ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 05 | May - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IMMUNOHISTOCHEMICAL EXPRESSION OF POLYCYSTIN-1 IN ODONTOGENIC KERATOCYST AND DENTIGEROUS CYST

Dentistry

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ABSTRACT

Aim: Immunohistochemical assessment of polycystin-1[PC 1] and to compare and correlate the expression of polycystin-1 in odontogenic keratocyst [OKC] and dentigerous cyst [DC]. **Materials and Method:** Retrospective study conducted by retrieving 60 cases of neutral buffered formalin fixed paraffin embedded blocks of OKC, DC, normal tissue, from the archives of the department of Oral pathology. 4µm sections are stained immunohistochemically using antibodies against polycystin-1, obtained from Mouse monoclonal antibody. Positive and negative stained cells will be counted with the help of Olympus BX51 rarefaction microscope and will be analyzed quantitatively using image analysis pro plus software. **Results:** Diffuse distribution pattern was observed with statistical significance in all the groups. The percentage of positive cells expressing PC1 within the strata was more in superficial layer of OKC and basal layer of DC with significant value. Overall immunoreactivity of PC1 was weak to strong in OKC and strong in DC. **Conclusion:** PC1 overexpression reflects the cystic progression thereby providing the pathologist to take necessary measure to curtail the process of cystogenesis.

KEYWORDS

Polycystin, odontogenic keratocyst, dentigerous cyst, Cilia.

INTRODUCTION

The proliferation rate, aggressive nature, neoplastic potential of odontogenic cysts (OC) are assessed by molecular markers. Cilia in mammals and its proteins are one such aids which are ignored till date by oral researchers. Polycystin 1(PC1), an integral transmembrane protein has been associated with primary cilium and is a major ciliary protein.[1] PC1 expression in odontogenic cystic epithelium helps us to assess the proliferative potential of the epithelium to link and evaluate the growth, expansion and invasion of the cyst thereby appreciating the biological behavior, molecular confirmation and early intervention of Ocs.

MATERIALS AND METHODS

This is a retrospective study conducted by retrieving previous records of 10% neutral buffered formalin fixed paraffin embedded tissue blocks of OKC and DC from the department of oral pathology. As controls, normal gingiva specimens are collected from department of oral and maxillofacial surgery from the patients who underwent surgical extraction of impacted third molars. The study sample included 70 histopathologically diagnosed cases which include 30 OKC and 30 DC, 10 normal gingiva. The set sample was further segregated demographically by age, gender, and lesion site and habit history from the records. The tissues were sectioned at 4 micrometers and mounted on positively charged slides. Deparaffinisation is done by incubating the slide for one hour at 60 degrees and subsequent two immersions in xylene for 10 minutes each. The tissue sections were rehydrated by placing in descending grades of alcohol i.e in absolute alcohol for 10 minutes and 89% alcohol for 10 minutes. Then the slides are washed with distilled water. Antigen retrieval was done by treating the slides with preheated tri sodium citrate buffer [ph-8.0] for 5 minutes in pressure cooker and allowed to cool to room temperature. Slides are then placed on immunohistochemistry (IHC) platform and are washed with Phosphate buffer saline (PBS) twice each for 5min. Sections are encircled with PAP pen & peroxide block is added for 10 min & wash. Then power block is added for 10 mins and wash with PBS. Slides are incubated with primary antibody for 1 hour and then wash with PBS for 2 times 5 min each. Primary antibody enhancer was added for 20 min & then washed with PBS for 2 times 5min each. Slides are incubated with secondary antibody Poly HRP for 30 min and then washed with PBS for 5 min. Slides are incubated with DAB substrate chromogen for 3min & washed in distilled water for 5min. Counterstaining was done with Harris Haematoxylin for 3min followed by bluing in running tap water. Dry the slides and dehydrate in absolute alcohol for 3 min followed by xylene 5 min. Dry and Mount with DPX. Stained slides are observed under light microscope.

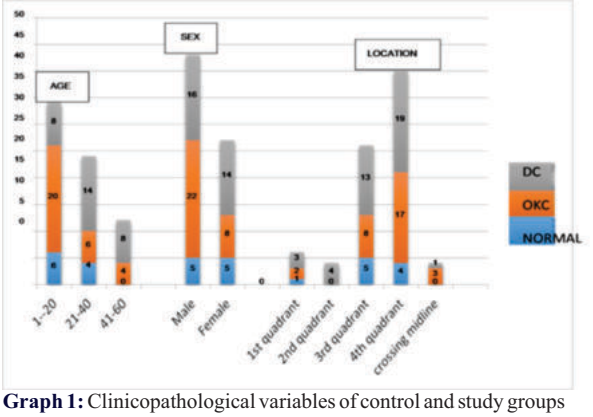
RESULTS

In our study 43 males and 27 females, total of 70 cases were included. Age incidence ranges from 1-60 years with predominant cases reported in 1st to 4th decade of life. A total of 44 cases of lesions were recorded in the lower jaw with 25 cases of OKC and 19 cases of DC. (Table 1and Graph 1)

Table1: Clinicopathological Variables Of Control And Study Groups

Variable	category	Group			Total	Chi square value	P value
		Normal	OKC	DC			
Age (n=70)	1-20	6 (17.6%)	20 (58.8%)	8 (23.5%)	34	12.033	0.017
	20-40	4 (16.7%)	6 (25.0%)	14 (58.3%)	24		
	41-60	0	4 (33.3%)	8 (66.7%)	12		
Gender (n=70)	MALE	5 (11.6%)	22 (51.2%)	16 (37.2%)	43	3.175	0.204
	FEMALE	5 (18.5%)	8 (29.6%)	14 (51.9%)	27		
Location (n=70)	1ST QUADRANT	1 (10%)	2 (6.7%)	3 (10%)	6	11.541	0.173
	2ND QUADRANT	0	0	4 (13.3%)	4		

	3RD QUADRANT	5 (50%)	8 (26.7%)	13 (43.3%)	26		
	4TH QUADRANT	4 (40%)	17 (56.7%)	9 (30.0%)	30		
	MANDIBLE	0	3 (10%)	1 (3.3%)	4		
	MAXILLA	0	0	0	0		



Graph 1: Clinicopathological variables of control and study groups

Among study and control groups, normal gingiva and DC showed diffuse staining distribution pattern for polycystin 1. In OKC 27 cases show diffuse staining distribution whereas 3 cases exhibited focal distribution. Comparison of distribution among all the groups did not show any statistical significance. (Table 2)

Table 2: Comparison Of Staining Distribution Pattern For Polycystin 1 Within Groups

Group	n	Distribution	
		FOCAL	DIFFUSE
Normal	10	0	10
OKC	30	3	27
DC	30	0	30
Chi square value=4.179		p value=0.124	

Comparision of intensity of polycystin 1 in different layers of control group and study group showed that 7 cases of OKC with moderate staining in the basal layer, 23 cases with superficial staining, 11 cases exhibiting moderate intensity and 12 cases exhibiting strong intensity. In DC strong intensity was seen in basal layer (n=13) and superficial layers (n=6). Comparison of intensity was statistically significant in OKC. The difference in staining pattern was significant with a p value of 0.015. (Table 3)

Table 3: Comparison Of Intensity Of Polycystin 1 In Different Layers Of Control Group And Study Groups

Group	Layers	N	weak	moderate	Strong	t value	p value
Normal	Basal	0	0	0	0	--	--
	superficial	10	0	10	0		
OKC	Basal	7	0	7	0	1	0.014
	superficial	23	0	11	12		
DC	Basal	24	3	8	13	2	0.114
	superficial	6	0	0	6		

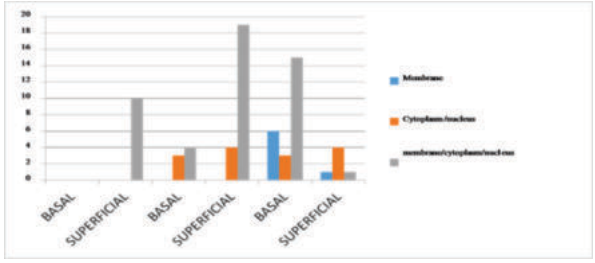
Moderate intensity was observed in control group, 18 samples of OKC and 8 DC. Strong intensity was observed in 12 cases of OKC, and 19 cases of DC. The difference in staining pattern was significant with a p value of 0.001.

Comparision of localization of polycystin 1 in different layers of control and study groups showed that OKC had predominance of PC1 in all membrane, cytoplasm and nucleus in both basal (n=4) as well as superficial layers (n=19). In DC localization of PC1 was seen in membrane, cytoplasm and nucleus among basal layers (n=15) and cytoplasm with nuclear localization was seen in superficial layers (n=4).The difference in localization pattern was statistically significant. with p value of (0.016).

Strong immunoreactivity was observed in 36.7% of cases of OKC and 53.3% of cases of DC.(Table 4,5 and Graph 2,3)

Table 4: Comparison Of Localization Of Polycystin 1 In Different Layers Of Control Group And Study Groups

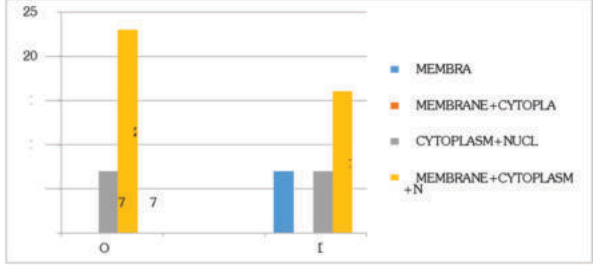
Group	Layers	n	Membran e	Membrane+ cytopla sm	Cytoplasm+nucleus	membrane +cytoplasm+nucleus	t value	p value
Normal	superficial	10	0	0	0	10		
OKC	Basal	7	0	0	3	4	1	0.163
	superficial	23	0	0	4	19		
DC	Basal	24	6	0	3	15	2	0.018
	superficial	6	1	0	4	1		



Graph 2: Comparison of localization of polycystin 1 in different layers of control group and study groups

Table 5: Comparative analysis of localization of PC1 between OKC and DC

Group	n	Membrane	membrane / cytoplasm	cytoplasm/ nucleus	membrane+ cytoplas m+ nucleus
OKC	30	0 0%	0 0%	7 23.3%	23 76.7%
DC	30	7 23.3%	0 0%	7 23.3%	16 53.3%
Chi square value =8.256		P value=0.016			



Graph 3: Comparative analysis of localization of PC1 between OKC and DC

The over expression of PC1 in DC and OKC is assessed for the percentage of positive cells, a quantitative analysis. This was performed by taking the mean of cells exhibiting PC1 expression in the superficial strata and throughout the thickness of epithelium in these cystic lesions.

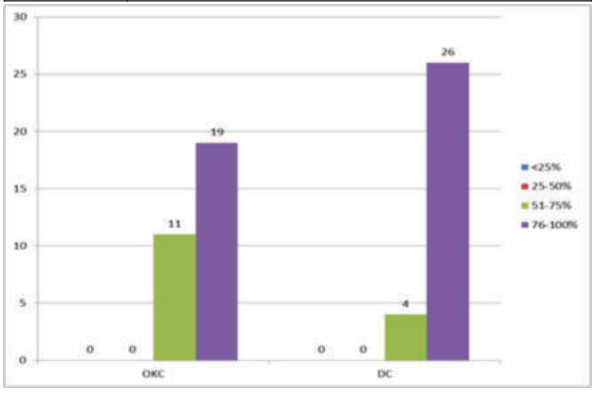
Table 6: Comparative analysis of average percentage of positive cells demonstrating between OKC and DC

Group	n	Mean + SD	<25%	25-50%	51-75%	76-100%
OKC	30	77.63±9.09	0	0	11 36.7%	19 63.3%
DC	30	1.00E2±0.00	0	0	4 13.3%	26 86.7%
Chi square value =4.356		P value=0.037				

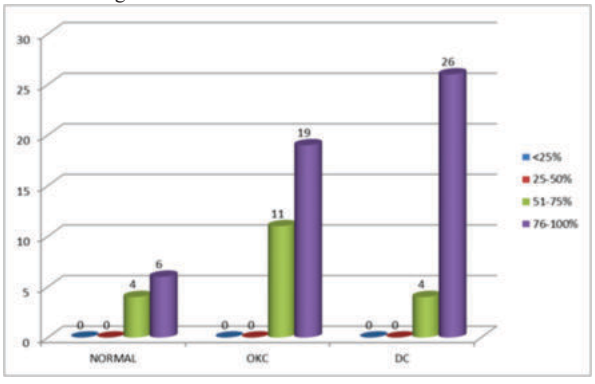
Table 7: Comparative Analysis Of Average Percentage Of Positive Cells Demonstrating Between Control And Study Groups

GROUP	n	Mean + SD	<25%	25-50%	51-75%	76-100%
Normal	10	83.00±10.83	0	0	4 40%	6 60%

OKC	30	77.63±9.09	0	0	11 36.7%	19 63.3%
DC	30	1.00±0.00	0	0	4 13.3%	26 86.7%
TOTAL	70		0	0	19 27.1%	51 72.9%
Chi square value = 5.105	P value=0.078					



Graph 4: Comparative analysis of average percentage of positive cells demonstrating between OKC and DC



Graph 5: Comparative analysis of average percentage of positive cells demonstrating between control and study groups

DISCUSSION

Human cells need complex communication systems to function effectively which contribute to histogenesis, morphogenesis and organogenesis.[2] Hh pathway determines cellular differentiation during embryonic and fetal development and remains active in the adult body and helps in maintenance of stem cell population.[3] Sonic hedgehog (Shh) represents a highly-conserved marker and plays a role in odontogenesis amongst the toothed vertebrates, SHh influences cell cycle regulation, morphogenesis and differentiation in the tooth germ.[4] Abnormal activation of the Hh pathway results in aberrations in odontogenesis causing the development of many odontogenic cysts and tumors. This abnormal activity is caused by mutations in the proteins like Patched gene (PTCH), Smoothened (Smo) and Glioblastoma (Gli). Studies show that loss of Ptch and Shh signaling pathways are involved in the cystogenesis of OKC and dentigerous cyst.[5] Shh is channelized through primary cilium an immotile structure on the plasma membrane. Primary cilia are found in tooth epithelium and mesenchyme cells at early stages of tooth development.[4]

It is established fact that cilia and ciliary proteins are involved in Hedgehog, Tumour growth factor beta (TGF-β), Wnt, Platelet derived growth factor receptor alpha (PDGFRα), Notch, and Hippo inter-cellular signalling systems.[6] Ciliary proteins play an important role in functional and metabolic aspects of cells.

Polycystin-1 is a modular membrane protein with a long extracellular N-terminal portion and an intracellular C-terminal portion with several phosphorylation signaling sites. This positioning of the protein allows it to interact with other proteins, carbohydrates, and lipids outside the cell and to receive signals that help the cell respond to its environment.[7] Polycystin-1 helps in regulation of cell growth and division, cell migration, and interactions with other cells.[5,6]

Loss of heterozygosity for PTCH gene is crucial for the development of dentigerous cysts and the same pathogenesis is observed in Basal cell carcinoma (BCC) and OKC.[5] Several authors revealed that PC1, a ciliary protein is an indicator to pathogenesis of DC and OKC involving Shh pathways to assess the cystic growth, proliferation and aggressive nature. Odontogenic keratocyst is known for its higher proliferative potential, high recurrence rate, aggressive nature and infiltrative behavior.This cyst was derived from the dental lamina or its remnants or even may be from the extensions of basal cells of the overlying epithelium[9] and has incidence of 13.8% worldwide and about 37.5% in Indian population.[10]

In the present study, OKC has peak incidence in 1st and 2nd decade with a male predilection. Maximum number of cases recorded in the mandible. DC the second most common developmental cyst after OKC showed peak incidence in 3rd and 4th decade with equal gender predilection and mostly seen in mandible which was similar to the studies conducted by Nigel R.Johnson et al,[7] Deepashri H. Kambalimath et al.[11]In the present study, OKC and DC were immunohistochemically stained with PC1 a ciliary protein which is believed to play a role in the cystogenesis of these OCs. The immunohistochemical staining done using anti PC1 (rabbit polyclonal antibody) presented 100% immunopositivity in 30 cases of OKC, 30 cases of DC and 10 cases of normal gingiva which is a control group. 27 cases of OKC and 30 cases of DC were showed diffuse staining pattern throughout the epithelium whereas 3 cases of OKC showed focal distribution.

Intensity of staining determines the activity and the concentration gradient of a protein.[4] In this study intensity was compared between strata of epithelium of OKC and DC. The control group exhibited moderate staining in the superficial strata whereas the basal layer did not show any immunopositivity for PC1. Usually cells in the basal layer are at a continuous stretch of division in order to replenish the turnover of the epithelium. Cilia are not detectable when the cell enters the mitotic phase. This discloses the loss of immunopositivity in the basal layer is due to the cells being present in a phase of mitosis[9] Moderate intensity of PC1 was observed in OKC whereas DC revealed strong intensity.Difference in intensity among the layers of OKC disclosed that there is strong intensity in the superficial layers when compared to the basal layers. The superficial layers of OKC are at a greater mechanical strain both from the proliferative and apoptotic stress imparted over them. Also certain genes like Bcl2 in the lower layer and BAD-BAX in the upper layers also make the cells strenuous therefore driving towards strong intensity of PC1.[12] When the intensity of PC1 was compared among the layers of DC, both the layers exhibited strong intensity. When the intensity was compared between study groups, there was a statistical significance with p value. This is the first study describing the patterns of intensity among OKC and DC. A two-hit model was proposed to explain the nature of the cyst by Lee and Somlo. The first hit, a germline mutation inherited from the affected parent, is predisposing but not sufficient for cyst formation. The second hit, a somatic mutation in an individual cell, inactivates the normal PC1 allele and causes abnormal focal proliferation and tissue remodeling, giving rise to cyst formation. Second hit mutation also affects the severity of cyst progression.[14]

Polycystins – PC1 and PC2 play a critical role in mechanosensing and subsequent signaling events. A mutation in PC1 gene has reported to impart an aberration in the normal signaling cascades that trigger towards enhanced cell proliferation. The pressure from the proliferation in these cystic lesions is sensed by the PC1 which forms a complex with PC2 resulting in an influx of calcium.[13,14] This results in modified cAMP activated MAP/ERK pathway and downstream proliferation of the lining cells that are prone to cyst formation.[16] From this evidence it is clear that defective mechanotransduction due to deregulation of polycystins, perturbs the cystic epithelial lining cells to promote cystogenesis.[15] There is greater proportion of positive cells in the control group. Study group have exhibited >75% of positive cells, however no significance was observed when the percentage of positive cells were compared in different layers of OKC and DC. Probably because the cells are in an active phase of cystogenesis. PC1 expression is concentration dependent, in our study the percentage of positive cells between OKC and DC showed a statistical significance of p value. Majority of cases in DC have shown greater proportion of PC1 positive cells when compared to OKC. In OKC, PC1 expression could not be elucidated in actively dividing cells and only few cases have greater proportion of PC1. The staining was higher in superficial

layer when compared to the cases which involved entire thickness of epithelium. This is because the basal cells of odontogenic epithelium contribute not only to rapid proliferation but also to the formation of daughter cysts within the connective tissue.[17,18,19,20,21] Immunoreactive score of PC1 in OKC and DC were achieved by comparing qualitative and quantitative scores. These are divided into Weak (1-3), Moderate (4-5) and Strong (6-7). Study groups finding revealed a predominantly strong immunoreactivity for PC1 in DC. Varied immunoreactive scores were observed in OKC ranging from weak to strong with a statistically significant difference. Strong immunoreactivity for PC1 in OKC and DC discloses the structural and functional demands of the cystic lining cells. Structurally PC1 maintains the architecture of the cystic lining of these odontogenic cysts. Strong expression of PC1 reflects that there is an increase in demand for the cell adhesion molecules to withstand the intracystic pressure. So both the pressure and increase in the cell adhesion molecules has caused for the enhanced intensity and overexpression of PC1 in these odontogenic cysts. The possible inference from the positive correlations in the study has brought the authors to understand a new molecular target, PC1 in the cystogenesis of OKC and DC. PC1 would be a novel immunotherapeutic target because of its availability in each and every cell. This availability of PC1 makes it unique from other molecular targets which are confined to certain specific locations.[21] The greater the availability, greater the chance of targeting and greater the fortunity to regulate and impede the cystic progression. As rapid and aberrant cell proliferation is hallmark of cancers both epithelial and odontogenic, the expression of PC1 in such cases would definitely provide diagnostic and therapeutic values. Our authors have concluded that PC1 would certainly act as a type of master regulator of cell function because it has an ability to assemble and connect different cellular signaling pathways which are required for the normal homeostasis of a cell.

CONCLUSION

From the overexpression of PC 1 the abnormal cilia structure or function may be the common link in the pathogenesis of these OCs. A thorough understanding of the molecular pathways leading to the cystogenesis will definitely help to design preventive measures that can retard cyst formation and progression. Our study also provides an insight to develop certain anti PC 1 agents which can inhibit the aberrant cellular proliferation.

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