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EVALUATION OF EXPRESSION AND DISTRIBUTION OF FIBRONECTIN ON THE ROOT SURFACE OF TEETH IN CHRONIC AND AGGRESSIVE PERIODONTITIS: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Background and Objectives: Periodontal disease is an inflammatory process which may result in damage and loss of tooth-supporting tissues, including periodontal ligament, cementum and bone. Significant evidence supports a strong correlation between periodontitis and diseased or altered cementum. Cementum contributes to the process of periodontal regeneration. The extracellular matrix (ECM) of cementum is a multicomponent three-dimensional structure composed of collagens, fibronectin, elastin, other non-collagenous proteins, and proteoglycans. Fibronectin is a dimeric glycoprotein found on the surface of cells, plasma, extracellular matrix and in the basement membrane. Fibronectin plays significant role in promoting the attachment of cells to one another, and to extracellular substrates. The present study aimed quanlitatively to analyze fibronectin on the root surface of extracted teeth of healthy, chronic and aggressive periodontitis patients. **Materials and Methods:** The study consisted of 45 extracted human teeth, 15 healthy teeth extracted for orthodontic purpose, 15 teeth affected by chronic periodontitis, and 15 aggressive periodontitis teeth with poor periodontal prognosis indicated for extraction were collected from patients, to analyze fibronectin on root surface using Immunohistochemistry technique. Periodontal evaluation included pocket probing depth, clinical attachment level and tooth mobility. Immediately

after extraction, teeth were fixed in 2% paraformaldehyde. Following demineralization with EDTA, longitudinal sections of tissue were made on saline coated slides and Immunohistochemical staining against fibronectin was done and analyzed. **Results:** The expression of Fibronectin on the root surface of chronic and aggressive periodontitis patients showed statistically significant difference ($p<0.001$) by using Chi square test. **Conclusion:** The expression of Fibronectin was higher on the root surface of healthy teeth compared to chronic and aggressive periodontitis. Expression of Fibronectin was greater in chronic periodontitis than in aggressive periodontitis.

KEYWORDS: Fibronectin, Chronic Periodontitis, Aggressive Periodontitis.

INTRODUCTION

Periodontal disease is an inflammatory process resulting in damage and loss of tooth-supporting tissues, including periodontal ligament, cementum and bone. Evidence supports a strong correlation between periodontitis and diseased or altered cementum. Cementum is believed to contribute to the process of periodontal regeneration. It may undergo alterations in structure as well as in the composition of its organic and inorganic components as a result of pathologic changes in the immediate environment. The extracellular matrix (ECM) of cementum is a multicomponent three-dimensional structure composed of collagens, fibronectin, elastin, other non-collagenous proteins, and proteoglycans.[1]

Cementum is a non-uniform, mineralized connective tissue. Several clearly different cementum varieties are found on human teeth. They differ with respect to location, structure, function, rate of formation, chemical composition and degree of mineralization. In fully formed and functioning teeth, cementum is firmly attached to the radicular dentin and covers the entire surface of the root. Cementum increases in thickness towards the apex and may extend partially into the apical foramen. Radicular cementum is unique, avascular, does not undergo continuous remodeling like bone.[2]

Cementum forms the interface where connective tissue of the periodontium is attached to the root surface. It serves as a substratum for cell adhesion and promotes cell spreading and cytoskeletal organization.[3]

Fibronectin was first described by Morrison et al in 1948. He described it as α-2-globulin with a molecular weight of 450,000 Daltons that is found in plasma at a concentration of 300

to 500 g/ml. In vivo, fibronectins are found in body fluids, soft connective tissue matrices, and basement membranes.[4] Fibronectins are synthesized by a wide variety of cells in vitro such as fibroblasts, endothelial cells, and other cell types like chondrocytes, myoblasts, macrophages, hepatocytes, amniotic cells, glial cell lines also produce fibronectin at lower levels.[5]

There are two types of fibronectin, termed plasma and cellular fibronectins. Cellular and plasma fibronectins, although distinguishable, are very similar in structure and properties. One major source of plasma fibronectin appears to be hepatocytes although endothelial cells and macrophages could also contribute, given their close association with the bloodstream.[5]

Fibronectins are large glycoproteins with a wide variety of cellular properties, involving the interactions of cells with extracellular materials. The properties include cell adhesion, morphology, cytoskeletal organization, migration, differentiation, oncogenic transformation, phagocytosis, and hemostasis.[5]

Fibronectin has affinity to the other main components of extracellular matrix, collagen and glycosaminoglycans. It also interacts with cell surfaces as shown by the fact that fibronectin-collagen complexes, enhances the attachment of various types of cells to such surfaces. Fibronectin plays significant role in promoting the attachment of cells to one another, and to extracellular substrates.[6] Plasma fibronectin levels are known to be affected by a variety of disease conditions.[7,8] Fibronectin has been found in whole saliva and also in gingival crevicular fluid.[9]

Studies have analyzed that fibronectin in GCF is degraded both in periodontal health and disease, the degree of fibronectin degradation increases with periodontal inflammation and decreases with periodontal treatment. In the diseased periodontal pocket, fibronectin may be further degraded as both supra- and sub gingival plaque, periodontopathogenic microorganisms are capable of degrading fibronectin.[10]

In one of the study several fibronectin (FN) fragments have been identified as markers for periodontal disease status, supporting their role as potential components of the pathogenesis of periodontitis. It is also demonstrated that degradation of fibronectin is a feature of periodontal disease in systemically healthy subjects.[11] There is significantly higher fibronectin fragment level in periodontitis than compared to clinically healthy tooth.[12]

Changes in cementum due to periodontitis include changes in the distribution and morphology of fibronectin. These changes may influence the ability for regeneration and connective tissue attachment onto periodontally affected root surface.[1] Hence it is hypothesized that the expression and distribution of fibronectin in cementum affected by periodontitis are altered compared to normal, non –diseased cementum.

Hence, the present study aimed to assess the distribution and expression of fibronectin on the extracted root surface of healthy, chronic and aggressive periodontitis.

METHODOLOGY

The present study was carried out in 45 teeth extracted due to poor periodontal prognosis or for orthodontic purpose, in patients with an age range of 18-60 years, reporting to the Department of Periodontology and Department of Oral and maxillofacial surgery.

The freshly extracted teeth were categorized into 3 groups, i.e healthy, chronic periodontitis and aggressive periodontitis teeth and stored in 2% paraformaldehyde solution until processing. The purpose of this study was explained to the patient verbally in a language that he or she could understand, and informed consent was obtained from the patient. The ethical clearance for the study was obtained from the institutional ethical committee.

The subjects included in the study were based on the criteria that patients were aged between 18-60 years, for healthy group- no sites with PPD >4mm or CAL >1mm, teeth extracted for orthodontic reasons, for chronic periodontitis group- CAL >5mm, radiographic evidence of bone loss on at least two teeth per quadrant, teeth with poor periodontal prognosis indicated for extraction poor prognosis indicated for extraction, for aggressive periodontitis group- PPD >5mm, generalized proximal attachment loss, the amount of microbial deposits inconsistent with disease severity, and patients with history of systemic diseases, smokers and alcoholics, grossly decayed, teeth with root resorption, patients on any medication taken within six months which may alter the periodontal status, pregnant and lactating women, patients who have undergone periodontal treatment within last 1 year were excluded from the study.

Each patient underwent a full mouth periodontal probing and charting. The loss of attachment and probing depth were measured prior to extraction of periodontally affected teeth. Chronic and aggressive periodontitis patients were diagnosed based on criteria given by American

Academy of periodontology classification of periodontal disease 1999. The selected teeth were divided into 3 groups as following Group 1 [control group] – 15 teeth extracted for orthodontic purpose. Group 2 [test group] – 15 teeth affected by chronic periodontitis, with poor periodontal prognosis indicated for extraction. Group 3 [test group] – 15 teeth affected by aggressive periodontitis, with poor periodontal prognosis indicated for extraction.

CLINICAL PARAMETERS

Clinical examination was performed by a single examiner. Clinical parameters such as Probing pocket depth (PPD), Clinical Attachment Level (CAL) and Tooth mobility were measured.

PREPARATION OF THE SLIDES FOR IMMUNOHISTOCHEMISTRY

Specimens obtained were fixed in buffered 2% formalin solution until processing, following by tooth demineralized in $5^0/_0$ nitric acid and processed in the series of alcohol and xylene solutions. The decalcified teeth were impregnated in paraffin wax and tooth embedding was done. Wax blocks of 5µm thick sections were obtained using semi-automatic microtome. The sections were placed on saline-coated slides used to prevent floating of the samples during incubation in the microwave oven for antigen retrieval. The slides were preserved in a slide-holding box until they were stained for Fibronectin.

IMMUNOHISTOCHEMISTRY (IHC) PROCEDURE

Fibronectin was detected by using immunohistochemical method. Tissue sections were made from formalin fixed, paraffin embedded specimens of 45 teeth taken in the study. From each specimen, one 4µm section was cut and placed on poly L lysine coated slide and subjected to IHC procedure, which were incubated overnight at 37˚C, deparraffinized on slide warmer for 10 minutes, deparaffinized in xylene (2 times, 10 minutes each), rehydrated in ethanol 100%, 95%, 70% and finally in distilled water. Antigen retrieval was performed by incubating slides in a jar containing Tris and EDTA buffer, pH 8.5-9 in preheated pressure cooker at 100-110˚C temperature and 15-20 Pascal pressure for 20 minutes, then cooled to room temperature. Slides were then rinsed twice in Tris buffer saline (TBS, pH 7.5-8), 5 minutes each. The slides were then transferred into a humidified chamber and incubated in 3% hydrogen peroxide for 10 minutes to block the endogenous peroxidase activities. The slides were washed with washed twice with wash buffer and were incubated with primary antibody against fibronectin (mouse monoclonal anti-p63, PathnSitu) for 60 minutes at room temperature followed by was with TBS for 3 minutes where excess was wiped off using a

blotting paper. Sections were incubated with secondary antibody StreptAvidin conjugated with horse radish peroxidase, HRP for 45 minutes at room temperature. Sections were washed with TBS for 5 minutes each. The sections were stained for 10 minutes in 3-diaminobenzidinetetrahydrochloride (DAB) and counterstained in Harris hematoxylin for 30 seconds. Slides were essentially dipped in 70%, 95%, 100% ethanol and finally in xylene. These were coated with DPX and covered with cover slips. Clinical procedural steps (Figure 1-7)

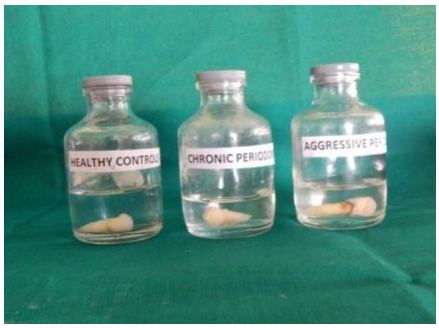


Figure 1: Samples Collected In 2% Formalin.

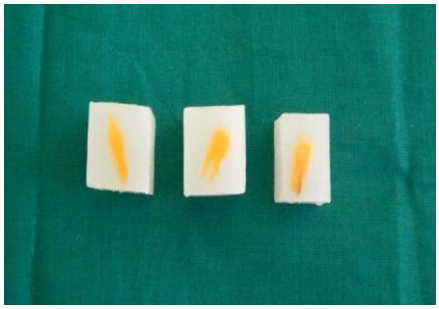


Figure 2: Teeth Mounted In Parafin Wax.

Figure 3: Tissue Processing.

Figure 4: Semi Automatic Microtome.

Figure 5: Slide Warmer.

Figure 6: Application of Dab.

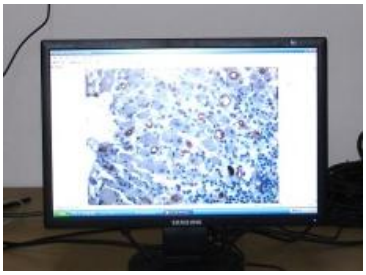


Figure 7: Research Microscope with Progres Speed*xt* Core 3. CAMERA Attached with Desktop Computer– For Photomicrography.

Statistical Methods: Descriptive and inferential statistical analysis has been carried out in the present study. Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. The Statistical software used was SAS 9.2, SPSS 15.0 were used for the analysis of the data**.**

RESULTS

The present study was undertaken to compare the expressive of Fibronectin on root surface of extracted teeth from healthy, chronic periodontitis and aggressive periodontitis subjects. The study population consisted of 45 subjects with 15 in each group.

Table 1: Qualitative Expression of Fibronectin on the Root Surface.

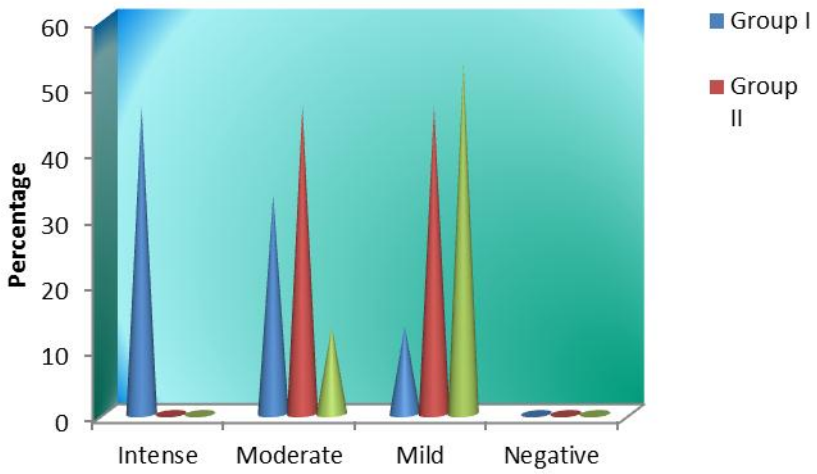
	Group I (n=15)		Group II (n=15)		Group III (n=15)		P value
	No	%	No	%	No	%	
Intense	7	46.7	0	0.0	0	0.0	<0.001**
Moderate	5	33.3	7	46.7	2	13.3	0.174
Mild	2	13.3	7	46.7	8	53.3	0.061
Negative	0	0.0	0	0.0	0	0.0	1.000

P<0.001**, Significant, Fisher Exact test

Group I-Group II: P=0.006**

Group I-Group III: P=0.002**

Group II-Group III: P=0.210



Graph 1.

Table 2: Comparison of Probing Depth (mm) in three groups studied.

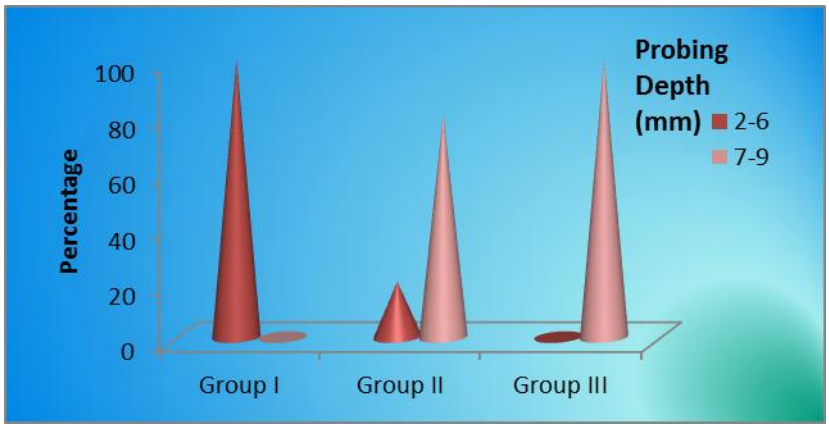
Probing Depth (mm)	Group I		Group II		Group III	
	No	%	No	%	No	%
2-6	15	100.0	3	20.0	0	0.0
7-9	0	0.0	12	80.0	15	100.0
Total	15	100.0	15	100.0	15	100.0
Mean ± SD	2.40±0.51		7.27±0.96		8.27±0.80	

P<0.001**, Significant, ANOVA test

Group I-Group II: P<0.001**

Group I-Group III: P<0.001**

Group II-Group III: P=0.003**



Graph 2.

Table 3: Comparison of CAL (mm) in three groups studied.

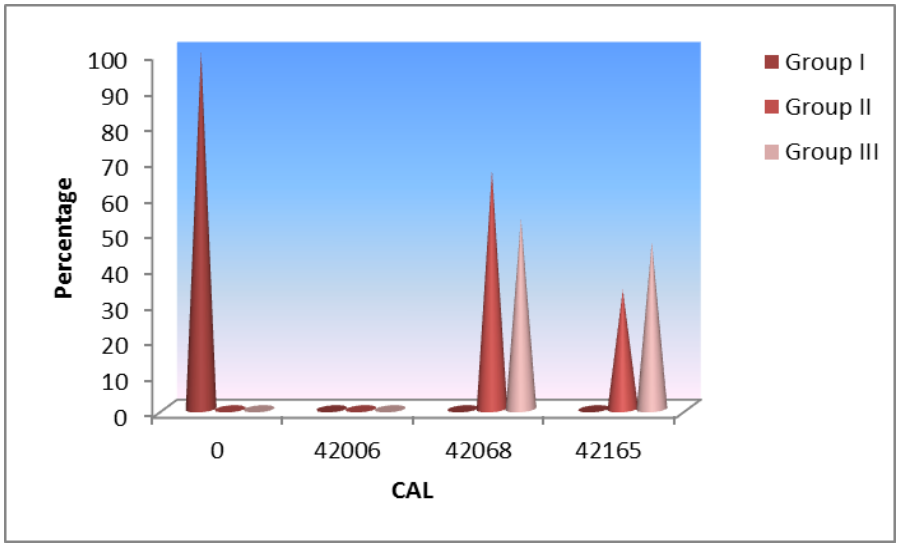
CAL	Group I		Group II		Group III	
	No	%	No	%	No	%
0	15	100.0	0	0.0	0	0.0
1-2	0	0.0	0	0.0	0	0.0
3-5	0	0.0	10	66.7	8	53.3
6-10	0	0.0	5	33.3	7	46.7
Total	15	100.0	15	100.0	15	100.0
Mean ± SD	0.00±0.00		5.27±0.96		5.47±0.92	

P<0.001**, Significant, ANOVA test

Group I-Group II: P<0.001**

Group I-Group III: P<0.001**

Group II-Group III: P=0.756



Graph 3.

Table 4: Comparison of Tooth mobility (grade) in three groups studied.

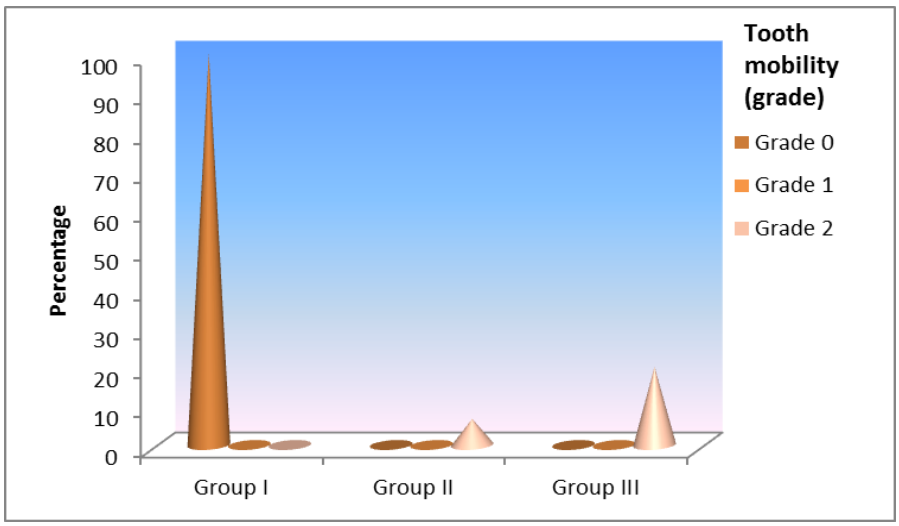
Tooth mobility (grade)	Group I		Group II		Group III	
	No	%	No	%	No	%
Grade 0	15	100.0	0	0.0	0	0.0
Grade 1	0	0.0	0	0.0	0	0.0
Grade 2	0	0.0	1	6.7	3	20.0
Grade 3	0	0.0	14	93.3	12	80.0
Total	15	100.0	15	100.0	15	100.0

P<0.001**, Significant, Fisher Exact test

Group I-Group II: P<0.001**

Group I-Group III: P<0.001**

Group II-Group III: P=0.598



Graph 4.

Mean age of subjects were, 23.80±2.68 years in healthy group, 43.73±4.62 years in chronic periodontitis group, 29.67±5.55 years in aggressive periodontitis group.

Among 45 subjects in healthy control group, 5 were females (33.3 %) and 10 were males (66.7%), in chronic periodontitis group 5 were females (33.3%) and 10 males (66.7%) and in aggressive periodontitis 9 were females (60 %) and 6 males (40%).

Distribution of Fibronectin on root surface (Table 1/ Graph 1)

Out of 45 subjects from all three groups the expression of fibronectin on root surface of healthy subjects showed that 7 (46.7%) samples stained intense for fibronectin with p value <0.001. Chronic periodontitis group showed 7 (46.7%) moderate stain, 7 (46.7%) mild stain with p value 0.174, Aggressive periodontitis group showed 2 (13.3%) moderate stain, 8 (53.3%) mild stain with p value 0.061 respectively. Intergroup comparison done using Fisher Extract test to find the significance the distribution of fibronectin on root surface compared between healthy and chronic periodontitis group was statistically significant p(0.006), healthy and aggressive periodontitis group was statistically significant p(0.002) and chronic periodontitis and aggressive periodontitis group was p(0.210), where no statistically significant difference was seen between the groups.

Comparison of Probing Depth (mm) in three groups studied (Table 2/ Graph 2)

Mean Probing depth in healthy controls, chronic periodontitis and aggressive periodontitis were 2.40±0.51, 7.27±0.96 and 8.27±0.80 respectively. In group I probing depth of 2-6 mm were measured in all 15 patients (100%), in group II probing depth of 2-6 mm were measured in 3 patients (20%), 7-9 mm in 12 patients (80%) and group III showed probing depth of 7-9 mm in all 15 patients (100%). Intergroup comparison of probing depth between the groups was statistically significant (p<0.001).

Comparison of Clinical attachment level (mm) in three groups studied (Table 3/ Graph 3)

Mean Clinical attachment level in healthy controls, chronic periodontitis and aggressive periodontitis were 0.00±0.00, 5.27±0.96 and 5.47±0.92 respectively. In Group I Clinical attachment level was 100%, in group II clinical attachment level of 3-5 mm were measured in 10 patients (66.7%), 6-10mm in 5 patients (33.3%), in group III clinical attachment level of 3-5 mm measurements in 8 patients (53.3%) and 6-10mm measurements in 7 patients

(46.7%). Intergroup comparison of clinical attachment level between the groups was statistically significant (p<0.001).

Comparison of Tooth mobility (Grade) in three groups studied (Table 4/ Graph 4)

Mean tooth mobility in group I showed grade 0 or no mobility in all 15 patients (100%, in group II grade 2 mobility was seen in 1 (6.7%) patient, grade 3 mobility in 14 (93.3%) patients, in Group III grade 2 mobility was seen in 3 (20%) patients and grade 3 mobility in 12 (80%) patients. Intergroup comparison of tooth mobility between the groups showed a statistically significant difference (p<0.001).

DISCUSSION

Periodontitis is an inflammatory disease initiated and maintained by bacterial plaque and its metabolic products that trigger the local infiltration of inflammatory cells associated with the breakdown of collagenous extracellular matrices.[12,13,14] The degradation of the gingival connective tissue during periodontitis could be a disturbance of cell-cell and cell-matrix interactions involving the production of enzymes, activators, inhibitors, and regulatory molecules such as cytokines and growth factors.[15]

A substantial portion of the volume of tissues in extracellular space, which is largely filled by an intricate network of macromolecules constituting the extracellular matrix [ECM]. The ECM is composed of two major classes of biomolecules: glycosaminoglycans (GAGs), most often covalently linked to protein forming the proteoglycans, and fibrous proteins which include collagen, elastin, fibronectin, and laminin.

ECM has been traditionally conceived of as a simple scaffold functioning mainly as a mechanical support for cells, a far more complex picture is emerging in which the ECM is a dynamic entity with physiological functions well beyond simple cell anchorage.[16,17,18] For example, fibronectin (Fn) matrices can act as a storage depot for a variety of growth factors, thus regulating cell growth and morphogenesis according to their timed release.[19,20,21]

Degradation products are also known to trigger numerous physiological responses, such as antibacterial activity and inflammation. Furthermore, cell interactions with the ECM can trigger a variety of signaling events *via* integrins, receptors that provide a molecular bridge between the external environment of the cell and its internal actin cytoskeleton.[22,23,24,25,26]

Fibronectin is connected to fibroblasts and helps them to migrate chemotactically to attach to the root surface. It plays a fundamental role in the early stages of healing, affecting clot formation and the formation of granulation tissue from fibroblasts and promoting cellular migration or tissue regeneration after periodontal treatment. Fibronectin constitutes a maturity index of the connective tissue, and its presence in cementum is disputed in diseased state.[27,28] Thus the present study was aimed at quanlitative analysis of fibronectin on the root surface of extracted healthy and periodontitis teeth.

Immediately after extraction the tooth were preserved in formalin without any root planning procedure because root planning may led to loss of cementum or any structural modification of proteins on root surface i.e fibronectin, the protein of interest in the present study.

Zappa et al pointed out that during thorough root planing of periodontally diseased teeth, the superficial layers of cementum (40 to 215 mm) are removed, and the pathologic variations in cementum are detected on these layers.[18]

The present study used imminohistochemical method to identitfy the specific fibronectin on the root surface using antibodies against fibronectin. In healthy control group it showed intense expression for fibronectin. Thus demonstrated the presence of fibronectin (positive immunoreaction) in and along the cementum surface in periodontally healthy human teeth.

In support of the present study, Guesdon et al showed the distribution and fibrillar form of fibronectin in normal cementum. Fibronectin in normal cementum was distributed uniformly, in the form of fibrils, on the whole cementum mass, from its external surface close to the PDL up to the cementum– dentin boundary.[28]

Connor et al studied the distribution of fibronectin in rat teeth with immunofluorescence and monoclonal antibodies; they reported the presence of fibronectin only in the cementum of molars.[31] Another study using immunofluorescence reported the absence of fibronectin in the cementum of non-erupted teeth inmice.[19]

Lukinmaa et al showed the localization of cellular fibronectin (cFN) was described in extracted human third molars. The expression of two matrix glycoproteins, tenascin and cellular fibronectin (cFN), has been studied in periodontal ligament, and alveolar bone, in both frozen and paraffin-processed material. Thus found the accumulation of tenascin and cFN in the interface zones between mineralized and non-mineralized tissue.[20]

Mc Allister et al showed full mandibular sections from a primate, the baboon, which indicate that fibronectin is widely distributed in the periodontium. In the presence of tissue inflammation in dog, fibronectin was localized to an amorphous material which was interpreted to be partially degraded fibers.[24]

On contrary Lukinmaa et al did not detect fibronectin in the cementum of human non-erupted third molars, which were studied without being demineralized. The observed differences in the distribution of fibronectin in the periodontally diseased cementum compared to normal cementum are remarkable. The distribution of fibronectin differed between teeth in the test group and control groups. These observed changes occurred from the external to the internal surface of cementum and from the cervical third to the apical third of the root surface. Thus various differences from the normal condition are seen, including amorphous cementum in diseased roots with deep pockets.[20]

Differences in the distribution of fibronectin were obvious when cementum apical to the pocket was compared to recession and pocket cementum. Major changes were found in pocket cementum, minor changes were found in recession cementum, and minimum changes were seen in cementum apical to the pocket.[20]

In the present study, the distribution of fibronectin on root surface compared between the groups showed statistical difference in expression for fibronectin, however there was no statistical difference between chronic periodontitis and aggressive periodontitis. The distribution of fibronectin in pocket cementum appears to have various immunoreactions. In the largest part of the pocket cementum mass, fibronectin has a non-uniform distribution, whereas on the external surface and middle mass it appears to have a negative immunoreaction Fibronectin in cementum of periodontitis group did not appear to have uniform fibrils as in normal cementum; fibronectin lost its fibrillar morphology or appeared to be completely amorphous in the whole cementum mass. On the root apex, the strongest immunoreaction of fibronectin was observed mainly on the external surface of cementum, close to the PDL.

Lopatin et al studied the concentration of fibronectin in sera and gingival crevicular fluid during various stages of periodontal disease; its concentration decreased when gingival inflammation increased. The study showed that the distribution of fibronectin as a structural element of cementum decreased in proportion to the severity of periodontal disease.[14]

Talonpoika et al suggested that crevicular fluid fibronectin is partially degraded in periodontal health and disease and that the degree of degradation increases with periodontal inflammation and decreases with periodontal treatment.[22]

In another study, Talonpoika et al suggested that the different molecular forms of fibronectin may affect the pathogenesis and healing of periodontal diseases because the biologic effect of the fragments of this molecule differ from those of the intact form. Fibronectin is an ECM molecule that is important in cell adhesion, migration, and wound healing. It plays a role in PDL cell–ECM interactions and, thus, in regenerating periodontal tissues.[23]

Kapila et al studied the response of PDL cells to fibronectin, reported that maximal proliferation and chemotaxis of these cells require specific fibronectin domains that are present on the intact molecule but not on its fragments. Because specific fibronectin fragments compromise PDL cell functions, the question that arises is whether these specific fragments are present in gingival crevicular fluid and can be used as markers for periodontal disease activity.[24]

Huynh et al identified several fragments as markers for periodontal disease status, supporting their role as potential components of the pathogenesis of periodontitis.[25]

Jee et al demonstrated that specific proapoptotic fibronectin fragments modulated proteinase expression in PDL cells and suggested that matrix-degrading proteinases may be involved in the apoptosis of these cells as part of a unique mechanism of periodontal tissue breakdown, in which proteinases may help to execute the dissolution of the ECM.[26]

Dai et al indicated that an altered fibronectin matrix, resulting from inflammation, induces ankylosis of PDL cells and resident cells and, thus may contribute to disease progression.

Ohshima et al. showed that PDL fibroblasts secrete laminin- and fibronectin-like molecules that are potent chemoattractants for gingival epithelial cells. They suggested that fibronectin molecules produced from PDL fibroblasts may be involved in the pathogenesis and progression of periodontitis by inducing the apical migration of epithelial cells.[29]

Stanley et al demonstrated that fibronectin fragmentation alters cell behavior. Upon fragmentation, matrix molecules may be recognized by other integrins and provide signals that vary from those of the intact molecule. The fragmentation process may expose cryptic

binding sites on the matrix molecules for interaction with additional integrins, thereby inducing different signaling events and, thus, altered cell behavior.[30]

Stanley et al demonstrated that degradation of fibronectin is a feature of periodontal disease in systemically healthy subjects. Murakami et al showed that Porphyromonas gingivalis fibrillin is one of the fibronectin-binding proteins.[31]

Larjava et al proposed that the fragmentation of fibronectin results from proteolytic cleavage by plaque and Porphyromonas gingivalis–derived proteases. Fibronectin is also degraded by a series of matrix metalloproteinases (MMPs).[32]

Hence, the present study demonstrated a statistically significant expression of Fibronectin on the root surfaces of healthy teeth compared to chronic and aggressive periodontitis.

CONCLUSION

The present study demonstrated a significantly higher expression of fibronectin on the root surfaces of healthy teeth compared to chronic and aggressive periodontitis. When compared between the groups the expression of fibronectin was greater in chronic periodontitis than in aggressive periodontitis.

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ETHICAL COMPLIANCE

1. **Source of Funding:** This study was self-funded by the authors and did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.
2. **Disclosure of Interest:** The authors declare that they have no conflict of interest.
3. **Informed Consent:** Informed consent was obtained from each individual participant involved in this study.
4. **Statement of Human Right:** This study was conducted in accordance with the 1964 Declaration of Helsinki and its subsequent amendments.
5. **Statement of Animal Welfare:** No animals were involved in the study

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