IMMUNOEXPRESSION OF TWIST IN PATIENTS PRESENTING WITH ODONTOGENIC KERATOCYST

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ABSTRACT

Objectives: *To evaluate the immunohistochemical expression of Twist in patients presenting with Odontogenic keratocyst.*

Materials and Methods: *This study was performed at the department of Pathology, "Khyber Medical University Peshawar" from January 2022 to June 2022.A total of 83 patients meeting the inclusion criteria were included. All the patients were diagnosed for OKC according to WHO criteria.Twist immunoexpression of OKC blocks were peroformed according to IHC protocol. SPSS version 22 was used for the analysis of data.*

Results: *Out of eighty three cases of Odontogenic keratocyst, 51 cases were positively stained for Twist (61.4%),while 32 cases were negatively stained (38.6%).Amongst the positively stained cells 17 cases (20.5%) were mild positive,16 cases (19.3%) showed moderate immunopositvity and eighteen cases (21.7%) exhibited strong positivity for Twist.*

Conclusion: *It is concluded that twist immunohistochemiacal analysis shows negative to strong positive results in odontogenic keratocyst, suggesting its possible neoplastic nature.*

Key words: *Odontogenic keratocyst, Twist gene, Epithelial mesenchyamal transition (EMT), impacted tooth*

INTRODUCTION

The most prevalent odontogenic intrabony pathology is odontogenic cysts.It affects the maxilla and mandible. Histologically, it is made up of an odontogenic epithelial remnant-derived lining and a fibrous wall[1]. Odontogenic Keratocyst was initially described by Philipsen in 1956. Okc develops from the dental lamina. It is located anywhere in the jaw, but posterior part of the mandible is the most common location[2]. Odontogenic keratocyst occur slightly more common in males. A ratio of 1.3 to 1 exists between men and women. Highest prevalance occur in twenty to thirty years of life[3]. Odontogenic keratocyst was reclassified by World Health Organisation in 2005 from an odontogenic cyst to an odontogenic tumour[4]. Again in 2017 WHO re-classified it from keratocystic odontogenic tumour into odontogenic keratocyst[5]. This controversial classification system of OKC shows the limited knowledge in the biological behavior of this lesion. There are two types of Odontogenic keratocyst,one is solitary or nonsyndromic and the other is multiple or syndromic Odontogenic keratocyst.Gorlin Goltz syndrome is associated with OKC[6].

"Odontogenic keratocyst" is notorious for its local aggressiveness and increased rates of recurrence[7,8]. 5-70 % is the range for the recurrence of OKC. Frequency of Odontogenic keratocyst is 12-14%[9].

Pain and swelling are the most common symptoms but in its early stages it is completely asymp-

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tomatic[10]. Lininig of Odontogenic keratocyst can be transformed into Squamous cell carcinoma[11].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. It is involved in invasion and tumour spread[12]. In EMT process lack of E-cadherin expression is the salient feature. Reduction of E-cadherin is linked with Twist up regulation. Twist acts as an oncogene, promote angiogenesis, drug resistance in tumour and action against apoptosis. Metastasis and invasion of different cancers like osteosarcoma,prostate cancer,breast cancer,and hepatocellular carcionoma are associated with Twist over expression[13]. Destruction of bone, local infiltration, and the capability of recurrence of odontogenic keratocyst are the factors by which we can assume that overexpression of twist may be responsible for the pathogenesis of odontogenic keratocyst. This study will help the clinicians to better forecast this lesion behavior and will help in planning for better management strategy. Targeting Twist can be used for odontogenic keratocyst therapy.This study will further aid in understanding the biologic behavior of this incompletely understood lesion, and will help in deciding its controversial classification system.

MATERIALS AND METHODS

This study was done on 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 institutional review board. Convenience non probability sampling was used to include all patients of all ages and both genders diagnosed clinically and histologically as odontogenic keratocyst , both the newly diagnosed and recurrent cases and both syndromic and non syndromic odontogenic keratocyst cases were included. Patients of odontogenic keratocyst having carcinomatous transformation and with incomplete medical record and history were exluded.

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. Tissue specimen were collected in properly labelled jars containing 10% neutral buffered formalin solution. Detailed gross examination was carried out. 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"14 were performed for the diagnosis of odontogenic keratocyst according to WHO criteria.6 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. Every 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 stain 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 %age 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)

The data was entered and analyzed 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.

RESULT

The age of the patient suffering from Odontogenic keratocyst ranged from 14 to 65 years with

the average age of 38.12 years ±10.13 S.D. "The mean"age of the male patients was 37.09 years ±11.26 S.D.The mean age for the female patient was 39.41 years ±8.49 S.D.

Most of the patients were from thirty-one to forty years of age (45.8%) followed by age group 41 to 50 years (27.7%). 2.4% of the patient were above 60 years,and this age group accounted for the smallest proportion.This data is shown in the Table 1.Male patients were more than females. 46 (55.4%) were male and 37 (44.6%) were females. Ratio of male and female was 1.2:1. This data is represented in Table 2.

The mandible was the most common site of involvement accounting for 57 cases (68.7%),while the maxilla was involved in 26 cases (31.3%). This is shown in Table 3. The Twist immunostain positivity was assessed as a percentage.In the reactive cells the "twist" was seen in cytoplasm and nuclei of the cells.

Out of eighty three cases of Odontogenic keratocyst, 51 cases were positively stained for Twist (61.4%),while 32 cases were negatively stained (38.6%).Amongst the positively stained cells 17 cases (20.5%) were mild positive,16 cases (19.3%) exhibited moderate immunopositivity and 18 cases (21.7%) showed the highest immunopositivity.This data is shown in Table 4 .

Table 1: Age allocation in 83 cases of Odontogenic keratocyst

Age in Groups(in years)	Frequency	%
Less than twenty years	3	3.6
Twenty -- thirty years	14	16.9
Thirty one – forty years	38	45.8
Forty one -- fifty years	23	27.7
Fifty one – sixty 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)

Sex	Frequency	%
Male	46	55.4
Female	37	44.6
Total	83	100

Table 3: Jaw distribution of OKC

Jaw	Frequency	Percent
Mandible	57	68.7
Maxilla	26	31.3
Total	83	100

DISCUSSION

Odontogenic keratocysts are derived from the odontogenic tissue in the mandible and maxilla.It is unicystic or multicystic and are lined by parakeratinized "stratified squamous epithelium". After the 2005 WHO classification OKC was the most common gnathic lesion[15]. There are many similarties between our and other studies.The gender & age allocation lie with in the calculated range of the study report performed by s.Mohanty in which male patients were more common in their fourth decade of life[16]. The age of the patient 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[17]. 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.Our study is in contrast with the study performed by Juliana campos et al,[18] 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 is 1.2:1.Twist is an endogenous protein which is necessary for the morphogenesis of embryo, and a transcription factor. For the ist time in animal model, the role of twist was observed in breast carcinoma.In our study we evaluated twist expression in 83 cases of odontogenic keratocyst.

Out of eighty three cases of Odontogenic keratocyst, 51 cases were positively stained for Twist (61.4%),while 32 cases were negatively stained

Table 4: Score of Twist expression in 83 cases of Odontogenic keratocyst

Score	Frequency	Percent
Negative (10% or Less cells positive)	32	38.6
Mild positive (11-25% cells positive)	17	20.5
Moderate positive (26-50% cells positive)	16	19.3
Strong positive (More than 50% cells positive)	18	21.7
Total	83	100.0

(38.6%).Amongst the positively stained cells 17 cases (20.5%) were mild positive,16 cases (19.3%) showed average immunoreactivity and 18 cases (21.7%) exhibed highest positivity.There is only one study in the world which shows the immunoexpression of twist in odontogenic keratocyst performed by Azadeh andisheh and co authors[19]. In this study 44.4% cases were positively stained for twist. Among the positively stained cases, 11% were mild positive, 16.7% were moderately positive and also 16.7% were strongly positive. 55.5% cases were negatively stained. Our study is the second study which shows twist expression.There is no detailed study which shows the association between histopathological morphology of OKC with the invasiveness and recurrent ability.

Therefore, the absence of research in this area adds to the widespread perception that lesions that express more twist are thought to be more aggressive as literature shows the worst outcome in many cancers like breast carcinoma and nasopharyngeal carcinoma with a high "twist" score[20,21].

The pathophysiology and clinical behaviour of OKC is significantly influenced by the epithelial lining. Tran et al in his study showed that Twist expression is associated with the survival and is highly suggestive of the recurrent disease[22]. As OKC is notorious for its recurrence, we can say that Twist is involved in its pathogenesis. Apart from this Twist is negatively correlated with p53 protein, it prevents apoptosis and enhance proliferation,Therefore in OKC Twist expression may induce high cellular multiplication and cause invasive and aggressive behavior.

Some of the vary aggressive tumours in the mandible also mimick as odontogenic keratocyst like carcinoma cuniculatum which is basically a low grade squamous cell carcinoma. It involves oral mucosa and can involve the underlying bone and presents as a cystic cavity. Many cases were presented in the mandible,and it pose a difficult challenge for the pathologist due to its overlapping clinical and pathological features with OKC[23]. Many cases were misdiagnosed as OKC.So our this study will further help to differentiate between these lesions.

CONCLUSION

We concluded from this study that increased rate of recurrence and pathophysiology of Odontogenic keratocyst is highly influenced by Twist expression and suggests its place in the neoplastic category.

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