram) x-ray was requested to see the multilocular or unilocular nature of the lesion. Clinical history proforma was filled out by the chief investigator with the information regarding patient biodata, clinical and radiographic characteristics of the lesion. Tissue specimen were collected in properly labelled jars containing 10% neutral buffered formalin solution. Detailed gross examination was carried out. The patient information on the requisition and on the specimen container was match in its entirety. The cassette number was checked with the specimen as well as with the requisition. The tissue sample size, colour, consistency and any ulcerated area were noted. After 24 hours of fixation, the representative tissues were collected after proper sectioning and were processed in automatic tissue processor through ascending grades of alcohol. The tissue was then cleared using xylene, embedded in paraffin, and sliced into pieces using a rotary microtome at a thickness of 4 micrometres. These sections were taken on albumenized slides and were stained. According to the standard protocol "Hematoxylin and Eosin staining"[18] was performed for the diagnosis of odontogenic keratocyst according to WHO criteria.[5] The Twist immunostaining was performed by commercially available antibodies (Elabscience Biotechnology Inc USA) Catalog No: E-AB—66494, Twist polyclonal antibody at a dilution of ratio 1:200. Each reagent was used in accordance with the manufacturer's guidelines. The positive control was done with the placental tissue. Tissue of normal oral mucosa was used as negative control. The Percentage of the Cells which showed immunoreactivity were counted and strength of the staining was estimated.

In the absence of background staining, distinct yellow-brown nuclei or cytoplasm was deemed affirmative and computed. The Twist reactive cells were calculated at "400 X magnification". Scoring of all the positively sections were done as a percentage as follows.

"Score 1: 10 percent or less cells +ve"
"Score 2: 11 percent—25 percent cells +ve"
"Score 3: 26 percent—50 percent cells +ve"
"Score 4: Over 50 percent cells +ve"

An OKC was assigned as twist positive if more than ten percent of the cells showed immune positivity (score 2-3-4)[19].

The data was entered and analysed using SPSS (version 22). Quantitative variables like age were presented as a mean and standard deviation. Qualitative variables like gender and Twist expression were described as frequencies and percentage by using chi square test". A p value of ≤ 0.05 was considered statistically important.

RESULTS

Majority of the patients reported were in their fourth decade of life, (45.8%) followed by 5thdecade (27.7%). 2.4% of the patient were above 60 years, and this age group accounted for the smallest proportion (Table 1).

Male patients were more common than females (55.4%) & (44.6%) respectively. Ratio of male and female was 1.2:1 (Table 2). Unilocular variety of odontogenic keratocyst was found in (48.2%) of cases, while multilocular variant was found in (51.8%) (Fig 1).

In (26.5%) an impacted tooth was associated with odontogenic keratocyst, while in (73.5%) there was no association with an impacted tooth (Fig 2). Syndromic odontogenic keratocyst was found in 3 cases (3.6%), while solitary or non-syndromic odontogenic keratocyst was found in 80 cases (96.4%) (Fig 3). In the positive cases, Twist was seen in cytoplasm and nuclei of the cells. Amongst the 43 cases of multilocular variant of odontogenic keratocyst, 41 cases (95.3%) were twist positive and 2 cases (4.75%) were negative, while amongst the 40 cases of unilocular variant of odontogenic keratocyst,10 cases (25%) were twist positive and 30 cases (75%) were negative (Table 3). All the three syndromic cases of odontogenic keratocyst were multilocular and twist positive. The calculated P value for the multilocular and unilocular variant of odontogenic keratocyst was less than .05 which shows a strong association between the locularity and twist immuno-reactivity.

Table 1: Age allocation in 83 cases of odontogenic keratocyst.

Age in Groups (in years)	Frequency	Percent
Less than 20 years	3	3.6
20-30 years	14	16.9

31-40 years	38	45.8
41-50 years	23	27.7
51-60 years	3	3.6
More than 60 years	2	2.4
Total	83	100

Table 2: Gender distribution in 83 cases of odontogenic keratocyst (OKC).

Gender	Frequency	Percent
Male	46	55.4
Female	37	44.6
Total	83	100

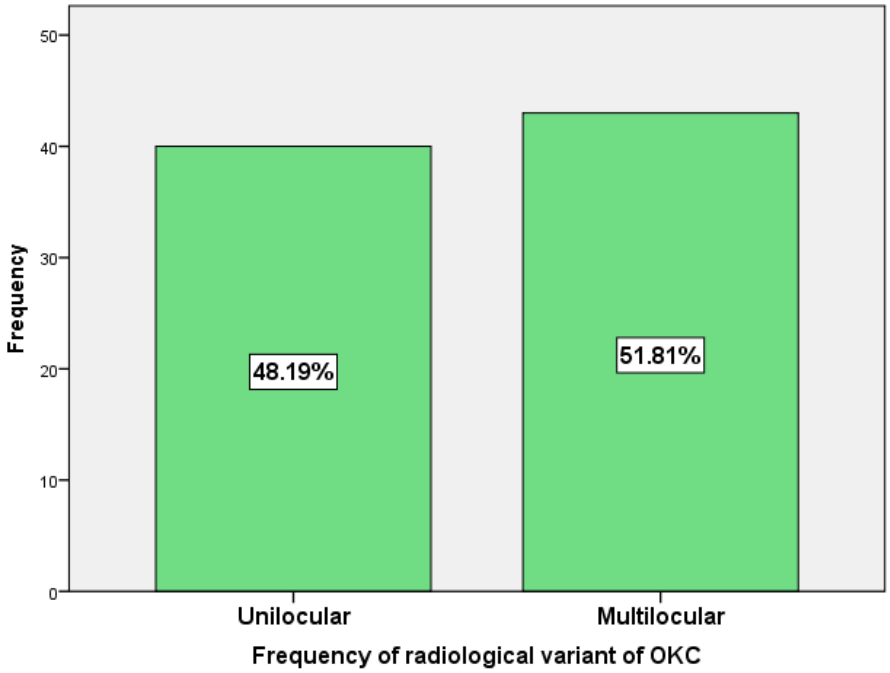

Figure 1: Multilocular variety of OKC was found more common than unilocular type.

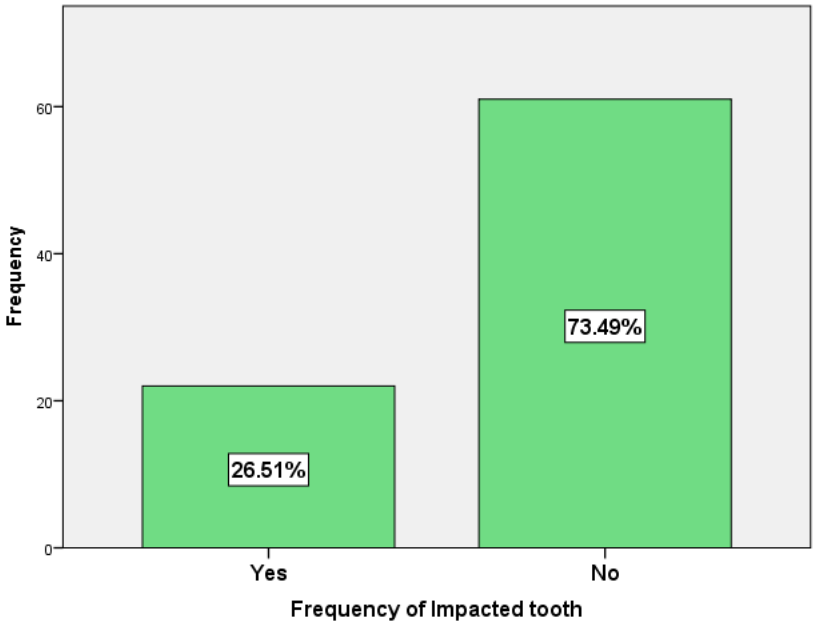

Figure 2: In 26.5 percent of cases OKC was associated with an impacted tooth.

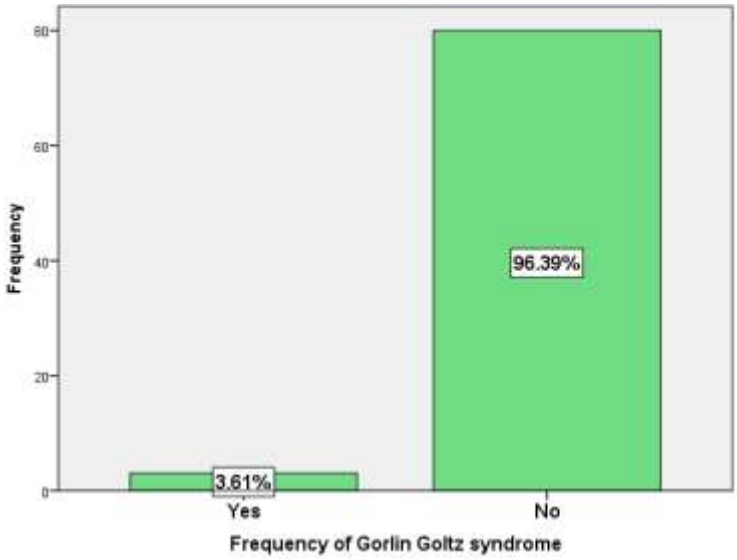

Figure 3: Solitary or non-syndromic OKC was more common than syndromic OKC.

Table 3: Association of Twist expression with radiological variant of OKC.

TWIST EXPRESSION	RADIOLOGICAL VARIANT OF OKC			
	UNILOCULAR		MULTILOCULAR	
	Frequency	Percent	Frequency	Percent
Negative	30	75.0	2	4.7
Positive	10	25.0	41	95.3
TOTAL	40	100.0	43	100.0

***Twist immuno-reactivity was more common in the multilocular type of OKC.**

DISCUSSION

Odontogenic keratocyst (OKC) originates from dental lamina. Radiographically, it presents as unilocular or multilocular radiolucency and are lined by parakeratinized stratified squamous epithelium with basal palisading. OKC is potentially a local infiltrative pathology having a debatable biological behavior.[20] The gender & age distribution lie within the calculated range of the study report performed by S.Mohanty in which male patients were more common in their fourth decade of life.[21] The age of the patients suffering from odontogenic keratocyst in our study ranged from 14 to 65 years, which is different from the study performed by N.Bushabu which shows age range from 7 to 81 years.[22] However the mean age in our study (38.12) was a little high as compared to this study (36.01).There is very less awareness about the oral health in our community. That is the reason why odontogenic keratocyst is diagnosed lately in our country. This study is in contrast with the study performed by Juliana campos et al,[23] in which they mention females were more commonly involved with a "female to male ratio of 1.09:1" compared to our study where the male to female ratio was 1.2:1.

In this study unilocular variety of odontogenic keratocyst was found in 40 cases (48.2%), while multilocular variant was found in 43 cases (51.8%), which shows that multilocular variety of OKC is slightly more common. This is in contrast with the study performed by Bushabu et al which shows that unilocular OKC is more common.[22] This difference may be due to genetic and ethnic variation. Our study is similar with the description draw by Khurshid, Samrina et al., where they found the multilocular variant to be the most frequent type.[24]

Amongst the 43 cases of multilocular variant of odontogenic keratocyst, 41 cases (95.3%) were twist positive and 2 cases (4.75%) were negative, while amongst the 40 cases of unilocular variant of odontogenic keratocyst, 10 cases (25%) were twist positive and 30 cases (75%) were negative.

To the best of our knowledge there is no single study which shows the association of Twist expression with the locularity of odontogenic keratocyst. This study shows high positivity in multilocular variant. All the three syndromic patients were of multilocular type and were recurrent and multiple OKC cases. It means that multilocular variant of OKC are

more aggressive and have more recurrent potential as compared to unilocular variety. This concept is explained by Avril L et al, in his study which shows that multilocular lesions are more aggressive and unilocular lesions are slow growing and benign.[25]Therefore multilocular OKC must be given special attention and needs meticulous follow up. Bruno R [26], in his study described that there is no clear-cut difference between the recurrence of female and male patients but there is a significant difference between multilocular and unilocular lesion. Another study found that multilocular OKC extend to intramedullary bone rather than the cortical bone, that's why causes more bone resorption and destruction.[27] This supports the findings that Twist are more positive in the multilocular lesions. Onur Yilmaz and coauthors also confirmed in their study that multilocular OKC have a high recurrence rate.[28]Another study also confirmed that in the case of complex histopathological assessment, multilocular appearance, irregular margins and cortical bone involvement, OKC must be consider for strict follow-up.[29] This supports our findings that multilocular OKC are more invasive.

In summary, Twist expression was evaluated in both unilocular and multilocular variant of OKC, and we found a diverse immune positivity of Twist proteins in these variants of OKC, with the unilocular type mostly showing Twist negativity and multilocular type showing more Twist reactivity.

CONCLUSIONS

We concluded from this study that in the multilocular variant of OKC, whether solitary or syndromic, its increased rate of recurrence and pathogenesis is greatly influenced by Twist expression and suggests its classification as odontogenic tumour.

1. **Author Contribution:**
   NK and BA conceived the study and developed the methodology. NAK, and BA contributed to data collection, analysis, and interpretation. NK, BA, AM, NAK, HRA, AST, SA, MAA, and FA supported the literature review and manuscript preparation. SA, AST, AM, NAK, HRA, MAA, FA critically revised the manuscript. All authors reviewed and approved the final manuscript.
2. **Funding:** No outside source of funding was used for this project.
3. **Institutional review board statement:** After approval from the Graduate Study Committee and the Advanced Study and Research Board of Khyber Medical University (approval no. KMU/IPDM/IEC/2021/47) the study was conducted in Institute of Pathology and Diagnostic Medicine (IPDM) Khyber Medical University, Peshawar.
4. **Ethical Approval:** Study was conducted after obtaining the ethical approval from Khyber Medical University (approval no.: KMU/IPDM/IEC/2021/47).
5. **Patient consent:** Samples and data were collected from the patients of Hayatabad Medical Complex, Khyber Medical University, Peshawar, who willingly participated in the study after providing informed consent.
6. **Declaration of available data:** The data will be provided on request by principal investigator, Dr Naveed Khan; drnaveedkhan81@yahoo.com.
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8. **Conflict of interest:** The authors declare no conflict of interest. All authors have reviewed and approved the manuscript for publication.

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