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Signalling molecules in jaw bones and gingival tissues of patients with Class II and Class III dentofacial deformities

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SUMMARY

Objectives. To detect signalling molecule specificities in jaw bone growth zones in skeletal class II and class III patients and compare them to those of a control group.

Material and Methods. Twenty skeletal Class II and 20 skeletal Class III patients who underwent orthognathic surgery treatment were in the study group and five skeletal Class I patients who had impacted third molars extracted were in the control group. During the orthognathic surgery, tissue samples were taken from the *tuber maxillae*, *ramus mandibulae* anterior and posterior part together with mucosa from the gingival transitory fold in the second molar region of the lower jaw. The samples were stained to detect TGF-β, BMP2/4, FGFR1, VEGF, OC, OP and MMP2 expression. The distributions of these factors were assessed semiquantitatively.

Results. We observed significant expression of TGF-β, BMP2/4, OC and OP in the bone tissue of the study group. FGFR1 expression was more pronounced only in mucosa. VEGF and MMP2 were found only in some tissue samples. More apoptotic cells were observed in the bone tissue and soft tissue of the control patients than in those of the skeletal Class II and Class III patients, in which apoptotic cell frequencies were relatively equal.

Conclusion. From bone tissue in *tuber maxillae* region the greater TGF-β and BMP2/4 expression is seen in Class III and control groups, comparing to Class II. In r*amus mandibulae* anterior part the expression of significant factors in bone tissue growth (TGF-β un BMP2/4) is higher in the control group and Class II patients, while in *ramus mandibulae* posterior part higher expression of TGF-β and BMP2/4 is in Class III patients, comparing to Class II, which indicates to a preserved growth potential in these jaw bone regions. More active bone extracellular matrix protein (osteocalcin and osteopontin) expression in *tuber maxillae* region both in class II and class III patient groups and different expression in *ramus mandibulae* anterior part, prove to the bone mineralization and metabolism activity changes, which, perhaps, characterize just these dentofacial deformations.

Key words: orthognathic surgery, bone, signalling molecules, immunohistochemistry.

INTRODUCTION

Most commonly, the skeletal morphology of dentofacial deformations involves jaw hypoplasia or hyperplasia (1, 2), which corresponds to Class II and Class III dentoalveolar malocclusion. Clinically, their jaw bone deformation manifestations are similar; however, X-ray cephalometry reveals these anomalies to have distinct skeletal morphologies (3), consistent with the multi-faceted aetiopathogenesis of these anomalies.

Both the form and size of bones are constantly developing during the bone tissue remodelling process, whereas at the cellular level, osteoclastic and osteoblastic cell lines function in coordination (4-6). Changes in the activities of these cell lines lead to deviations from the accepted norms of bone form and size (7). At the molecular level, the activity of these cells is induced and maintained by growth factors (8). Growth factors are main signaling molecules for growth and development in ante– and postnatal period. They are involved in cellular proliferation, differentiation and morphogenesis of tissues and organs during embryogenesis, postnatal growth and adulthood. The effect of growth factors is mediated through surface receptors on the target cells by activating intracellular phosphorylating enzymes, which in turn induce an intracellular signaling pathway by aggregation of co-factors and other proteins which migrate to the nucleus. Together with other tran-

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scription factors they activate a set of genes, which then exert the specific changes in cellular activity or phenotype (9).

The main growth factors important in bone growth and turnover are transforming growth factor β (TGF-β), bone morphogenetic proteins (BMP), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). Transforming growth factor-β belongs to a large superfamily of related proteins that also includes bone morphogenetic proteins, growth and differentiation factors, activins and inhibins. During the early stages of bone formation, the action of TGF-β is to recruit and stimulate osteoprogenitor cells to proliferate, providing a pool of early osteoblasts (10). Quite the opposite, during later phases of osteoblast differentiation, TGF-β blocks differentiation and mineralization (11). Bone morphogenetic proteins have the unique functions of inducing the differentiation of cells of the osteoblastic lineage, therefore increasing the pool of mature cells and of enhancing the differentiated function of the osteoblast. Fibroblast growth factors during embryonic development play a critical role in morphogenesis by regulating cell proliferation, differention and cell migration but in adult organism FGF have an important role in the control of the nervous system, in tissue repair, wound healing and in tumor angiogenesis (12, 13). Vascular endothelial growth factor takes part in osteogenesis by recruitment of new blood supply, which provides the necessary osteoprogenitor cells (14, 15).

An important aspect of the structure of bone tissue lies in the composition, formation and degradation of intercellular material known as the extracellular matrix (16). MMP (matrix metalloproteinases) are secreted from connective tissue cells, can break down many structural macromolecules in the connective tissue matrix. MMP activity is associated with physiologic and pathologic conditions that involve matrix degradation and remodeling.

Cell death is equally important because under physiological conditions, cells end their life-cycle by means of apoptosis (17, 18).

The aim of this study was to assess the distribution of signalling molecules important for bone remodelling in jaw bone growth zones of skeletal Class II and Class III patients in comparison with a control group.

MATERIAL AND METHODS

Twenty skeletal Class II and 20 skeletal Class III patients who needed combined orthodontic and orthognathic surgery were included in the study. The mean age of the patients was 20.64±3.27 years. In the Class II group, there were 13 women and 7 men, whereas in the Class III group, there were 14 women and 6 men. Patients with a severe general disease at the time of the operation or the anamnesis, with a lip and/or palatal cleft, with a dentofacial syndrome or with skeletal asymmetry were excluded from the study. Biopsy samples were taken during orthognathic surgery from the jaw osteotomy sites in the upper (*tuber maxillae*) and lower jaw (*ramus mandibulae* anterior and posterior part) and from the lower jaw gingival transitory fold in the second molar region.

The control group included 5 patients who underwent extractions of impacted third molars with skeletal and dentoalveolar Class I and with no tooth row anomalies. The control group patients had not had any orthodontic treatment and lacked general illnesses, lip clefts, palatal clefts, dentofacial syndromes, and any clinical signs of inflammation or pain prior to extraction. The mean age of the patients was 19.4±2.7 years; 3 were women, and 2 were men. Tissue samples were taken from *tuber maxillae* and the anterior part of the *ramus mandibulae*, but considering the extraction method and in order not to cause extra tissue damage to the patient, tissue samples were not obtained from the posterior part of the *ramus mandibulae*.

The tissue samples for the study were used in conformity with the permission of the Ethics Committee of Riga Stradins University.

Immunohistochemistry. Tissue were fixed for a day in mixture of 2% formaldehide and 0,2% picric acid in 0.1 M phosfate buffer (pH 7.2). After samples were rinsed in thyroide buffer, containing 10% sacharose for 12 hours, then embedded into paraffin and cut in 6-7 μmthin sections. The primary antibodies utilized in immunohistochemistry were specific for transforming growth factor β (TGF-β, ab1279, Cambridge Science, UK), bone morphogenetic factor 2/4 (BMP2/4, av1024011; RD Systems, UK), fibroblast growth factor receptor 1 (FGFR1; ab10646; Abcam, UK), vascular endothelial growth factor (VEGF, M7273; Dako, Denmark), matrix metalloproteinase 2 (MMP2, AF902; RD Systems, UK), osteocalcin (OC, ab13418; Abcam, UK) and osteopontin (OP, ab8448; Abcam UK).

Immunoreaction was visualized by the avidin-biotin (LSAB) immunoperoxidase method using an LSAB kit (DakoCytomation, DK), and DAB (diaminobenzidine) solution (Dako, DK) was used as chromogen, and hematoxylin was used as the counterstain.

TUNEL reaction was used for detection of apoptosis (19). *In situ Cell Death Detection*, POD (Roche Diagnostics) and DAB substrate (Vector) were used. Deparafinised sections (xylol 2×4 min, 99% ethanol 2×2 min, 95% ethanol 2×2 min and 70%

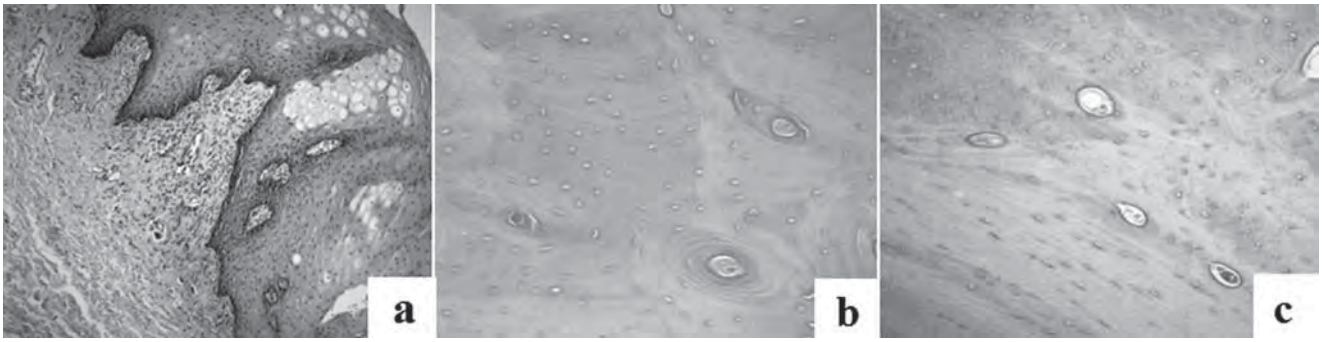

Fig. 1. Microphotograph of oral mucosa from the region of the lower second molar in a class III patient showing gingival epitheliocyte vacuolisation and basal cell hyperplasia (a), bone from the ramus mandibulae anterior part of a Class II patient with, irregular connective tissue ingrowths and blood vessel sclerotisation in the osteon canals (b), bone with irregular mineralisation from the tuber maxillae of a Class III patient (c)

ethanol 2×2 min) were rinsed with water (7-10 min) and transferred to PBS (pH 7.5) for 10 min. Subsequently slides placed into 50 ml PBS solution with 500 µl 30% hydrogen peroxide for 30 min on shaker to block the endogenous peroxidases. Afterwards tissue samples were washed with PBS (3×5 min), placed into 0.2 M boric acid (pH 7.0), placed into microwave (700 W) for 10 min for fixation of antigen, cooled to room temperature and rinsed with PBS. After that, slides were kept in refrigerator in 0.1% BSA (bovine serum albumin) solution with PBS for 10 min and then incubated in TUNEL mix (Tdt – mix of terminal deoxynucleotide transferase and DIG-labeled deoxynucleotide) for 1h at +37°C. Then the slides were rinsed with PBS 1:10, and incubated for 30 min at +37ºC with POD (sheep anti-digoxygenin antibogy coupled with horseradish peroxidase Fab fragment). Then the slides were washed with PBS, covered with DAB (diaminobenzidine chromogen) for 7 min, and then rinsed with running water for 5 min. For visualisation, a routine histological method was used and the samples were routinely stained with haematoxylin and eosin and microscopically analysed with a Leica BME microscope.

To record the relative frequency of indices detected by immunohistochemistry, a semi-quantitative counting method widely used in the literature was employed (20, 21). The appearance frequency of factors was analysed in three visual fields of each slice. To process the data statistically, the number of cells observed in each visual field was coded (0 – no positive structures in the field; 1 (0/+), rare occurrence of positive structures in the field; 2 (+), a few positive structures in the field; 3 (+/++), few to average positive structures in the field; 4 (++), average to many positive structures in the field; 5 (++/+++), above average to abundant positive structures in the field; and 6 (+++), abundant positive structures in the field).

The data were analysed using descriptive and analytical statistical methods. The mean values and standard deviations (SDs) were calculated. For hypothesis testing, appropriate parametric (t test and dispersion analysis (ANOVA)) and non-parametric (Kruskal-Wallis and Mann-Whitney) methods were used. To compare two or more variables, correlation analysis methods were used. Correlation coefficients of range scale values were calculated with the Spearman range correlation coefficient, whereas those of measured values were calculated with the Pearson correlation coefficient (22).

RESULTS

Morphological findings in bone tissue and mucosa

Routine review picture of the soft tissue revealed gingival epitheliocyte vacuolisation and basal cell hyperplasia in the epithelium of both Class II and Class III patients, together with irregularly thickened basal membrane and inflammatory cell infiltration into the subepithelial connective tissues (Fig. 1a).

Bone material from the anterior *ramus mandibulae* and posterior trabecules contained chaotically localised collagenous fibres and unevenly localised osteon structures. In the osteon canals, irregular connective tissue ingrowths and blood vessel sclerotisation were observed (Fig. 1b). *Tuber maxillae* bone tissue also had irregular bone mineralisation, and osteon canal obliteration and connective tissue proliferation were observed (Fig. 1c).

The bone and mucosal tissue of the control group were without marked structural changes and presented a histological picture corresponding to the common standard.

Analysis of the expression of the studied growth factors, bone extracellular matrix proteins and degeneration enzymes in the jaw bones and surrounding soft tissues in skeletal Class II, Class III and control group patients (Table 1) revealed that the expression of these biological markers is rather variable; however, in all

the study groups, the expression of those markers mainly responsible for bone remodelling was pronounced. We observed significant expression of TGF-β (Fig. 2a), BMP2/4 (Fig. 2b), OC (Fig. 3a) and OP (Fig. 3b) in the bone tissue of the *tuber maxillae* and the anterior and posterior *ramus mandibulae* of the study group.

The expression the growth factor receptor FGFR1 was found to be more pronounced only in the soft tissues from the lower jaw gingival transitory fold in the second molar region in comparison to its expression in bone tissue. Vascular endothelial growth factor and the degeneration enzyme MMP2 were found only in two Class II patients gingival tissues, whereas in the control group, no positive signals for VEGF or MMP2 were observed in any of the tissue samples. In the control group, the expression of biological markers in the *tuber maxillae* and anterior *ramus mandibulae* is rather similar. However, in Class III patients, higher expression is observed only in the bone tissue from the *tuber maxillae* and *ramus mandibulae* posterior parts, whereas in the Class II tissue samples, higher expression was observed in the *tuber maxillae* and *ramus mandibulae* anterior part.

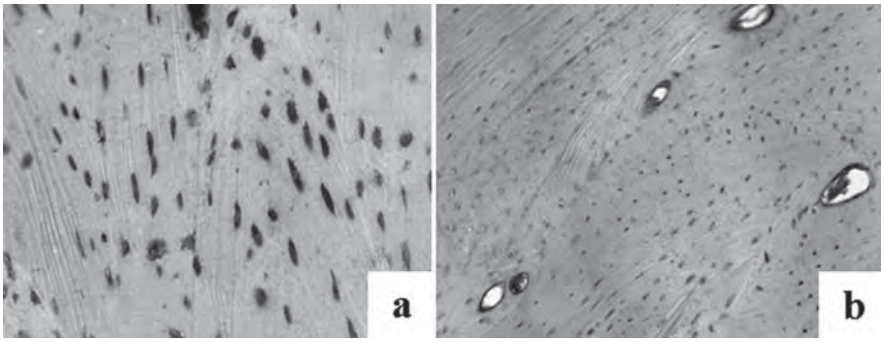

Fig. 2. Microphotograph of significant TGF-β expression in ramus mandibulae anterior part of a control group patient, IMH, ×400 (a), abundant BMP2/4 positive structures in ramus mandibulae posterior part of a Class II patient, IMH, ×250 (b)

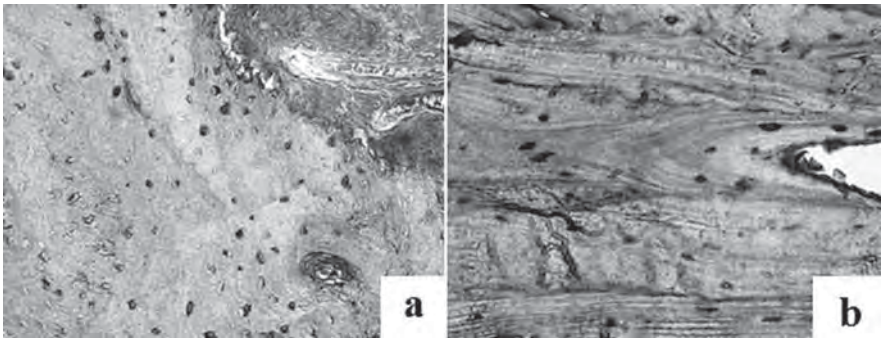

Fig. 3. Microphotograph of osteocalcin expression in the tuber maxillae of a Class III patient, IMH, ×200 (a), osteopontin expression in the tuber maxillae of a Class III patient, IMH, ×200 (b).

Analyzing the mean number of apoptopic cells in the bone tissue from *tuber maxillae* class II, class III and in the control patient groups, we found that a

Table 1. Mean protein expression indices of growth factors, bone extracellular matrix proteins and degeneration enzymes in the jaw bones and soft tissues in the patient groups

	Class II					Class III					Control (Class I)				
				Mucosa					Mucosa					Mucosa	
	TM	RMAP	RMPP	Epithelium	Connective tissue	TM	RMAP	RMPP	Epithelium	Connective tissue	TM	RMAP	RMPP	Epithelium	Connective tissue
TGF-β	+/++	+/++	+	+++	+++	++/+++	+	+/++	+++	++	+++	++/+++	-	+++	+++
BMP2/4	0/+	+	+	0	+	+	+	+/++	+	+/++	+	++	-	0	0/+
FGFR1	+*	+	+	++*	+++	++*	+	+/++	+++*	+++	+	-	-	-	-
VEGF	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0
OC	+++	++	+	-	-	+++	+	-	-	-	++/+++	+++	-	-	-
OP	+/++*	+/++	+/++	-	-	++*	0/+	-	-	-	+++*	+++	-	-	-
MMP2	0	0/+*	0	0	0	0/+	0*	0	0	0/+	0	0*	-	0	0

0 – no positive structures in the visual field; 0/+ – rare occurrence of positive structures in the visual field; + – a few positive structures in the visual field; +/++ – few to average positive structures in the visual field; ++ – average to many positive structures in the visual field; ++/+++ – above average to abundant positive structures in the visual field; and +++ – abundant positive structures in the visual field; TM – tuber maxillae, RMAP – ramus mandibulae anterior part, RMPP – ramus mandibulae posterior part. * – expression is significantly different between groups, $p \leq 0.05$.

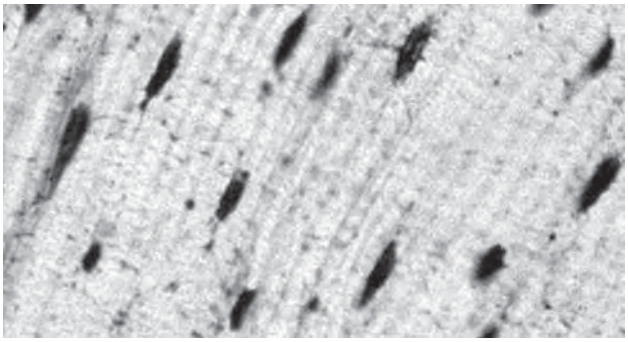

Fig. 4. Microphotograph of apoptotic cells in ramus mandibulae anterior part of a Class III patient (TUNEL, ×250)

greater number of apoptotic cells were in the control group (mean value 25.89±11.34 cells in the visual field). In class II and class III patients the number of apoptopic cells is practically equal and less than in the control group (mean values respectively are 9.42±19.56 and 9.25±9.53). Also in the tissue material from *ramus mandibulae* anterior part the apoptopic cell number in the control group is the greatest (mean value 28.44±6.45), but in class II and class III patients the apoptopic cells are seen to be in a lesser amount (mean values respectively are 10.84±10.52 and 11.86±13.38). Number of apoptotic cells in *ramus mandibulae* posterior part in class II and class III patient groups mean values respectively were 7.27±11.74 and 5.73±5.19 cells.

Analyzing the cross-correlations in *tuber maxillae* (see Table 2) we found out that there existed a moderately close statistically significant correlation between the transforming growth factor β (TGF-β) and the bone morphogenetic protein 2/4 (BMP2/4) expression (p=0.035; r=0.415), as well as between TGF-β and osteocalcin expression (p=0.036; r=0.512). With the increase of the number of apoptotoc cells in *tuber maxillae* bone tissue, there in parallel increases also the fibroblast growth factor's receptor 1 (FGFR1) amount in the tissues, which gives the evidence to a moderately close statistically significant positive correlation (p=0.025; r=0.615).

Table 2. Cross-correlations between the growth factors, bone extracellular matrix proteins, degeneration enzymes and apoptotic cells expression in the *tuber maxillae*

		TGF-β	BMP2/4	FGFR1	OC	MMP2
BMP2/4	r	**0.415**	1			
	p	***0.035**				
FGFR1	r	-0.070	0.077	1		
	p	0.764	0.748			
OC	r	**0.512**	0.288	-0.449	1	
	p	***0.036**	0.279	0.166		
MMP2	r	0.087	0.308	-0.309	0.270	1
	p	0.701	0.175	0.213	0.397	
TUNEL	r	0.287	0.249	**0.615**	0.272	-0.296
	p	0.249	0.352	***0.025**	0.393	0.325

TGF-β – transforming growth factor β, BMP2/4 – bone morphogenetic protein 2/4, FGFR1 – fibroblast growth factor receptor 1, OC – osteocalcin, MMP2 – matrix metalloproteinase 2, TUNEL – apoptotic cells expression, r – correlation coefficient; p – statistical significance, * – p≤0.05.

Analyzing the correlations in the tissue material from *ramus mandibulae* anterior part (see Table 3), we found out statistically significantly close positive correlation between TGF-β and BMP2/4 (p=0.008; r=0.427). With the increase of TGF-β expression, there increases the number of apoptotic cells, since both of these indices correlated between each other with a statistically significant close correlation (p=0.003; r=0.548). We also found that in the tissues from *ramus mandibulae* anterior part there exists a statistically significantly close positive correlation between the number of apoptotic cells and BMP2/4 expression (p=0.009; r=0.502). The amount of the growth factor TGF-β in the studied patients' bone tissue from *ramus mandibulae* posterior part (see Table 4) statistically significantly moderately closely correlates positively with FGFR1 expression (p=0.041; r=0.595). No statistically significant or moderately close positive correlations were observed for any of the studied morphological parameters in the mucosal epithelium acquired from the second molar region.

DISCUSSION

The mucosal changes found in skeletal Class II and Class III patients, who are characterised by epitheliocyte focus-type vacuolisation, cell hyperplasia, basal membrane changes and inflammatory cell infiltration, likely reflect oral hygiene difficulties. Kurol *et al.* (23) found similar gingival morphological changes in patients with fixed appliances during orthodontic treatment. In the bone tissue of the patients under study, irregular mineralization with vascular sclerosis and connective tissue proliferation in osteon canals was observed, which could be associated with insufficient blood supply to jaw bones, thus affecting upper and lower jaw growth in patients with dentofacial deformations**.**

Our results indicate that patients with skeletal dentofacial deformations have a greater amount of relative positive structures for important growth factors that are well recognised in bone tissue morphogenesis (such as TGF-β and BMP2/4) and bone extracellular matrix proteins (OP and OC), with slight differences between the study groups, indicating the importance of these morphological markers in supportive tissue remodelling and the development of bone tissue in

patients with dentofacial deformations. In all tissue samples, TGF-β expression was most evident, possibly because this protein belongs to the TGF-β superfamily, which includes TGF-βs themselves, activins and BMP. BMP 2/4 showed high levels in tissue samples from the anterior and posterior *ramus mandibulae* and in the *tuber maxillae*, which partially supports the data of Suttapreyasri *et al.* (24).

In the present study, OP was the most dominant noncollagenous extracellular bone matrix protein in the control group. OP is an early marker of bone matrix, whereas OC is a late marker (25). Sasano *et al.* (26) suggested that bone matrix proteins continue to accumulate in the matrix as embryonic osteogenesis proceeds, and bone expands its area with more intense OP deposition at the periphery, which consists of immature matrix components, and lower OP levels in the central parts of bone that are more mature. These data correspond to the findings in our study, as OP expression was relatively uniform throughout the bone, with increased staining near periosteum.

OC is produced by mature osteoblasts, odontoblasts and chondrocytes. In a study with OC knockout mice, Ducy *et al.* (27) reported no abnormal phenotype until 6 months of age because there was markedly higher bone mass and bones of improved functional quality. The findings of the current study demonstrate very inconsistent OC expression, suggesting that there could be some disturbances in osteocalcin-depending bone remodelling.

Similar and marked expression of FGFR1 in the oral mucosa of all groups' tissue samples indicates the importance of this growth factor receptor in soft tissues. Therefore, we can conclude that oral mucosa has sufficient regenerative potential, which is beneficial because skeletal Class II and Class III patients undergo orthognathic surgery with intraoral incisions, and this factor's dominant expression should promote rapid convalescence of the oral mucosa after the operation. The expression of FGFR1 in the *tuber maxillae* and *ramus mandibulae* posterior part was less pronounced in Class II patients than in Class III patients, which could reflect defective bone remodelling processes. This possibility is consistent with the results of Jacob *et al.* (28), who demonstrated that signalling through FGFR1 in osteoblasts is necessary to maintain the balance between bone formation and remodelling through a direct effect on osteoblast maturation. They suggest that the predominant role for FGFR1 signalling in mature bone is to decrease anabolic activity and slow the terminal differentiation (mineralisation) of osteoblasts.

Weak MMP2 expression and a virtual lack of VEGF expression in the patients' tissues indicates that that neither have a significant role in skeletal Class II and Class III patients' bone remodelling.

Jilka *et al.* (29) suggested that the prevalence of osteoblast apoptosis is much lower in human bone than in mouse bone. This difference results from two reasons: human osteoblasts live longer and are fewer in number compared to their murine counterparts because bone turnover in humans is lower. This difference could also be associated with the total number of osteoblasts; the more osteoblasts observed in a biopsy section, the more apoptotic cells were also observed. In our study, the greatest numbers of apoptotic cells were observed in the bone and soft tissues of the control patients, whereas the lower and relatively

Table 3. Cross-correlations between the growth factors, bone extracellular matrix proteins, degeneration enzymes and apoptotic cells expression in *ramus mandibulae* anterior part

		TGF-β	BMP2/4	FGFR1	OC	MMP2
BMP2/4	r	**0.427**	1			
	p	****0.008**				
FGFR1	r	-0.037	-0.073	1		
	p	0.836	0.691			
OC	r	**0.522**	0.246	-0.057	1	
	p	***0.018**	0.309	0.835		
MMP2	r	0.083	**0.486**	-0.064		1
	p	0.708	***0.019**	0.794	.000	
TUNEL	r	**0.548**	**0.502**	**0.036**	0.130	-0.251
	p	****0.003**	****0.009**	**0.865**	0.595	0.387

TGF-β – transforming growth factor β, BMP2/4 – bone morphogenetic protein 2/4, FGFR1 – fibroblast growth factor receptor 1, OC – osteocalcin, MMP2 – matrix metalloproteinase 2, TUNEL – apoptotic cells expression, r – correlation coefficient; p – statistical significance, * – p≤0.05; ** – p≤0.001.

Table 4. Cross-correlations between the protein expression of growth factors, bone extracellular matrix proteins and degeneration enzymes in *ramus mandibulae* posterior part

		TGF-β	BMP2/4	FGFR1	OC
BMP2/4	r	0.121	1		
	p	0.694			
FGFR1	r	**0.595**	0.052	1	
	p	***0.041**	0.867		
OC	r	0.943	0.500	0.866	1
	p	0.057	0.667	0.333	
TUNEL	r	0.045	0.515	-0.136	0.730
	p	0.890	0.105	0.674	0.161
TUNEL	r	0.548	0.502	0.036	0.130
	p	**0.003	**0.009	0.865	0.595

TGF-β – transforming growth factor β, BMP2/4 – bone morphogenetic protein 2/4, FGFR1 – fibroblast growth factor receptor 1, OC – osteocalcin, TUNEL – apoptotic cells expression, r – correlation coefficient; p – statistical significance, * – p≤0.05.

equal numbers of apoptotic cells in skeletal Class II and Class III patients could indicate an imbalance between cell proliferation, differentiation and apoptosis.

CONCLUSIONS

From bone tissue in *tuber maxillae* region the greater TGF-β and BMP2/4 expression is seen in Class III and control groups, comparing to Class II, which, probably, suggests to a potentially probable, yet not expressed bone growth in these region. In r*amus mandibulae* anterior part the expression of significant factors in bone tissue growth (TGF-β un BMP2/4) is higher in the control group and Class II patients, while in *ramus mandibulae* posterior part higher expression of TGF-β and BMP2/4 is in Class III patients, comparing to Class II, which indicates to a preserved growth potential in these jaw bone regions. FGFR1 similar expression and practically lack of VEGF expression in patients' bone issue confirm both as insignificant factors in skeletal Class II and Class III patients bone remodelling. The same can be referred also to MMP2 unpronounced expression in the Class II, Class III and control groups. More active bone extracellular matrix protein (osteocalcin and osteopontin) expression in *tuber maxillae* region both in Class II and Class III patient groups and different expression in *ramus mandibulae* anterior part, prove to the bone mineralization and metabolism activity changes, which, perhaps, characterize just these dentofacial deformations.

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