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RESEARCH ARTICLE

ROLE OF FACTOR XIIIA IN PATHOGENESIS AND BIOLOGIC BEHAVIOR OF PERIAPICAL LESIONS

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ABSTRACT

Objectives: The role of collagen in the pathogenesis and behavior of odontogenic cysts has been a matter of renewed interest. Factor XIII a plays an important role in fibrosis by stimulating fibroblasts and also by interacting with inflammatory mediators. Understanding its role in periapical lesions may help in predicting biological behavior and define the treatment approach. The aim of the study is to evaluate the expression of factor XIIIa in periapical granulomas and cysts, and to assess the role of fibrosis in their pathogenesis.

Study Design: The study was an in-vitro study which was performed on archival tissue samples using immunohistochemistry. 30 cases of periapical granuloma and 30 cases of periapical cyst were examined for factor XIIIa immunoexpression and the total number of positive cells in the two groups were evaluated. Also, the positivity was compared between three layers of periapical cyst capsule. Statistical anlaysis was performed using SPSS (20) software. Student t-test was used to compare the expression between periapical granuloma and cyst ($p<0.05$) while ANOVA and Tukeys test were used to evaluate the expression between the three layers ($p<0.05$)

Results: Factor XIIIa showed a non-significant increase in periapical cysts than granulomas. The expression was significantly increased in the intermediate layer and around the areas of inflammation. This suggested that factor XIIIA is expressed in cells prior to marked fibrosis and is higher in areas of inflammation.

Conclusion: Factor XIIIa plays an important role in limiting the size of lesion and warranting a more conservative therapeutic approach.

INTRODUCTION

Epithelial mesenchymal interactions occurring during odontogenesis give rise to teeth at one end and bewildering varieties of pathologies in the form of odontogenic cysts and tumors at another. (Aggarwal *et al* (2011). The proposed histogenetic differences and the clinical behavior of odontogenic cysts have prompted studies aimed at characterizing possible differences between their fluid aspirates and epithelial linings. Little research has been done with respect to their connective tissue walls. (Singh HP *et al* (2012) Despite the fact that proliferation of epithelial cells is an indispensable ingredient for cyst formation, connective tissue may be regarded as a functional part of cyst and not just a structural support. (Aggarwal *et al*(2011)

Many authors have concluded that dissimilar pattern of collagen arrangement in periapical cyst as compared to other odontogenic cysts suggests a different biologic behavior and thus a more conservative approach. (Aggarwal *et al*(2011) The role of collagen as a major component of extracellular matrix (ECM) in the pathogenesis and behavior of epithelial odontogenic lesions has been studied suggesting intricate relationship between epithelium and mesenchyme but still there is lack of understanding in the precise role of mesenchyme in behavior of odontogenic lesions. Immunohistochemistry has wide application in studying the cystic capsular fibres and the process of fibrosis giving a better understanding of the role of mesenchyme in the pathogenesis of the lesions.

Factor XIIIa, other than being a clotting factor, is also a cellular enzyme with implications beyond the clotting system. Beck *et al* (1961) as well as Lebovich and Ross reported the stimulatory effect of factor XIIIa on fibroblast proliferation. (Back *et al*(1961) It has been shown by several investigators that factor XIIIa containing interstitial cells increase in number in various human tissues associated with fibrosis such as liver cirrhosis, granulation of gastric ulcer, systemic nodular pannuculitis, fibrous stroma of various salivary gland tumors and various oral and maxillofacial fibrous lesions.(toida *et al*(1990). An immunohistochemical study using factor XIIIa was performed to understand the effect of fibrosis as a component of ECM in periapical cyst, its association with inflammation and its influence on biologic behavior.

MATERIALS AND METHODS

A total of 60 cases including 30 periapical granulomas and 30 periapical cysts were studied. The archival samples were selected based on microscopic examination of H and E stained slides along with clinical and radiographic details.

IHC expression of Factor XIIIa was performed using primary antibody of anti-factor XIIIa, polymer IHC detection kit with Diaminobenzene as Chromogen. (Biogenex Pvt. Ltd.) Standard IHC procedure was followed. Counterstaining was done with haematoxylin and slides were mounted with DPX.

All cases were evaluated for the cellular expression of factor XIIIa in the connective tissue. Brown staining of the cytoplasm was considered positive. The number of positive cells was evaluated in each layer of the fibrous capsule of the periapical cyst namely inner granulomatous layer, intermediate layer and outer fibrous layer. The inner granulomatous layer was seen as the edematous layer with sparse fibrous components, varying degree of vascularity and inflammatory cell infiltrate. The connective tissue layer with numerous dense bundles of collagen was considered as outer fibrous layer and the intermediate layer was slightly to moderately fibrous[4]. Comparative analysis of factor XIIIa was performed to identify the difference among the three layers in periapical cyst capsule. Shape of the immunostained cells as well as their distribution in fibrous zones and inflammatory areas was assessed. Further, a general distribution of Factor XIIIa positive cells was compared between periapical granuloma and periapical cyst.

Statistical Analysis

Parametric and Non-parametric tests were applied as required for data analysis using SPSS software and a p-value of less than 0.05 was considered to be significant. The expression of factor XIIIa was compared between periapical granuloma and periapical cyst using independent t-test (parametric test). Data for the factor XIIIa expression amongst the three layers separately was first analyzed using ANOVA (parametric test), following which a Tukey's multiple comparison test was performed to determine the exact difference between the three layers of periapical cyst

RESULTS

The independent t-test (parametric test) suggested an increased expression in periapical cyst but no statistical significance was observed (0.995) (Table 1, fig. 1) The pattern of distribution of factor XIIIa positive cells in periapical granuloma was an overall uniform pattern with most cells being stellate shaped (fig. 2.) Also the frequency of factor XIIIa positive cells was increased in areas of dense inflammation.

Table 1 Comparison of Factor XIIIa expression between periapical granuloma and periapical cyst (independent student t- test).

Study groups	N	Mean ± SD	p value
Group I Periapical cyst	30	20.17 ± 5.51	0.995 *
Group II Periapical granuloma	30	6 ± 5.54	

*Not significant

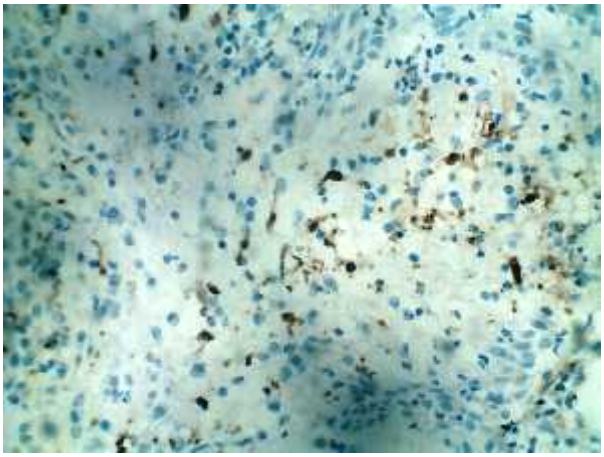

Fig 1a

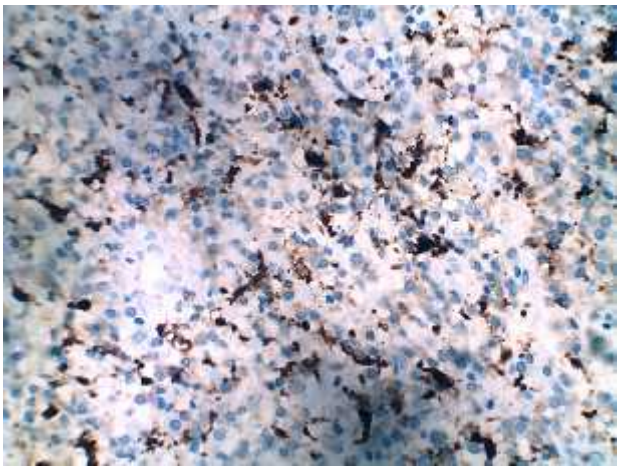

Fig1b

Figure 1 Increased expression of factor XIIIa in periapical cyst as compared to periapical granuloma. (a: periapical granuloma, b: periapical cyst)

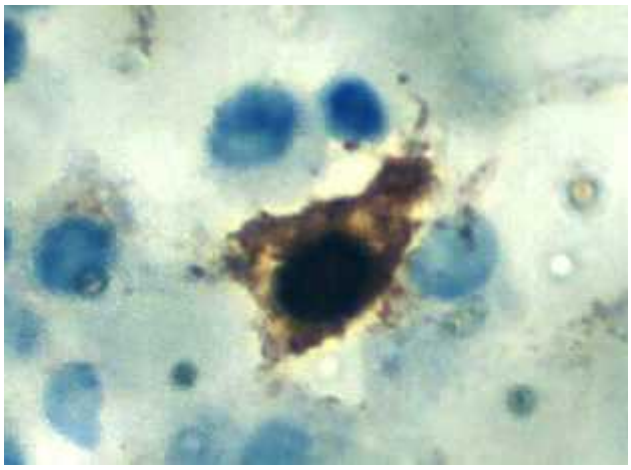

Figure 2 Factor XIIIa positive stellate shaped cell.

ANOVA (parametric test) which gave a p-value as less than 0.001 suggested a highly significant difference between the numbers of positive cells in the three layers (Table 2).

Table 2 Comparison of Factor XIIIa expression between the three layers for periapical cyst (ANOVA test)

Group	n	Mean ± SD	p- value
Inner layer	30	19.88 ± 4.81	
Middle layer	30	29.97 ± 9.74	0.00*
Outer layer	30	10.88 ± 4.08	

*highly significant.

Factor XIIIa positive cells in periapical cyst were predominantly seen in the intermediate or middle layer, followed by the inner layer, and scarcely in the outer fibrous layer (Table 2, fig. 3). Cells in the middle and inner layer were more stellate shaped whereas those in the outer fibrous layer were more spindled (fig. 4).

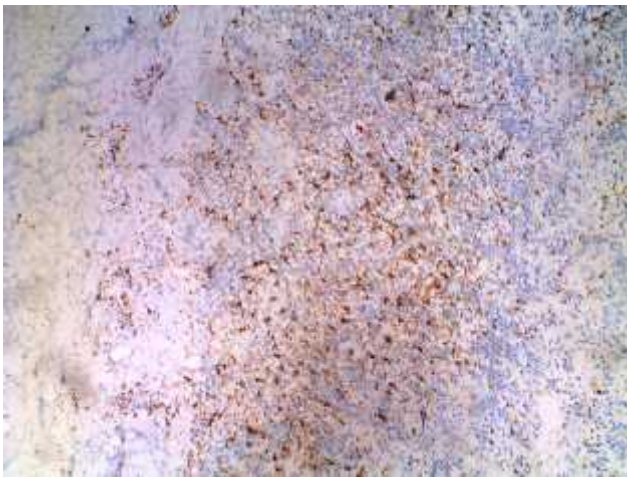

Figure 3 Differences in positive cell distribution within three layers of periapical cyst capsule with maximum expression in intermediate layer*

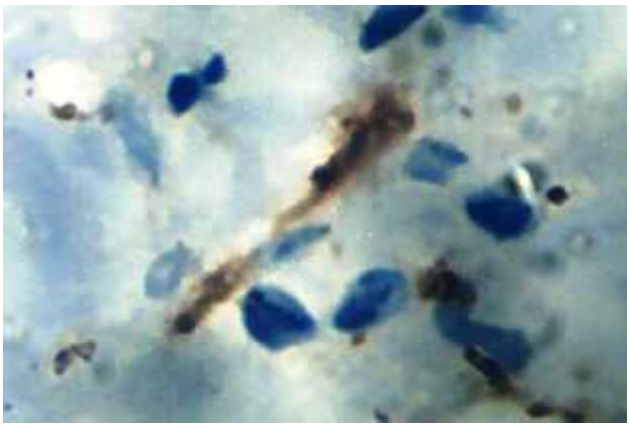

Figure 4 Factor XIIIa positive spindle shaped cell.

A Tukey's multiple comparison test was performed to determine the exact difference between the three layers of periapical cyst (Table 3).

Table 3 Tukey's multiple comparison test for Factor XIIIa expression in periapical cyst capsule.

GROUPS	p- value
Inner layer V/S Middle layer	0.000*
Outer layer V/S Inner layer	0.000*
Middle layer V/S Outer layer	0.000*

*highly significant

The comparison between the inner layer and the intermediate layer gave a negative difference with a p-value of less than 0.001 suggesting a highly significant difference between the numbers of the cells amongst the two layers, with the inner layer having lesser number of positive cells as compared to the intermediate layer. The comparison between intermediate and outer layer gave a positive difference with p-value of less than 0.001 suggesting a highly significant difference with the intermediate layer having more number of positive cells as compared to the outer layer. Further, comparison between inner and outer layer gave a positive difference with a p-value less than 0.001 suggesting a highly significant difference with inner layer having more number of positive cells as compared to outer layer.

DISCUSSION

Bagoli *et al* (2012) state that the first finding suggesting the cross linking of fibrin was reported 75 years ago. Twenty years later Robbins confirmed the existence of 'a serum factor" which was thermolabile and non-dialyzable, and named the protein "fibrin stabilizing factor". The factor then was purified by Loewy and colleagues and its enzymatic nature was revealed and characterized as Factor XIII. (Bagoly *et al* (2012)

Plasma factor XIII is a zymogen consisting of two potentially active catalytic A subunits (FXIIIA) and two protective/ carrier subunits (F- XIIIB). FXIII-A is synthesized in cells of bone marrow origin and FXIII-B is synthesized and secreted by hepatocytes in excess to FXIII-A. The two subunits form a tight tetrameric complex (FXIII-A2B2) in the plasma; practically all FXIII-A is in complex, while about 50% of FXIII-B circulates in free form. A cellular dimeric form of FXIII-A (cFXIII) is also present in the cytoplasm of platelets and monocytes/macrophages.[5] It was realized that factor XIII plays an important role in regulation of fibrinolysis and the demonstration of the cellular form present in macrophages and monocytes including tissue macrophages revealed its presence in most body tissues. Thus, it became clear that factor XIII in addition to being a clotting factor, is also a cellular factor with implications far beyond the clotting system[6] such as wound healing, maintenance of pregnancy and proliferation of fibroblasts.[1]

Factor XIIIa expressing cells were noted in periapical granulomas as well as periapical cysts. Expression was increased in areas of inflammation with stellate shape of cells. Natah *et al* (1994)[10] stated that factor XIIIa positive cells were quite frequent within mononuclear cell-rich inflammatory cell infiltrates. Other findings have described that factor XIIIa expressing dendrocytes may have a minor role in antigen processing, phagocytosis, and fibroplasias(Regezi JA *et al* 1993) thus suggesting that Factor XIIIa expression in periapical lesions is a part of the inflammatory process leading to fibrosis.

Periapical lesions are a result of inflammation and destruction of the periapical tissues as a sequence of various insults to the dental pulp, including infection, physical and iatrogenic trauma, following endodontic treatment, the damaging effects of root canal filling materials. (Graunaite I *et al*, 2011) These insults elaborate host defenses consisting of several classes of cells, intercellular messengers, antibodies, and effect or molecules. (Wantanabe H *et al*, 2008) This results in an encounter between microbial factors and host defense forces, destruction of periapical tissue and formation of various periapical lesions. Factor XIIIa is one of the many effector molecules that form a link between inflammation and fibrosis.

Factor XIIIa expression is a facultative function induced by certain undefined local factors(Quatresooz P *et al*, 2008). A viscious cycle ensues as transforming growth factor-beta (TGF-β), released by Factor XIIIa, regulates the maturation of the dendritic cells leading to further recruitment and activation of dendrocytes and more TGF- β production is associated with tissue fibroplasias. (Quatresooz P *et al*, 2008)

It also has been suggested that T-cell derived cytokines may concomitantly up-regulate the production of connective tissue growth factor (TGF- β), with stimulatory and proliferative effect on fibroblasts and the vasculature in the pathogenesis of periapical granulomas. Regezi *et al 1992* [12] found Factor XIIIa positive dendrocytes in characteristic distributions: collagen associated, vessel associated and lymphoid associated. Further a direct proliferation effect of factor XIIIa on fibroblasts has also been described. [13]

Factor XIIIa labeling was found in mast cell granules. Mast cells, dermal dendrocytes, and endothelial cells have in common that all three express FXIIIa, belong to the microvascular unit, and are increased in number during angiogenesis and in fibrovascular processes.[13] $FXIIIa^{+}$ dendrocytes increased when mast cells degranulated and released Tumor Necrosis Factor-α on cell activation. Factor XIIIa positive dendrocytes can intiate or promote proliferation of CD34+ uncommitted mesencymal cells in co-operation with mast cells. [14] Also, factor XIIIa has been shown to regulate the proliferation of fibroblasts and some tumor cells in vitro. Mast cells are present in both inflammatory infiltrate and fibrous area of periapical lesions with degranulation a frequent finding in these zones. [15]

The present study found that factor XIIIa positive cells were seen variably in the three layers of the periapical cyst capsule. The cells had maximum expression in the intermediate layer, followed by the inner cellular layer whereas the outer fibrous layer showed sparsely positive cells. These findings are in accordance with those noted by Toida *et al* in 1990. It is a known fact that factor XIIIa positive cells differentiate prior to fibrosis [4,7] and show decreased expression in areas of dense fibrosis. The periapical granuloma and periapical cyst are part of a continuum. A periapical granuloma in response to continued irritation ultimately transforms into a cyst with formation of the cystic lumen and organization of the connective tissue to form a well condensed cystic capsule composed of three layers namely the inner cellular layer, the intermediate granulomatous layer and the outer fibrous layer.[19] As per Lee *et al* (2010), activity of factor XIIIa in dermatofibroma, continuously diminishes with aging of the lesion and is completely absent in atrophic variant.[20] Thus, as the lesion matures fibrosis increases and factor XIIIa expression is reduced. This fibrosis helps to limit the size of the lesion and walls off the infection indicating that factor XIIIa could be one of the factors that influence the biologic behavior of periapical lesions.

Toida *et al* (1989) have suggested that factor XIIIa containing cells belong to the macrophage / monocyte cell lineage including tissue macrophages.[7] Whereas Natah *et al* (1994) suggest that localization to perivascular areas/inflammatory cell infiltrates which could possibly explain the increased expression in the inflammatory zones of the cyst capsule. Also, authors have suggested that this localisation would be compatible with a role in antigen presentation.[10] A study conducted by Derrick *et al* (1993)[16] to localize factor XIIIa positive dendritic cells and found that factor XIIIa positive dendritic cells were associated in large numbers with epithelial structures in lung and kidney and only rarely observed in liver, thyroid, testis and spleen. They concluded that owing to its distinctive distribution, these dendrocytes have an important role in immune responses at those sites. Though factor XIIIa cells in the periapical cyst capsule are thought to be histiocytic in origin, a minor role in antigen presentation and thus involvement of the cells in evoking an immune response cannot be ruled out.[9]

CONCLUSION

Periapical lesions remain the most commonly encountered lesions in the oral cavity. Although epithelial proliferation forms the basis of cyst formation, the connective tissue component may be regarded as the functional element rather than just providing structural support. The present study demonstrated that factor XIIIA is expressed in cells prior to marked fibrosis and its expression is significantly higher in areas of inflammation as opposed to scarcity in areas with fibrosis. Connective tissue organization is essential in the transformation of periapical granuloma into a periapical cyst, thus walling off the infection and warranting a more conservative treatment approach. Thus, Factor XIIIa plays an important role not only in pathogenesis but also in determining the biologic behavior of lesions.

References

1. Aggarwal P, Saxena S. Stromal differences in odontogenic cysts of a common histopathogenesis but with different biological behavior: A study with picrosirius red and polarizing microscopy. *Indian J Cancer*. 2011; 48:211-5.
2. Singh HP, Shetty DC, Wadhwan V, Aggarwal P. A quantitative and qualitative comparative analysis of collagen fibers to determine the role of connective tissue stroma on biological behavior of odontogenic cysts: A histochemical study. *Natl J Maxillofacial Surg*. 2012; 3:15-20
3. Beck E, Duckert FE, Ernst M. The influence of fibrin stabilizing factor on the growth of fibroblast in vitro and wound healing. *Thromb Diath Haemorrh* 1961; 6:485-91.
4. Toida M, Tsai C-S, Okumura Y, Tatematsu N, Oka N. Distribution of Factor XIIIa-containing cells and collagenous components in Radicular Cysts: histochemic and immunohistochemic studies. *J Oral Pathol Med* 1990; 19:155-9.
5. Bagoly Z, Katona E, Muszbek L. Factor XIII and inflammatory cells. Thromb Res 2012; 129:S77–S81.
6. Muszbek L, Yee VC, Hevessy Z. Blood Coagulation Factor XIII: Structure and Function. *Thromb Res*. 1999; 94:271–305.
7. Toida M, Wanatabe F, Tsai CS, Okutomi T, Tatematsu N, Oka N. Factor XIIIa containing cells and fibrosis in oral and maxillofacial lesions: An immunohistochemical study. *Oral Surg Oral Med Oral Pathol*. 1989; 68:293-9.
8. Regezi JA, Macphail LA, Richards DW, Greenspan JS. A study of macrophages, macrophage-related cells, and endothelial adhesion molecules in recurrent aphthous-ulcers in HIV-positive patients. *J Dent Res*. 1993; 72:1549-53.

9. Regezi JA, Zarbo RJ, Daniels TE, Greenspan JS. Oral traumatic granuloma: Characterization of the cellular infiltrate. *Oral Surg Oral Med Oral Pathol*. 1993; 75:723–727.
10. Natah SS, Häyriner-Immonen R, Hietanen, J, Malmström M, Konttinen YT. Factor XIIIa-positive dendrocytes are increased in number and size in recurrent aphthous ulcers (RAU). *J Oral Pathol Med*. 1994; 26:408–13.
11. Quatresooz P, Paquet P, Hermanns-Lê T, Piérard GE. Molecular mapping of Factor XIIIa-enriched dendrocytes in the skin. *Int J Molec Med*. 2008. 22(4):403-9.
12. Regezi JA, Nickoloff BJ, Headington JT. Oral submucosal dendrocytes: factor $XIIIa^+$ and $CD34^+$ dendritic cell populations in normal tissue and fibrovascular lesions. *J Cutan Pathol*. 1992; 19:398–406.
13. Stashenko P, Teles R, D'souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral biol Med. 1998; 9:498-521.
14. Ide F, Kusama K. Solitary Fibrous Tumor Is Rich in Factor XIIIa+ Dendrocytes. *Am J Dermatopathol*. 2002; 24:449-450.
15. Ledesma-Montes C, Graces-Ortiz M, Rosales-Garci G, Hernandez-Geurrero JC. Importance of Mast Cells in Human Periapical Inflammatory Lesions. *J Endod* 2004; 30:855-9.
16. Derrick EK, Barker JNWN, Khan A, Price ML, Macdonald DM. The tissue distribution of factor XIIIa positive cells. *Histopathology* 1993; 22: 157–162.
17. Graunaite I, Lodiene G, Macuilskiene V. Pathogenesis of Apical Periodontitis: a Literature Review. *J Oral Maxillofac Res.* 2011; 2(4):e1.
18. Nair PNR. Pathogenesis of Apical Periodontitis and the causes of Endodontic failures. *Crit Rev Oral Biol Med* 2004; 15:348-81.
19. Watanabe H, Ishida Y, Nagashima K, Makino T, Norisugi O, Shimizu T. Desmoplastic fibroblastoma (collagenous fibroma). *Journal of Dermatol*. 2008; 35:93–7.
20. Lee HI, Lee JW, Han TY, Li K, Hong CK, Seo SJ *et al*. A case of dermatofibroma of the upper lip. *Annals of dermatology*. 2010; 22(3):333-6.