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Specific signaling molecule expression in periodontal ligaments in different age groups: pilot study

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SUMMARY

Introduction. Orthodontic teeth movement is accompanied by remodeling of alveolar bone, including the interradicular septum and periodontal ligaments (PDL). Periodontal signaling molecules have important functions during tooth movement and they are active in the bone remodeling process. Patients involved in orthodontic treatment belong to different age groups: therefore age must be considered as a contributing factor compromising the remodeling potential of periodontal tissues. The aim of the current study was to investigate the specific expression of signaling molecules in the PDL of interradicular septum in patients from different groups of age.

Materials and methods. The study group included 25 patients to whom extractions of teeth was recommended as a part of further orthodontic treatment. 25 patients (10 males and 15 females) were divided into three groups as follows: 1) 12-14 years old; 2) 15-22 years old; and 3) 23 years old or older. The routine histological method was followed and samples were stained with hematoxyline-eosine. According to literature data in current immunohistochemical study were included and examined expression of NGFR (nerve growth factor receptor), TGF-β (transforming growth factor β), bFGF (basic fibroblast growth factor), FGFR1 (fibroblast growth factor receptor), IL-1 (interleukin 1), IL-6 (interleukin 6), IL-8 (interleukin 8), MMP-1 (matrix metalloproteinase 1), MMP-2 (matrix metalloproteinase 2), MMP-8 (matrix metalloproteinase 8), MMP-9 (matrix metalloproteinase 9), MMP-13 (matrix metalloproteinase 13) in PDL of interradicular septum. The distribution of these factors was evaluated semi quantitatively.

Results. Expression levels of FGFR1, bFGF, MMP 8 and 9, and IL-6 in PDL of interradicular septum structure were determined in all samples. Decreases in the mean values of signaling factors relevant to age were statistically significant in bFGF.

Conclusions. Analyzed data suggest that bFGF, FGFR, IL-6, MMP 8 and 9 were determined as signaling factors in PDL of interradicular septum. Mean expression level decrease with age of FGFR1, IL-6, MMP-8, MMP-9 was non- statistically significant. The mean expression level of bFGF decreased with age, and this decrease was statistically significant. In younger patients, signal molecule expression is higher because of increased PDL metabolic activity. Increased PDL metabolic activity is a reason for higher expression of signal molecule in younger patients. Activity of remodeling process of periodontal tissue decreases with the aging and expression of signaling molecule decreases in adults.

Key words: aging, bFGF, FGFR, MMP-8, MMP-9, IL-6.

INTRODUCTION

Tooth movement by orthodontic force application is characterized by remodeling changes in dental and periodontal tissues, including dental pulp, periodontal ligament (PDL), alveolar bone, and gingiva. The tissues, when exposed to varying degrees of magnitude, frequency, and duration of mechanical loading, express extensive macroscopic and microscopic changes. Orthodontic tooth movement differs markedly from physiological dental drift or tooth eruption. The former is uniquely characterized by the abrupt creation of compression and tension regions in the PDL [1].

Remodeling changes in the process of orthodontic treatment of alveolar bone and PDL induce

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production of various cell mediators or enzymes that can be used as biomarkers of orthodontic treatment [2,3]. PDL and alveolar bone cells are exposed to physical forces in vivo in response to mastication, parafunction, and orthodontic tooth movement [4].

Current evidence suggests that downstream from the initial mechanotransduction event at focal adhesions which links an the extracellular matrix of the cytoskeleton, mechanically induces remodeling, mediated by a complex feedback mechanism involving the synthesis of cytokines, such as interleukin-1 (IL-1), interleukin-6 IL-6 and receptor activator of nuclear factor κ B ligand by cells of the osteoblast and/or fibroblast lineages. These factors in turn act in an autocrine/paracrine fashion regulates an expression of transcription factors, cytokines, growth factors, enzymes, and structural molecules involved in the differentiation, proliferation, and function of mesenchymal and other cell types [5].

Oral environment is constantly exposed to stress. PDL cells play essential role in following biomechanical context: they are subjected to mechanical stress and participate in remodeling, repair, and regeneration of periodontal tissues. PDL cells respond to force by alteration in cell proliferation and differentiation to control various cell populations in the PDL and to reflect the specific biomechanics [6,7]. PDL is considered to be one of the most highly metabolically active tissues in the body [8]. Generally known that, proliferation is one of the most basic physiological activities of cells, and mechanical stimulation can induce proliferation or inhibition of cells [9].

Fibroblasts are considered to be a mixture of various types of cells of "spindle shape" and as such there are no clearly defined biomarkers of fibroblasts. The target cells of fibroblast growth factor FGFs include fibroblasts, endothelial cells, myoblasts, chondrocytes, and osteoblasts. Base fibroblast growth factor bFGF is sequestrated in the cells responsible for their synthesis and is released only when there is a disruption of the plasma membrane [10]. IL-6 is secreted not only by macrophages and inflammatory cells but also by cells of mesenchymal origin [6,7].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases comprised of over 25 enzymes regulating many biological processes, including development, morphogenesis, and wound healing [11]. MMP-9 is known to degrade type 4 collagen, a major structural component of basement membrane [12]. MMP-8 is produced by gingival fibroblasts, bone and plasma cells. MMP-8 is most effective in hydrolyzing type I collagen and is the major interstitial collagenase in inflammed human gingival [13].

The aim of the current study was to investigate specific expression of signaling molecules in PDL of interradicular septum in different age groups and to investigate the presence of correlation between expressed signaling factors and patient age.

MATERIALS AND METHODS

The study group included 25 patients to whom extractions of teeth was recommended as a part of further orthodontic treatment. Exclusion criteria were apical periodontal and periodontal inflammation of teeth. The 25 patients (10 male and 15 female), were divided in to three groups as follow:

Table1 Analysis of specific signaling molecules in two patients from each group (6 patients together). The levels of factors were determined semi-quantitatively by counting the number of positive structures in the visual field.

Group	Age	Sex	IL-1	IL-6	IL-8	TGF-α	bFGF	FGFR1
1st	12	F	0/+	++/+++	0/+	0	+++	+++/++++
1st	14	F	0	++	0	0	++	+++/++++
2nd	15	F	0	0/+	0	0	+++/++	+++
2nd	16	M	0	0/+	0	0	+++	++/+++
3rd	45	M	0/+	0/+	0/+	0	+	++/ +++
3rd	49	F	0	0	0	0	+	++
Group	**Age**	**Sex**	**NGFR**	**MMP-1**	**MMP-2**	**MMP-8**	**MMP-9**	**MMP-13**
1st	12	F	0	0	0	++	+	0/+
1st	14	F	0	0	0	++	0/+	0
2nd	15	F	0	0	0	++/+	+	0
2nd	16	M	0	0	0	+	+	0
3rd	45	M	0	0	0	+	++	0
3rd	49	F	0	0	0	+	+/++	0/+

1) 12-14 years old, 2) 15-22 years old and 3) 23 years old or older. Tooth extractions were performed by four surgeons. Tissue samples from the interradicular septum were collected after the surgical extraction procedure. The tissue samples were fixed in 2% formaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.2). Fixed tissue samples were washed in phosphate-buffered saline for 12 hours, embedded in paraffin and cut into 6 to 7 μm thick sections. To determine the levels of the specific signalling molecules, immunohistology was used to analyse two samples from each group, to determine which signalling molecules were present (see Table 1). The following signalling molecules were selected: NGFR (nerve growth factor receptor) [ab3125; Abcam; 1:150, Cambridge UK], bFGF (basic fibroblast growth factor) [ab16828; Abcam; 1:200, Cambridge UK], FGFR1 (fibroblast growth factor receptor) [ab10646; Abcam; 1:200, Cambridge UK], TGF-β (transforming growth factor β) [ab27969; Abcam; 1:1000, Cambridge, UK], IL-1 (interleukin 1) [B-7 SC-9983; 1:50, Santa Cruz Biotechnology, Inc. California, US], IL-6 (interleukin 6) [NYRhIL6:SC-1269; 1:50, Santa Cruz Biotechnology, Inc. California, US] IL-8 (interleukin 8) [C-19 SC-1269; 1:50, Santa Cruz Biotechnology., Inc., California, US], MMP-1 (matrix metalloproteinase 1) [3-B6 SC-21731; 1:100, Santa Cruz Biotechnology, Inc., California, US], MMP-2 (matrix metalloproteinase 2) [DUB03; R/D; 1:100, Germany], MMP-8 (matrix metalloproteinase 8) [6-19Z: SC-80206, 1:50, Santa Cruz Biotechnology, Inc., California, US], MMP-9 (matrix metalloproteinase 9) [H-129:SC-10737, 1:250, Santa Cruz Biotechnology, Inc., California, US], MMP-13 (matrix metalloproteinase 13) [M-66: SC-81547, 1:100, Santa Cruz Biotechnology, Inc., California, US] (see Table 1). After deparafinisation, the expression levels of, matrix metalloproteinase 8, matrix metalloproteinase 9, and Il-6, βFGF and FGFR1 staining patterns in the tissues were examined using biotin- streptavidin immunohistochemistry (IMH) [14]. The routine histological method with hematoxyline-eosine staining was used. The expression levels were detected semi-quantitatively by counting the number of positive structures in the visual field ("0/–" – occasional, "+" – few, "++" – moderate, "+++" – numerous, "++++" – abundant positive structures in the visual field) [14]. Then be semi-quantitative results were digitized as "0" – 0; "0/+" – 0.5; "+" – 1; "+/++" – 1.5; "++" – 2; "++/+++" – 2.5; "+++" – 3; "+++/++++" – 3.5; and "++++" – 4. The study protocol for work with human materials was approved by the Committee of Ethics, Riga Stradins University (2010).

Data were analyzed using descriptive and analytical statistical methods. The mean values and standard deviations (SD) were calculated for all signaling molecules in each group. The statistical significance of the differences among the mean values of the different age groups was tested by means of one-way ANOVA with the Bonferroni correction. The correlation between age and signaling molecules' expression was assessed by using Spearman's correlation coefficient. The association between signalling molecules and age was also assessed by means of ANOVA analysis, where age was included in the model as a continuous variable. A level of significance of 5% was chosen (i.e., p value 0.05).

RESULTS

Patients were categorized in following groups of age: 1st group (12 – 14 years old) – eight patients, mean age 12.6 years; 2nd group (15–22 years old) – seven patients, mean age 17.4 years; 3rd group (23 years old or older) – ten patients, mean age 33.1 years. All data were summarized and are grouped according to patients age (see Table 2). Results for the PDL of interradicular septum were as follows: FGFR1 (see Fig. 1) was found in all groups and its expression was moderate in periodontal structures; bFGF (see Fig. 2) was highly expressed in the 1st group, and in the 2nd group its expression was lower than in the 1st group, a significantly lower level of expression was found in the 3rd group; IL-6 (see Fig. 3) expression was higher in 1st and 3rd group than in 2nd group. Results for matrix metalloproteinase-8 were similar (see Fig. 4), but matrix metalloproteinase-9 expression was higher in the 3rd group than in the 1st and 2rd groups - the mean expression level

Table 2. Mean expression levels of base fibroblast growth factor, fibroblast growth factor receptor, interleukin 6, matrix metalloproteinases 8 and 9 by age group

Variable	I group (n=8)		II group (n=7)		III group (n=10)	
	Mean	SD	Mean	SD	Mean	SD
Age	12.6	0.74	17.4	2.57	33.1	8.79
FGFR1	3.2	0.26	2.7	0.69	2.8	0.79
bFGF	2.4	0.42	1.8	0.81	1.0	0.71
IL-6	2.6	0.49	1.9	1.05	2.2	0.43
MMP8	2.4	0.52	1.4	0.61	1.6	0.73
MMP9	1.3	0.59	1.3	0.91	1.5	0.85

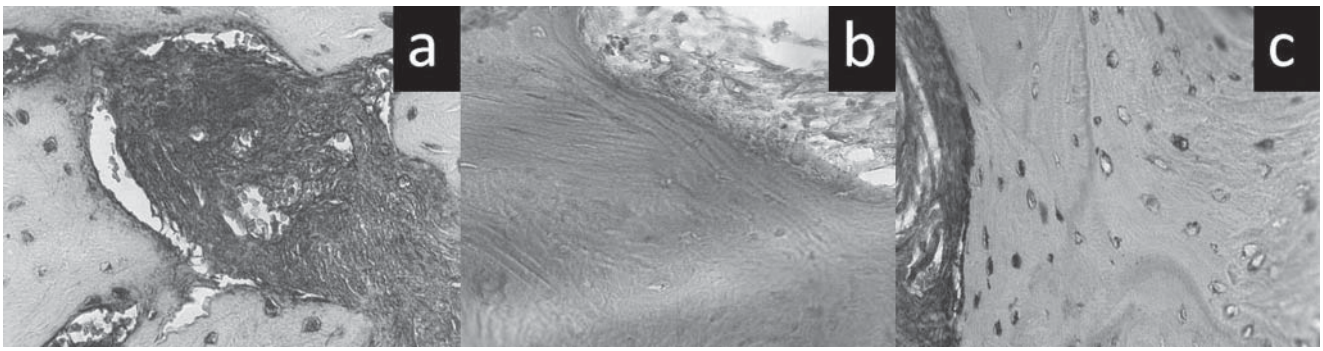

Fig. 1. Microphotograph of FGFR1 expression in bone from the interradicular septum in 12, 21, and 45-year-old patients, IMH, ×400

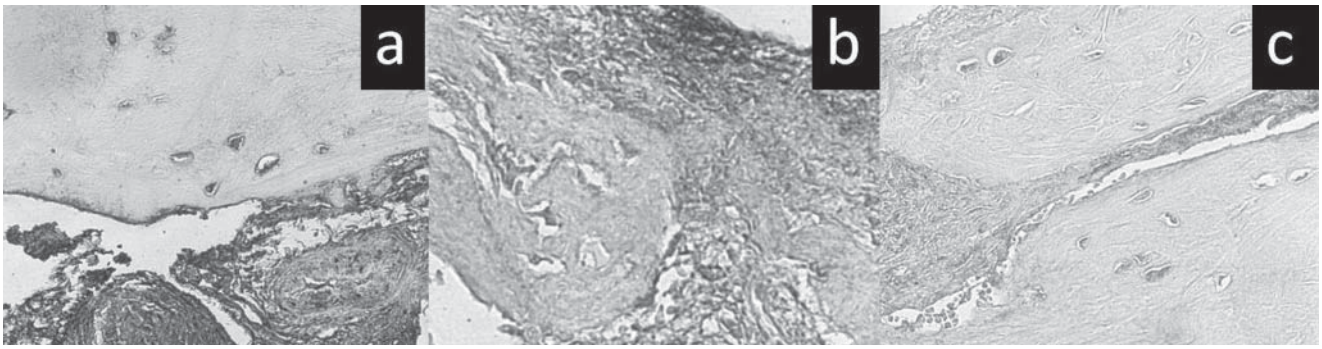

Fig. 2. Microphotograph of bFGF expression in bone from the interradicular septum in 12, 21, and 45-year-old patients, IMH, ×400

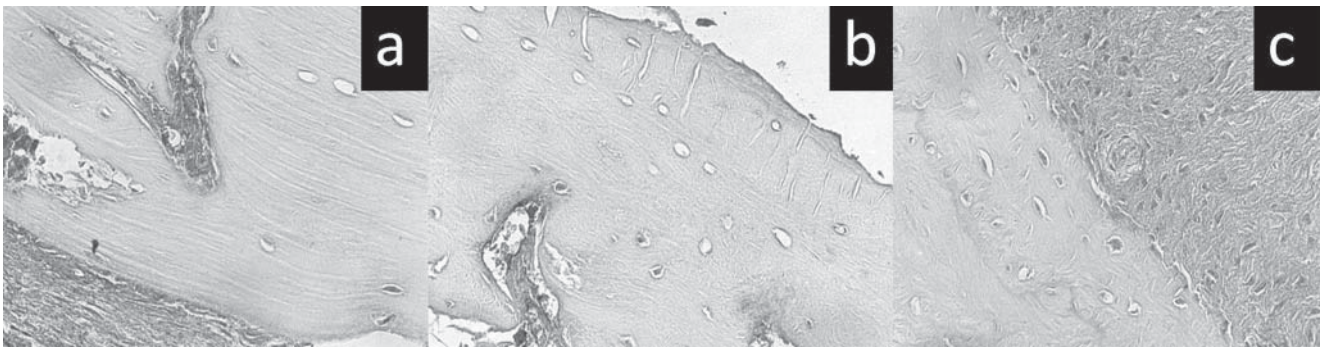

Fig. 3. Microphotograph of IL-6 expression in bone from the interradicular septum in 12, 21, and 45-year-old patients, IMH, ×400

was similar in the 1st and 2nd groups, (see Fig. 5) expression was higher in the 1st and 2nd groups than in the 3rd group. There was a statistically significant relationship between age and bFGF expression, decreasing with age (see Table 2). Spearman's correlation coefficient also revealed a moderate correlation between age and mean bFGF (see Table 3).

DISCUSSION

For current immunohistological study signaling molecules were selected according to literature data. Specific factors were determined after sample staining. Expression level of IL-1, NGFR, MMP-1, MMP-2, TGF-β, MMP-13 were independent from patient age and these were excluded. Expression levels of bFGF, FGFR1, IL-6, MMP-8, MMP-9 were low, moderate and highly dependent to patient age, therefore further evaluation was performed.

Literature states that periodontal tissue ligaments are considered to be in the range of the most highly metabolically active tissues in the body. Metabolic activity of periodontium depends on quantity of sig-

Table 3. The correlation between age expression of signaling molecules (Spearman's rank correlation coefficient (rho))

	rho	p value
FGFR1	-0.2340	0.26*
bFGF	-0.6851	0.0002
IL-6	-0.1836	0.39*
MMP-8	-0.4312	0.04*
MMP-9	-0.0079	0.97*

* – non-significant

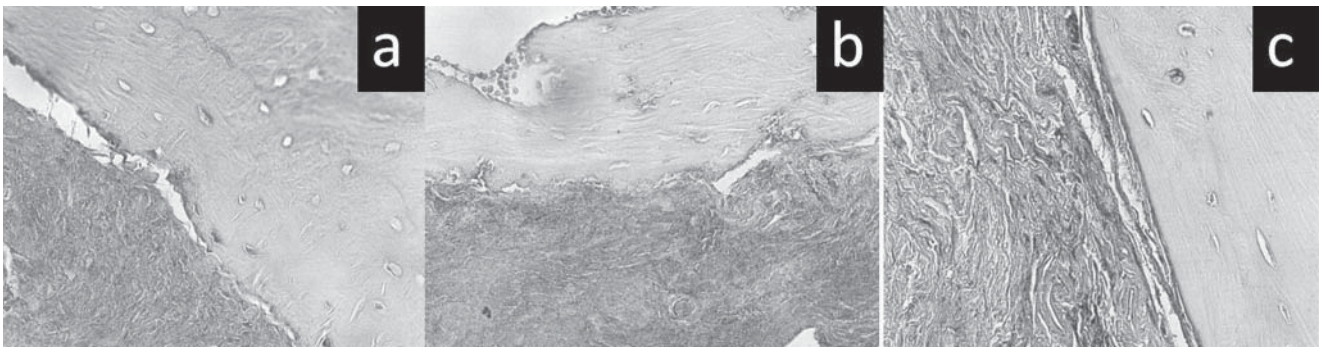

Fig. 4. Microphotograph of MMP-8 expression in bone from the interradicular septum in 12, 21, and 45-year-old patients, IMH, ×400

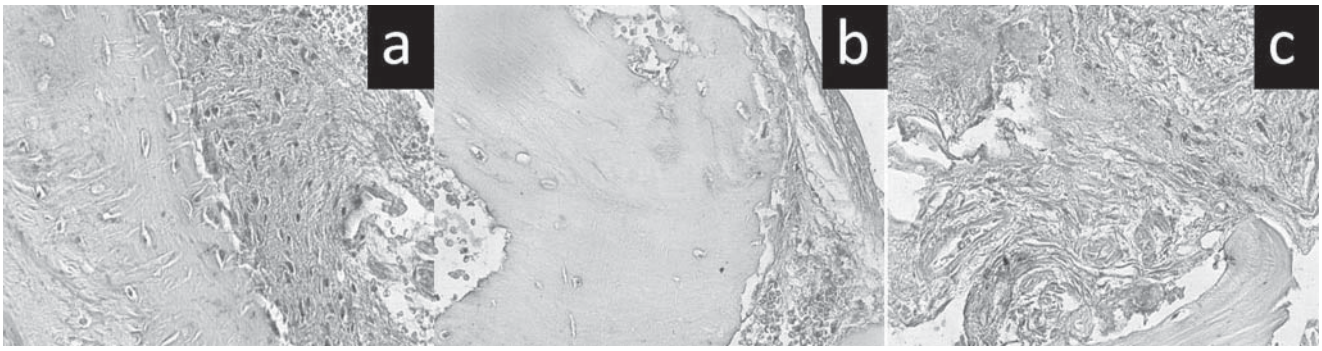

Fig. 5. Microphotograph of MMP-9 expression in bone from the interradicular septum in 12, 21, and 45-year-old patients, IMH, ×400

naling molecules being in ECM. It is known, that PDL like other tissues in human body are influenced by aging [15]. All signaling factors are secreted in autocrine and paracrine manner in the extra cellular matrix (ECM). ECM is primarily a collection of fibrous proteins embedded in a hydrated polysaccharide gel. This important tissue component mainly contains macromolecules such as collagen and glycosaminoglycans (GAGs), secreted at a local level by cells such as fibroblasts, osteoblasts, and chondroblasts. In the ECM, GAGs link to a protein with a covalent bond to form proteoglycans. The GAG and proteoglycan molecules make a gel-like ground substance, in which other fibers such as collagen are embedded. This gel allows diffusion of nutrients and hormones, whereas collagen strengthens the matrix. ECM has following roles: to provide a physical framework for the cells responsible for its production, to function as a medium regulating cellular identity, position, proliferation, and fate. It has been reported that all connective tissues in the body undergo a constant remodeling by synthesizing and degrading the macromolecular components of their extracellular matrix [10].

The fact that in our study we have got only statistically significant decrease of expression level with aging of bFGF comparing with FGFR1 could be explained as follows: Receptor quantity on cell surface was approximately the same in all patient groups, but fibroblast growth factors played a major role in fibroblast cell population regulation. Globus et al [16] reports that bone cells can synthesize and secrete bFGF into the surrounding ECM, where it might act as an autocrine or a paracrine signal.

Alhashimi et al [17] in their study found that IL-6 is secreted by many cells such as endothelial cells, basophils, epithelial cells, and fibroblasts. It explains IL-6 expression pattern in all 3 groups, because of cytokine presence in remodeling and also in inflammatory processes. Respectively, in young patients its expression depends on remodeling and more in oral inflammatory events in adults.

MMP-8 and MMP-9 expression in periodontal tissues is associated also with remodelling and inflammation. Similar finding were found in Kim et al [18] study on rats. In adults PDL is exposed to mechanical and inflammatory stress agents more than in young patients, but in young patients PDL remodelling, according to growth processes, occurs with ECM degradation and its renewal.

Small number of patients in every group does not created adequate power for statistical assumptions. Higher number of included patients in all study groups is reasonable for continuous study with adequate statistical power. Semi-quantitative evaluation method has subjective issues because of random visual fields that are selected manually and also by evaluation that is made by two examiners. Last issue brings some human factor in results. On

the other hand, we found strong correlation between bFGF decrease with aging ($p<0.0002$).

CONCLUSIONS

Analyzed data suggest that bFGF, FGFR1, IL-6, MMP-8 and -9 were determined as signaling factors in PDL of interradicular septum. FGFR1, IL-6, MMP-8 and MMP-9 mean expression level decrease with age was statistically non significant. The mean expression level of bFGF decreased with age, and this decrease was statistically significant. In younger patients, signal molecule expression is higher because of increased periodontal tissue metabolic activity. With aging, the periodontal tissue remodeling process becomes less active, and in adults, signalling molecule expression is decreased.

Obtained data will be used in our further studies.

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