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Expression Of Nuclear Factor IC And Detection Of Calcium During Odontogenesis And Odontogenic Tumors

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Abstract
Background: *Tooth development is a complex and well-coordinated developmental process that is achieved through a series of reciprocal interactions between dental epithelium and neural crest-derived ectomesenchyme. The dental epithelium gives rise to the outer and inner enamel epithelium from which ameloblasts differentiate, whereas ectomesenchymal cells differentiate into odontoblasts. Nfic has a specific function as a key regulator of dentin formation. Nfic signaling modulates late odontoblast differentiation and mineralization. Both dental papilla cells and dental follicle cells have extensive proliferation ability, expressed similar cell surface antigens and were capable of forming hard tissue. But dental papilla cells had diverse calcium expression than that in dental follicle cells. Most of the literatures have focused the influence of Nfic and OPN in tooth germs. There is no clear evidence to elicit their influence in odontogenic tumours.*
Aims and objective: *The present study was intended to evaluate expression and elicit the influence of Nfic and calcium binding protein (OPN) in odontogenesis and odontogenic tumors.*
Materials and methods: *The study comprised of 20 tooth germs from human foetuses of terminated pregnancies with age ranging between 10 to 36 weeks and 30 paraffin embedded blocks of histopathologically diagnosed odontogenic tumours. Five micrometer thick sections were taken and stained immunohistochemically with Nfic and OPN and evaluated for their expression in tooth germs and in odontogenic tumors.*
Results: *In tooth germs, Nfic showed strong expression in odontoblasts and OPN showed strong expression in enamel matrix. Nfic showed positive expression in odontogenic tumours and OPN showed strong expression in calcification foci in AOT.*
Conclusion: *The present study was an attempt to assess the influence of Nfic during crown formation. Expression of Nfic in tooth germs confirmed its influence in crown formation. Expression of OPN suggested that it promotes mineralization in tooth germs, bone formation and calcification focus in odontogenic tumours.*
Keywords: *Calcium binding protein, Nfic, Odontogenesis, Odontogenic tumours, OPN, Ameloblast, Odontoblast.*

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I. Introduction:

Tooth development is a complex physiological process that includes the bud, cap and bell stages, tooth root development and tooth eruption. The interaction between dental epithelial and neural crest derived mesenchymal cells is essential for tooth development.[1] The dental epithelium gives rise to the outer and inner enamel epithelium from which ameloblasts differentiate, whereas ectomesenchymal cells differentiate into odontoblasts, cementoblasts, osteoblasts and fibroblasts.[2]

Nfic is a member of the nuclear factor I family, which includes Nfia, Nfib, Nfic and Nfix. The four nuclear factor I members function independently.[1] To assess when and where Nfic is expressed during tooth development, studies were done in situ hybridization with Nfic-specific probe.[3] Nfic functions as a key regulator during dentin formation. Nfic signaling modulates odontoblast differentiation. During tooth bud development,

Nfic is expressed strongly in the mesenchymal cells of the dental papilla and weakly in the epithelial components. Nfic is also expressed strongly in most tissues of the tooth, including ameloblasts, odontoblasts, surrounding mesenchymal tissues, including the stellate reticulum and the dental papilla of the molars and incisors.[3] Odontogenic tumors also exhibit haphazardly arranged dentin. Both dental papilla cells and dental follicle cells have extensive proliferation ability, express similar cell surface antigens and are capable of forming hard tissue. There is a difference between them such as dental papilla cells have diverse calcium expression than that in dental follicle cells.[3]

To our knowledge there are no studies regarding the analysis of Nfic and OPN in odontogenesis and odontogenic tumors. The measurement of their role may be important for better understanding of odontogenesis and pathogenesis of odontogenic tumours. They can be measured by quantification of cells and extracellular matrix immunolabelled by specific antibodies against Nfic and osteopontin respectively. By this approach, it may be feasible to correlate odontogenesis and pathogenesis of odontogenic tumours.

II. Materials And Methods:-

The present study was carried out in the Department of Oral and Maxillofacial Pathology, St. Joseph Dental College and Hospital, Eluru. Thirty paraffin blocks of histologically diagnosed odontogenic tumours were retrieved from archieves of the department and twenty tooth germs from human fetus in cases of terminated pregnancies were collected with parent consent from Siddhartha medical college and hospital, Vijayawada and the present study was approved by the Ethical Committee.

The study consisted of the following groups:
GROUP I: 20 tooth germs from human foetuses of terminated pregnancies with age ranging between 12 to 36 weeks.
GROUP II: 30 histopathologically diagnosed odontogenic tumours.

A total of 100 samples were treated with two different primary antibodies namely Nuclear Factor ic (50) and Osteopontin (50). Thirty paraffin blocks of odontogenic tumours were retrieved from archives of the department of Oral and Maxillofacial Pathology. After obtaining parents consent, human foetuses ranging from 12 – 36 weeks were carefully dissected for tooth germs. After sufficient fixation in 10% formalin, specimens were grossed and labeled for identification. This was followed by processing and embedding in paraffin wax. Formalin fixed paraffin embedded tissue samples were sectioned at 4-5 microns thick sections using semi-automatic microtome. Sections were placed on poly-Lysine coated slides and a total of 100 samples were treated with two different primary antibodies namely NFic and OPN markers. Stained IHC slides were analyzed under Trinocular Olympus Bx53Progress CT research microscope.

After immunohistochemical staining, expression of Nfic and OPN were observed in tooth germs and odontogenic tumors. Particular areas like ameloblasts, stratum inetrmedium, stellate reticulum, enamel matrix, odontoblasts, dental papilla, osteoblasts and dental follicle in tooth germs and epithelial islands, cystic lining and connective tissue in odontogenic tumors were observed under magnification 40X to determine the localization and intensity of staining.

III. Results:

The present study comprised of 20 tooth germs from human fetus and 30 cases of odontogenic tumours. All the sections were treated immunohistochemically with Nfic and OPN antibodies and examined to determine their influence in odontogenesis and odontogenic tumors.

Tooth germs in the advanced bell stage showed strong expression of Nfic in odontoblasts in dental papilla near dentinoenamel junction, but the mesenchymal cells in dental papilla showed moderate expression of Nfic. The ameloblasts, stratum intermedium and stellate reticulum in enamel organ showed moderate expression of Nfic. Mesenchymal cells also showed weak expression in dental follicle (Table 1). Strong expression of OPN was observed in ameloblasts, stellate reticulum and enamel matrix where as the other components of enamel organ like stratum intermedium showed negative expression. Moderate expression was observed in odontoblasts, mesenchymal cells of dental papilla whereas osteoblasts in dental follicle showed weak expression (Table 2).

The studied odontogenic tumour specimens showed strong expression of Nfic in the cuboidal epithelial cells of plexiform ameloblastoma (PA), peripheral columnar epithelial cells in follicular ameloblastoma (FA), stellate reticulum like cells in follicles showed weak expression. In adenomatoid odontogenic tumour (AOT), the columnar cells in epithelium forming duct like structures showed strong expression and two thirds of basal epithelium in OKC also showed strong expression but the cells in underlying connective tissue showed weak expression. In desmoplastic ameloblastoma (DA), moderate expression was observed in fibroblasts of the connective tissue. In unicystic ameloblastoma (UA), cystic lining showed moderate expression and cells in connective tissue showed weak expression. The stellate shaped fibroblasts in mesenchyme of OM showed weak expression and negative impression was observed in epithelial islands. OPN showed strong expression in

stellate reticulum like cells in PA but in cuboidal epithelial cells, weak expression is observed. In AOT, calcification foci showed strong expression of OPN. Moderate expression of OPN is observed in connective tissue within the follicles in FA. In UA, cystic lining showed moderate expression of OPN and the underlying connective tissue showed negative expression. The basal and parabasal layers of OKC showed moderate expression of OPN where as the connective tissue cells showed negative expression. In DA, weak expression of OPN was observed in connective tissue of DA and negative expression was observed in epithelial islands. OPN showed weak expression in stellate shaped fibroblasts in mesenchyme of OM but negative impression is seen in epithelial islands (Table 3).

TABLE 1: Immunohistochemical profile of Nfic in tooth germs

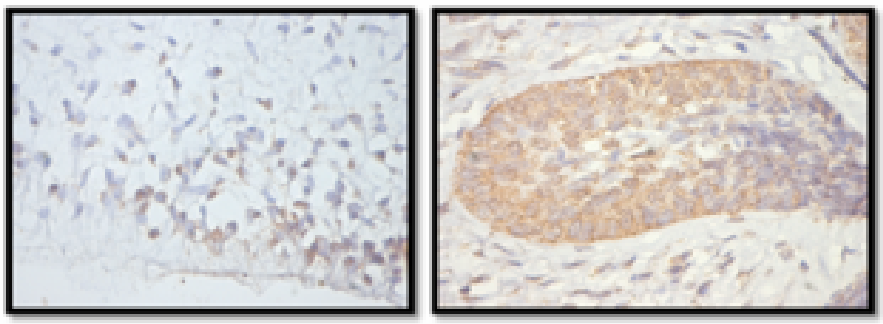
Location	Nfic		
	Strong	Moderate	Weak
Enamel organ	–	Ameloblasts Stratum Intermedium Stellate reticulum	–
Dental papilla	Odontoblasts	–	–
Dental follicle	–	Osteoblasts	Mesenchymal cells

TABLE 2: Immunohistochemical profile of OPN in tooth germs

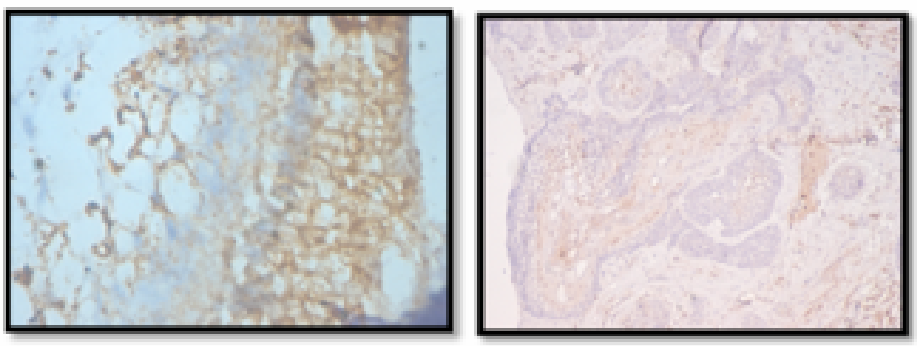
Location	OPN		
	Strong	Moderate	Weak
Enamel organ	Enamel matrix Ameloblasts Stellate reticulum	–	–
Dental papilla	–	Odontoblasts	Mesenchymal cells
Dental follicle	–	Osteoblasts	–

TATABLE 3: Immunohistochemical profile of Nfic and OPN in odontogenic tumours

IHC Markers	Strong	Moderate	Weak
Nfic	PA FA AOT OKC	DA UA	OM
OPN	PA AOT	FA UA OKC	DA OM



Immunohistochemical expression of Nfic in Tooth germ
Immunohistochemical expression of Nfic in FA



Immunohistochemical expression of OPN in tooth germ
Immunohistochemical expression of OPN in FA

IV. Discussion:

Nfic in tooth germs:

In the present study, the results showed strong expression of Nfic in odontoblasts, moderate expression in ameloblasts, stratum intermedium and stellate reticulum and weak expression in mesenchymal cells in dental follicle. Therefore, it is suggested that Nfic is expressed at multiple stages of tooth development and strongly in odontoblasts during advanced bell stage. Our results were in accordance with the study done by **Steele-Perkins** where they also found that Nfic is expressed in most of the tissues of the tooth, including ameloblasts, stellate reticulum, odontoblasts, surrounding mesenchymal tissues and in the dental papilla.[3] According to **Xiao Feng Huang**, Nfic appeared to play an important role in root, but not crown dentin formation. The possible reason is lack of dentin formation in Nfic knocked out mice in the dental mesenchyme. The present study also elicited the expression of Nfic in mesenchymal cells of dental follicle. These mesenchymal cells might be progenitors of osteoblasts which have expressed the transcription replication factor called Nfic. It was suggested that Nfi-c controls bone formation during the postnatal stages. Osteoblast activity was determined in femur of 6-week-old mice. Nfic -/- mice showed decreased number of osteoblasts and proliferation rate, which possibly impaired osteoblast function during bone formation.[4]

Nfic in odontogenic tumors:

Nfic in ameloblastoma:

In the present study, the results showed strong expression in cuboidal epithelial cells of plexiform ameloblastoma and columnar epithelial cells of follicular ameloblastoma. But its expression was moderate in stellate reticulum like cells in FA, connective tissue of desmoplastic ameloblastoma and cystic lining of unicystic ameloblastoma. **Leider et al** suggested that ameloblastomas may recapitulate some of the cell types and developmental events, observed in the developing enamel organ, but the tumour cells fail to synthesize enamel matrix protein. The suggested tissues for origin of this tumour are enamel organ, remnants of dental lamina, cell rests of malassez, epithelial lining of odontogenic cysts and oral epithelium.[5] The evidence of expression of Nfic in ameloblastoma would suggest the synthetic activity of ameloblast like cells. Ameloblastomas express growth factors like FGF and its receptors. It might be because of Nfic regulating FGF expression in ameloblast like cells. Therefore, the strong expression of Nfic in epithelial component of ameloblastoma would suggest the increased cell synthetic activity of ameloblast like cells in tumour islands. Moderate expression of Nfic is present in stellate reticulum like cells explains the fact that these cells have less synthetic activity when compared to ameloblast like cells. Therefore Nfic expression is stronger in plexiform and follicular type of ameloblastoma where the epithelial component is vastly present.[5] Moderate expression of Nfic in connective tissue of desmoplastic ameloblastoma could be explained by the presence of extensive stromal desmoplasia and low epithelial cell population. Moderate expression of Nfic in epithelial cells of unicystic ameloblastoma would support the rationale that Twist, a helix transcription protein which is essential in embryological morphogenesis showed higher expression in solid ameloblastoma as compared with unicystic ameloblastoma.[6]

Nfic in Adenomatoid OdontogenicTumour:

In the present study, the results showed strong expression in epithelium forming duct-like structures resembling ameloblasts. The evidence of its expression suggests that there is a link between AOT and Nfic during tumour progression as Nfic is well known to implicate cell regulation and proliferation. Though Nfic is a transcription – replication factor contributing for proliferation of cell, it can be considered as co-regulator of cell proliferation.

Further, the presence of fine filamentous layer and finger like cytoplasmic processes in epithelial cells are similar to those in normal tooth germ at the beginning of predentin formation. The tumour cells lining the duct like structures and small eosinophilic areas seem to be comparable to the ameloblasts of differentiating stages. But in this tumour, mesodermal cells, which have the potential to form dentin, are not present. Due to this, the ameloblast like cells could not further differentiate and development is thus arrested at the stage before enamel matrix formation.[7]

Nfic in Odontogenic Keratocyst:

In the present study, the results presented strong and moderate expression of Nfic in two thirds of epithelium and connective tissue respectively. But there is no evidence in literature eliciting the profile of Nfic in OKC.

Since expression of Nfic indicates cell proliferation activity, strong expression of Nfic in epithelial component of OKC would suggest the increased cell proliferation activity of ameloblast like cells in tumour overlying epithelium. These ameloblasts with similar function of those in tooth germs exhibit hyperchromatic nuclei expressing Nfic as they are highly active in proliferating and synthesizing glycoproteins like Nfic.

Therefore, it can be proposed that Nfic has dual character to play as accessory regulator in synthetic activity and replication.

Nfic in Odontogenic Myxoma:

In the present study, the results in our study showed weak expression of Nfic in the mesenchymal tissues. The possible reason behind could be the inability of mesenchymal cells to differentiate. Another reason could be because of low expression of TGF which inturn is regulated by Nfic in OM.[8] Therefore Nfic is downregulated in OM.

Another reason could be that the degenerated fibroblasts in odontogenic myxoma expressed FGF at lower levels, therefore Nfic which regulates FGF might have shown weak expression.[9]

Osteopontin in tooth germs:

The present study also showed positive expression of OPN in odontoblasts and mesenchymal cells of dental papilla. The evidence of OPN expression in odontoblasts suggests that OPN is a protein secreted by odontoblast which is later released into dentrix matrix. OPN in dentin matrix functions as a nucleator for mineralization. Positive expression of OPN in mesenchymal cells of dental papilla suggests that OPN expression is present even in the progenitors of odontoblasts which might secrete OPN into dentin matrix. Moderate expression of OPN was observed in osteoblasts in dental follicle. Varying degrees of OPN expression in developing rat mandible was observed in pre-osteoblasts, osteoblasts, and osteocytes.

OPN in odontogenic tumours:

OPN in ameloblastoma:

In the present study, strong expression of OPN was seen in the cytoplasm of peripherally arranged cuboidal epithelial cells in PA and moderate expression in nests of columnar and spindle shaped epithelial cells in FA. In case of UA moderate expression was seen in cystic lining and luminal growth. But weak expression was observed in the connective tissue of DA. The results of our study are consistent with **Masloub** study which revealed that in both FA and PA, cytoplasm of ameloblast like cell and stellate reticulum were OPN positive. The evidence of strong expression of OPN in PA suggests the ability of cuboidal cells to differentiate into ameloblast like cells.[10] In FA, OPN protein is probably synthesized and secreted by stellate reticulum like cells, which is later picked up by ameloblast like cells and released into the stromal tissue in ameloblastoma surrounding tumour.[10] The difference in expression of OPN in PA and FA can be attributed to stellate reticulum. The moderate expression of OPN in FA can be due to degeneration of stellate reticulum within the follicle and the inability of ameloblasts to secrete OPN whereas; the degeneration of stellate reticulum in PA is minimal which can be the reason for strong expression in PA.

The immunohistochemical results of the present study showed moderate expression of OPN in cystic lining and in luminal growth in UA. The results of **Wang and Liu** study were consistent with our study. In their study, they observed that distribution pattern of OPN expression was different in variants of UCA and in the intraluminal cases, OPN immunoreactivity was observed in neoplastic epithelial cells with no stromal reaction surrounding the tumor.[10] The present study also expressed OPN in neoplastic epithelial cells in luminal variant with surrounding stroma showing weak expression. In the mural cases of **Wang and Liu** study, the OPN expression was prominent in neoplastic epithelial cells and peritumoral stromal tissue. This difference in OPN localization in luminal and mural UCA might explain the difference in the biological behavior of these variants.[10] Weak expression of OPN was observed in connective tissue of DA. The possible reason could be due to extensive stromal desmoplasia, there might be less availability of epithelial component to express OPN.

Osteopontin in AOT:

In the present study, the results showed strong expression in columnar epithelial cells forming rosettes and foci of calcification also showed strong expression. This finding suggests that OPN is secreted by columnar epithelial cells which form into rosette like structures with minimal stromal connective tissue.

The lumen contained variable amount of eosinophilic amorphous material in the centre of the rosette. The amorphous eosinophilic material was reported as heterogenous masses consisting of thin collagen fibrils, electron-dense fibrils and amyloid filaments. This amorphous eosinophilic material is considered as non-calcified tumor droplets. He suggested that this amorphous eosinophilic material represent some form of enamel matrix.[7] Expression of OPN within the lumen of rosette in our study might be because of this enamel matrix. Irregular calcified bodies representing dystrophic calcification are also present in areas of loose connective tissue expressing OPN. Thus, the evidence of OPN in AOT suggests a possible participation of this glycoprotein in its biological behavior.

Osteopontin in Odontogenic Keratocyst:

The present study showed moderate expression of OPN in the basal and parabasal layers of epithelium in OKC. Results of **Yi Ping Wang** study were in consistent with our study.[12] In the present study, OPN expression was observed in basal and parabasal layers which would suggest that OPN is probably synthesized and secreted by basal cells which resemble preameloblasts. Expression of OPN was found to be weak in connective tissue because very minute amounts of OPN might be released into connective tissue. Probably OPN is not secreted by cells in the fibrous capsule, but released into the subepithelial connective tissues of OKCs via basal cells, by transcytosis. The evidence of OPN in OKC suggests that OPN plays a chief role in the spread of OKC lining epithelial cells.

Osteopontin in OM:

In the present study, the results in our study showed weak expression of OPN in the mesenchymal tissues. The possible reason behind, could be the inability of mesenchymal cells to differentiate, due to absence of epithelial mesenchymal interactions. Since the cells of dental papilla being immature, showed weak expression of OPN, this could suggest that OPN might be absent in undifferentiated cells. Another reason as proposed by **Adekeye** could be that OM represents a degenerative form of odontogenic fibroma.[13]

V. Conclusion:

Odontogenesis is a complex physiological process of tooth development that is regulated by sequential and reciprocal interactions between epithelial and mesenchymal tissues. In the present study, tooth germs showed strong expression of Nfic in odontoblasts and odontogenic tumours stating that Nfic has a role in embryogenesis and in pathogenesis of odontogenic tumors due to its proliferative activity. OPN showed strong expression in synthesizing cells and calcification foci of odontogenic tumors suggesting that it plays a chief role in mineralization and in biological behavior of odontogenic tumors. Presence of Nfic expression confirmed its influence during crown formation, bone formation in tooth germs and increased cell proliferation activity in odontogenic tumours. Expression of OPN suggested its influence during mineralization in tooth germs, bone formation and calcification focus in odontogenic tumours. Thus our study demonstrates a clear association of Nfic and OPN with odontogenesis and odontogenic tumours. Future studies should be directed at molecular and ultrastructural level to assess the influence of Nfic and OPN in the pathogenesis of odontogenic tumours.

References:

[1] Huang Xf, Chai Y. Molecular Regulatory Mechanism Of Tooth Root Development. International Journal Of Oral Science. 2012;4:177–81.

[2] Lee Ds, Park Jt, Kim Hm. Nuclear Factor I–C Is Essential For Odontogenic Cell Proliferation And Odontoblast Differentiation During Tooth Root Development. J Biol Chem. 2009;284(25):17293–303.

[3] Steele-Perkins G, Butz Kg, Lyons Ge. Essential Role For Nfi-C/Ctf Transcription Replication Factor In Tooth Root Development. Mol Cell Biol. 2003;23(3):1075–84.

[4] Roh Sy, Park Jc. The Role Of Nuclear Factor I-C In Tooth And Bone Development. J Korean Assoc Oral Maxillofac Surg. 2017;43:63-9.

[5] Kaur J, Pannu Tp, Kaur R, Singh R, Goel J, Kumari R. Pathogenesis Of Odontogenic Tumors Of Epithelial Origin- A Review. Journal Of Advanced Medical And Dental Sciences Research. 2015;3(1):106-15.

[6] Zhong Y, Guo W, Wang L, Chen X. Molecular Markers Of Tumor Invasiveness In Ameloblastoma: An Update. Annals Of Maxillofacial Surgery. 2011;1(2):145-9.

[7] Reichart P, Barnes L, Everson Jw. Odontogenic Tumours. In: Barnes L, Everson Jw, Reichart P, Sidransky D. World Health Organization Classification Of Tumours Pathology And Genetics Of Head And Neck Tumour. Lyon: Iacr Press. 2005;283–327.

[8] Heikinheimo K, Happonen R, Pnivi J. Miettinen P. Transforming Growth Factor ,B2 In Epithelial Differentiation Of Developing Teeth And Odontogenic Tumors. J. Clin. Invest. 1993;19:1019-27.

[9] Adekeye Ed, Edwards Mb, Goubran Gf. Ameloblastic Fibrosarcoma, Report Of A Case In A Nigerian. Oral Surg. 1978;46:254–9.

[10] Masloub M, Adel M, Ehab S. Cd10 And Osteopontin Expression In Dentigerous Cyst And Ameloblastoma. Diagnostic Pathology. 2011;6:44-6.

[11] Filipe M, Michelle T, Martins T, Suzana O. Expression Of Extracellular Matrix Proteins In Adenomatoid Odontogenic Tumor. J Oral Pathol Med. 2010;39:230–5.

[12] Wang Yp, Liu By. High Expression Of Osteopontin And Cd44v6 In Odontogenic Keratocysts. J Formos Med Assoc. 2009;108(4): 286-92.

[13] Molina R, Taylor A, Oslei A, Rizo O, Mata G. Peripheral Desmoplastic Ameloblastoma: Histopathological And Immunohistochemical Profile Of A Case. Med Oral Patol Oral Cir Bucal. 2010;15(6):846-9.