RIGA STRADINS UNIVERSITY
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FUNCTIONAL MORPHOLOGY OF THE CLEFT LIP AND PALATE AFFECTED TISSUE IN THE MATERIAL OF OPERATIONS

(Speciality - Morphology)

SUMMARY of the DOCTORAL THESIS

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Topicality of the Research

The facial clefts refer to one of the most commonly encountered human congenital conditions. In Latvia the cleft lip and cleft palate occur on the average in one child in 700 - 800 newborn infants, which is the second widely met pathology in embryos and fetus (Akota etc., 2001; Pilmane and Šūmahers, 2006). Clefts can entail esthetical disorders and cause heavy functional deviations, which already on the first days and years of life may adversely affect the child's physical and mental development. These deviations affect the child's basic life functions – wholesome eating, teeth and jaw functions, language development and sometimes also further social integration of an individual. The cleft lip and cleft palate treatment includes multiple plastic surgical corrections and repeated visits to different specialists. Besides, the cleft correction, which is followed by wound closure and formation of scar tissues, may adversely affect the growth of facial and oral cavity tissues (Van Beurden et al., 2005; Burdi, 2006).

The development of the face and the oral cavity is based on the successive merger of five facial creases, which occurs within a period from the fourth to the twelfth week of the embryonic development. This is a cascade of precisely coordinated processes that involves not only the cell growth, differentiation, cell-to-cell and cell-extracellular matrix interactions, but also the programmed cell death. The before-said transformations in the embryonic tissues are caused and regulated by various factors. A significant place among them is directly related to the growth factors and the importance of the genes identified, whose study has been intensified within the recent two ten years and is still actively going on. Besides, the lack of just one definite factor or its excessive presence can cause irreversible changes, resulting in congenital abnormality, i.e., facial cleft.

The growth factors refer to proteins, which work as signal molecules and relate with the target cell surface receptors. The molecular aspects of their work during the development of facial and oral cavity structures are not fully clear yet. Recently the studies on the role of apoptosis during the secondary palate development have become very topical. It is known that primordial epithelium during fusion disappears; however, there are several theories to explain the mechanism of this process (Dudas et al., 2007).

The fibroblast growth factors (FGF) are known to refer to the polypeptide group, which plays a determinative role in migration of neural crest cells, as well as in regulation of interaction of epithelium and mesenchyma during the development of the face and oral cavity (Szebenyi and Fallon, 1999; Greene and Pisano, 2004). They form complexes with four fibroblast growth factor receptors (FGFR), which have an impact on normal cell growth and differentiation (Hughes, 1997; Greene and Pisano, 2004). The FGFR plays a significant role in the development of a normal mammalian palate (Crisera et al., 2008). The transforming growth factor – beta (TGF-β) is a multifunctional cytokine, which regulates the development of the structure of oral cavity (Bodo et al., 1999; Meng et al., 2009) and directly affects the development of the secondary palate (Greene and Pisano, 2004).

Nonetheless, irrespective of many studies**,** the data on distribution of various growth factors and genes and location in various human cleft-affected tissues are still not clear. Although the significance of an individual factor is being viewed quite often (Kohama et al., 2002), there should be a team approach used for the analysis of results. In literature there are no integrated data on the position of various genes, growth factors and their receptors and distribution thereof in the cleft-affected tissues.

Recently the studies on the significance of growth factors in the successful tissue regeneration and healing during the postoperative period have become specifically topical (Werner and Grose, 2003; Akasaka et al., 2004; Ono et al., 2007). The data as to the mentioned local factors, their receptors and gene expression, as well as on a relative abundance of apoptosis in the cleft-affected tissues are important for a successful or less successful therapeutic prognosis.

Aim of research

To make an immunohistochemical study of the growth factors, the growth factor receptors and the gene relative abundance and location, determination of cell death and data correlation in the cleft-affected tissues in children with unilateral and bilateral cleft lip and cleft palate in the ontogenetic aspect with the detection of factors, who are main characteristic in morphopathogenesis of clefts.

Hypothesis of research

1. Abundance and expression of the growth factors, the growth factor receptors and genes are determined in the processes of regeneration and degeneration of the tissue local response.
2. Every cleft type has its own most significant factors that determine the cleft development.

Objectives of research

1. To determine the relative abundance and distribution of basic FGF, FGFR1, NGF, NGFR, TGFβ, PGP 9.5 and Barx1 in the cleft disordered tissues of children with unilateral and bilateral cleft lip and palate, applying the immunohistochemical method.
2. To determine relative abundance and distribution of apoptotic cells in the unilateral and bilateral cleft disordered tissues, applying the TUNEL method.
3. To determine the relative abundance and distribution of basic FGF, FGFR1, NGF, NGFR, TGFβ, PGP 9.5 and Barx1 in tissues of the control group, applying the immunohistochemical method.
4. To determine relative abundance and distribution of apoptotic cells in tissues of the control group, applying the TUNEL method.
5. To determine possible cross – correlation of the obtained data.
6. To determine possible correlation of the obtained data in the age aspect.
7. To determine the relative abundance and distribution of basic FGF, FGFR1, NGF, NGFR, TGFβ, PGP 9.5 and Barx1 in the cleft disordered tissues after repeated surgical operation in children with unilateral and bilateral cleft lip and palate.
8. To determine the diagnostic and prognostic factors in the development of various facial clefts and therapeutic prognosis.

Novelty of research

Applying the immunohistochemical and TUNEL methods, there were studied the tissues from the cleft correction area of 52 patients with unilateral and bilateral cleft lip and palate. There has been proved and documented in pictures the bFGF, FGFR1,

NGF, NGFR, TGFβ, PGP 9.5 and Barx1 gene expression and the relative abundance of immunoreactive cells in the tissue material taken not only during the first, but also during the repeated surgical operation. The data obtained during our study show, that the unilateral and bilateral cleft lip and palate cleft-affected tissues are characterized by increased bFGF and FGFR1, while by a reduced NGF local expression, increased cell apoptosis, variable NGFR, TGFβ and Barx1 expression and relatively different quantity of DNES structures, are completely new. The increased cell apoptosis and the relatively more variable expression of factors in severe abnormalities are found in the bilateral lip and palate affected tissues.

The structure and volume of the doctoral thesis

The thesis for PhD degree is written in Latvian. The thesis consists of 7 parts: introduction, review of literature, materials and methods, results, discussion, conclusions, the list of references. The volume of the thesis amounts to 113 pages, including 18 tables and 2 figures. 76 micro photos are enclosed in appendix. The list of references consists of 246 titles. There are 22 publications on the doctoral thesis.

Material and Methods

The research is based on the material of cleft lip and cleft palate patients, which was gathered within a period of time from 2003 to 2006 at the Cleft Lip and Palate Centre of the Institute of Stomatology of the Riga Stradins University.

Permission of the Ethics Committee: decision of the RSU Ethics Committee dated 22nd May 2003.

The research involved 52 patients with cleft lip and palate at the age of three months to 12 years and six months. 37 children had unilateral cleft lip and palate, but 15 children had bilateral cleft lip and palate. A part of the patient material was taken not only during the primary, but also the repeated plastic surgery correction. Depending upon the age and diagnosis, the patients were divided into four groups:

The 1st group: children with unilateral cleft lip and palate in age before and primary dentition,

The 2nd group: children with unilateral cleft lip and palate in mixed dentition age,

The 3rd group: children with bilateral cleft lip and palate in age before and primary dentition,

The 4th group: children with bilateral cleft lip and palate in mixed dentition age.

The control material was obtained from 11 patients with oral - facial trauma and cleft – unrelated surgical operations. The material was gathered at the Institute of Stomatology of the Riga Stradins University within a period of time from 2004 to 2006.

Tissue was proceeded for detection of basic fibroblast growth factor (bFGF), the first fibroblast growth factor receptor (FGFR1), the nerve growth factor (NGF), the nerve growth factor receptor p75 (NGFRp75), the transforming growth factor - beta (TGFβ), the Barx1 gene (Barx1) and the protein gene product 9.5 (PGP 9.5) expression by use of biotin-streptavidin immunohistochemistry (Table 1).

Table 1. Data on antibodies applied in immunohistochemistry.

N°	Factor	Code	Obtained from	Working dilution	Manufacturer
1	bFGF	ab16828	a rabbit	1:200	Abcam, UK
2	FGFR1	ab10646	a rabbit	1:100	Abcam, UK
3	NGF	ab6199	a rabbit	1:500	Abcam, UK
4	NGFR	M3507	mice	1:150	DakoCytomation, Denmark
5	TGF beta	ab1279	mice	1:1000	Abcam, UK
6	PGP 9.5	Z 5116	a rabbit	1:150	DakoCytomation, Denmark
7	BarX1	ab26156	a rabbit	1:250	Abcam, UK

The TUNEL method was applied for determination of apoptosis in the tissues using in situ cell death detection kit (POD Cat. No. 11 684 817 910 Roche Diagnostic).

In order to visualize the scene, the routine histology method was used, staining the samples with haematoxylin and eosine.

For marking of relative frequency of the immunohistochemically determined structures we used a widely applied semi-quantitative counting method (Tobin et al., 1990; Pilmane, 1997**)** used in the scientific literature. The quantity of structures was analyzed in five visual fields of one section. Explanatory notes on the applied markings are given in Table 2.

Table 2. Marking of relative frequency of the immunohistochemically determined structures.

Applied markings	Explanatory notes
-	No positive structure seen in the visual field
0/+	Rare positive structures seen in the visual field
+	Few positive structures seen in the visual field
+/++	Few to moderate number of positive structures seen in the visual field
++	Moderate number of positive structures seen in the visual field
++/+++	Moderate to numerous positive structures seen in the visual field
+++	Numerous positive structures seen in the visual field
+++/++++	Plenty of positive structures in the visual field

For the group description we used the generally accepted descriptive statistical methods (Altman, 1991; Altman, 2000; Teibe, 2007). For data evaluation we applied the nonparametric statistical methods. For comparison of two independent groups, applying the ranking values, we used the Mann-Whitney test. Results are considered to be plausible, when $p<0.05$. For comparison of a number of samples we used the Kruskal Wallis test.

The correlation coefficient *r* as a quantitative indicator of coherence tightness between two or more variables calculated for the ranking values (Spearman's Rank Correlation Coefficient). In the study the qualitative coherence tightness between variables, on the grounds of the correlation coefficient value, was assessed as weak, average or tight. The distribution of the correlation coefficient was as follows: $r = 0 – 0.3$, low, insignificant correlation; $r = 0.4 – 0.7$, average correlation; $r = 0.7 – 0.9$, tight correlation.

A statistical analysis was carried out by means of the SPSS (SPSS Inc., USA).

Results

Routine Histology Findings

In tissues of oral cavity of **the control patients** we saw, mainly, a normal histological picture, i.e., unchanged epithelium of masticatory and lining mucosa of various thickness, indistinct basement membrane, unchanged below connective tissues, as well as normal cartilaginous and bone tissues. Only in rare circumstances we observed indistinct infiltration of inflammatory cells into soft tissues.

In the material of **the patients with unilateral and bilateral cleft lip and cleft palate** we saw the morphologically changed mucosa of oral cavity and lip tissues. The oral cavity mucosa is formed by stratified squamous epithelium, basement membrane and underlying connective tissue. We found the most pronounced changes exactly in epithelium.

Thickness of both stratified squamous nonkeratinized and stratified squamous parakeratinized epithelium in tissues of different patients differed markedly. In some children with clefts we saw thin and atrophic, while in other children we observed thick epithelium with pronounced proliferation of basal cells therein. Besides, in the majority of the patients the epithelial tissues were formed as a long outgrowth by jut out connective tissues. In epithelium of children with unilateral and bilateral clefts we observed also more or less pronounced vacuolization of cells in the stratum spinosum.

In the oral connective tissues of the patients with facial clefts we often observed the pronounced densely and at times very chaotically arranged bundles of collagen fibers with rare connective tissue cells between them. In some children we saw very many small, but often sclerotic blood vessels, which mainly localize around the mentioned epithelial outgrowths and around practically unchanged secretory portions and ducts of the small salivary glands. In the majority of the patients we observed infiltration of inflammatory cells both into the mucous epithelium and the underlying connective tissues.

Mainly in the lip skin of patients with unilateral and bilateral cleft lip and cleft palate we saw an unchanged histological picture – typical epidermis and derma, sebaceous glands and hair follicles. In tissues of some children we observed atypical hair follicles and chaotically arranged and sclerotic muscle fibers.

In the hyaline cartilage of the patients with unilateral and bilateral cleft lip and cleft palate we observed an abundant quantity of chondroblast in the zone of proliferation, relatively small isogenous groups and plenty of hypertrophic chondrocytes. In some cases we saw the degenerative cartilage fragments in fibrous tissues. In the tissue material of some patients we found practically unchanged cartilage.

In the tissue material of patients with unilateral and bilateral cleft lip and palate we often saw degenerative bone fragments. They are characterized by small quantity and irregular location of osteons, as well as few and often empty lacunae between the bone lamellae.

Immunohistochemical study and apoptosis findings

Control group findings

Expression of basic FGF was detected in the tissue material of nine patients (Table 3). In two cases we observed few positive structures – neutrophils and connective tissue fibers, but in four cases - rare positive structures in the visual field. The mentioned growth factors in these cases were expressed mainly in rare inflammatory cells, epithelial cells, vascular wall cells and connective tissue fibers. In three tissue samples relative number of bFGF containing structures in various tissues was a bit different. Thus, in the patient N°2 we saw few immunoreactive cells in the middle epithelial layer and rare positive connective tissue fibers. But in the patient N°1 the bFGF was expressed by few epithelial cells and connective tissue fibers, but no immunoreactivity was observed in the bone.

Expression of FGFR1 was observed in the tissues of all control group patients. In tissues of two patients we observed numerous positive structures. An abundant expression of the mentioned growth factor receptors was seen in mucosal epithelial cells (mainly the cells of the middle layer). In four children the relative number of immunoreactive structures in various tissues varied. In the tissue of patient N°6 we saw many immunoreactive connective tissues cells, but a moderate number of epithelial cells. In the patient N°1 we stated an abundant FGFR1 expression in osteocytes, but a moderate number of positive epithelial cells and few positive vascular smooth muscle cells. But in the tissue of patient N°7 the expression of FGFR1 was pronounced in epithelial and connective tissue cells, but less in the bone cells. In three cleft disordered tissue samples we found a moderate number of positive structures, where in two cases there were connective tissue cells and in one case – bone cells. Only in tissue of two patients we found few and rare FGFR1 - containing connective tissue and bone cells. In parallel, in certain cases we observed an expression of the receptors in vascular wall endotheliocytes and in the cells and fibers of perineurium, localized around the bundles of nerve fibers.

Expression of NGF we observed in tissues of the nine patients of control group. The relative number of mentioned factor - containing structures varied from rare to moderate. In the tissue material of patient N°7 we saw moderate number of

Table 3. Semiquantitative distribution of immunoreactive structures in the material of control group patients

N°	Code	bFGF	FGFR1	NGF	NGFR	TGFβ	Barx1	PGP 9,5	TUNEL
1	K10	- - +	+ - +++	0/+ - ++	++	+ - +++	+ - +++	+	+ - +/++
2	K7	0/+ - +	+++	-	+ - +++	++ - +++	0/+	0/+	+ - ++
3	K6	0/+	++	+	+/++	+++	-	+	-
4	K8	+	++	+	++	+ - ++	+ - ++	+++	++
5	K5	0/+	+++	0/+ - ++	+ - +++	++ - +++	-	+	0/+ - +/++
6	K1	+	++ - +++	0/+ - ++	+ - +++	++/+++ - +++	+ - +++	+/++	++
7	K11	- - 0/+	+/++ - +++	++	+	+ - +++	- - ++	+	++ - +++
8	K2	-	+ - ++	0/+	++	+++	- - 0/+	-	+/++
9	K9	-	0/+	0/+	++	+ - ++	- - +	-	- - ++/+++
10	K3	0/+	++	-	-	- - +++	- - 0/+	-	+/++
11	K4	0/+	+	+	++ - ++/+++	0/+ - ++	- - +	0/+	0/+

- no positive structure seen in the visual field; 0/+ rare positive structures seen in the visual field; + few positive structures seen in the visual field; +/++ few to moderate number of positive structures seen in the visual field; ++ moderate number of positive structures seen in the visual field; ++/+++ moderate to numerous positive structures seen in the visual field; +++ numerous positive structures seen in the visual field; +++/++++ plenty of positive structures seen in the visual field

immunoreactive inflammatory cells. In two tissue samples we also observed an average pronounced factor expression in the inflammatory cells, but rare positive cells in the middle layers of mucous epithelium. Material, taken from patient N°5, showed a moderate number of immunoreactive epithelial cells, but rare positive connective tissue cells. In three cases we observed few, but in two - rare positive structures. In these cases the NGF was expressed in epithelial cells, inflammatory cells – neutrophils, macrophages, mast cells and in one case in the nerve fibers, which were localized in subepithelium.

Expression of NGFR was observed in the tissue material of ten patients. In one case we saw few, in one – few to moderate number and in four cases a moderate number of positive structures. Yet, in four people we observed a different expression of the mentioned growth factor receptors in epithelial and nerve tissues. Besides, in all patients there was a characteristic NGFR expression in the basal epithelial cells. In eight of them we found immunoreactive nerve fibers, which were localized in the walls of blood vessels and in the bundles of nerve fibers.

The expression of TGFß was observed in the tissues of all control group patients. In eight samples we found many positive structures, which in five cases were bone cells, in two – epithelial cells and in one case – cells of epithelium and connective tissue. It is interesting that in the tissue of patient N°10 we saw many immunoreactive osteocytes, but we did not see any positive reaction in soft tissues. The relative number of immunohistochemically detected structures in the largest part of the patient epithelium, connective tissue and bone varied from few to many. In four cases we observed the TGFß - containing cells in the wall of blood vessels.

The expression of Barx1 gene was observed in the tissue material of nine patients. We found many positive structures in two cases. In one case these were bone cells, but in the second – epithelial cells, though we mainly observed rare and few positive structures in epithelium, mucous connective tissues and in bones. In the tissue of eight patients we saw comparatively indistinct and most frequently regional expression of said gene in epithelium. In four cases the Barx1 gene was seen in the inflammatory cells.

PGP 9.5 - containing nerve fibers were found in the tissue of eight patients. In one case we saw many, in one - few to moderate number, in four – few and in two – rare PGP 9.5 immunoreactive nerve fibers and bundles of nerve fibers. In three cases the mentioned structures localized in the walls of blood vessels and around them.

Apoptotic cells were found in the cleft disordered tissue material of ten patients. In five cases their relative number differed in the prepared various tissues of one patient. In one of them apoptosis was seen in numerous and yet in one in moderate to numerous connective tissue cells, but comparatively less or practically none in cells of the bone. In the tissue of patient N°1 and N°5 we saw few to moderate number of apoptotic regionally localized epithelial cells, as well as the connective tissue cells, but less pronounced number of apoptotic cells in the bone. Tissue of child N°2 showed more prominent apoptosis in the epithelial cells, but less - in the connective tissue cells, among them, in lymphocytes. In six cases we observed apoptosis in cells of epithelium and connective tissue. In general, we found averagely pronounced and most frequently regional apoptosis in epithelium of nine patients.

Findings in the group of children with unilateral cleft lip and palate in the age before and primary dentition

The youngest group of the patients with unilateral cleft lip and palate was formed from 15 children in age before and primary dentition. In seven patients the material was taken repeatedly.

We found **the basic FGF - containing** structures in the cleft disordered tissues of nine patients primary taken tissue (Table 4). In one case we observed rare, in one – rare to few, and in three cases – few positive structures, which, in general, were inflammatory and epithelial cells. In tissue of two patients a moderate number of immunoreactive structures were found. In tissues of these children we observed the bFGF already in the mentioned inflammatory cells, vascular smooth muscle cells and in perineurium around the bundles of nerve fibers. In two patients the fraction of the immunohistochemically determined structures in various tissues considerably varied (N°2, N°10), varying from the total average number of factors in other patients. In the first case we saw few positive in epithelium infiltrated lymphocytes, whereas in the connective tissues was saw many different immunoreactive inflammatory cells. Also

Table 4. Semiquantitative distribution of immunoreactive structures in the material of children with unilateral cleft lip and palate in age before and primary dentition.

N°	Code	bFGF	FGFR1	NGF	NGFR	TGFβ	Barx1	PGP 9,5	TUNEL
1	73	-	+++	+	0/+	+++	++	++/+++	+++/++++
2	38	+ - +++	+++	+++	+++	+/++	++/+++	+	+++/++++
	46	-	+++	-	0/+	+/++	-	++	+
3	149	-	+++	0/+	+	++	0/+	++	++/+++
	178	-	++ - +++	-	+ - ++	++ - +++	-	+	++/+++
4	52	+	+++	0/+	+	++	0/+	+++	+++
	80	-	+++	-	++	++	-	+	++
5	129	0/+ - +	+++	-	-	+++	-	-	+++
	144	+++	+++	-	+/++	-	-	+	+/++
6	166	++	++	-	++/+++	++/+++	-	+	+ - +++
	182	++	+++	+	++	++/+++	-	+	+/++
7	17	+	++	-	-	+/++ - +++	-	0/+	+++
8	37	++	+++	0/+	++	++	++	++	++/+++
	160	+	+++	0/+	++/+++	+++	-	+	+
9	59	-	++	-	+	+++	-	+	0/+
10	25	+ - ++	+++/++++	0/+	++	+++	-	++/+++	-
	104	0/+	++/+++	-	++	+++	-	+	++
11	143	-	++	-	+++	++/+++	+	+	++
12	119	-	+	-	++	+ - ++	+	+	+/++
13	151	-	+	-	-	+	0/+	+	0/+
14	153	0/+	++	-	+/++	+/++	0/+	+/++	++
15	170	+	+/++	-	+++	+++	+++	+/++	++

-- no positive structure seen in the visual field; 0/+ rare positive structures seen in the visual field; + few positive structures seen in the visual field; +/++ few to moderate number of positive structures seen in the visual field; ++ moderate number of positive structures seen in the visual field; ++/+++ moderate to numerous positive structures seen in the visual field; +++ numerous positive structures seen in the visual field; +++/++++ plenty of positive structures seen in the visual field

in the second case the bFGF was slightly expressed in epithelium, but a moderate number of positive structures we saw in sebaceous glands, in the bundles of nerve fibers and vascular smooth muscle cells. An average number of the bFGF - containing structures in the taken tissues was slightly larger than in the material of the control group patients, however, this difference was not statistically significant.

In the repeatedly taken material we observed the basic FGF expression in tissue of four patients. In one case the relative number of positive structures was considerably increased (N°5), and we could observe many immunoreactive inflammatory cells. In the tissues of other patients (N°2, N°4, N°8, N°10) we discovered an opposite trend. Comparing the number of the bFGF positive structures in the repeatedly taken tissues with the findings of the primary taken and control material, no statistically significant differences were found.

FGFR1 expression was found in the samples of primary taken tissues of all patients. We observed plenty FGFR1 positive structures in one, many – in six, moderate number – in the tissue material of five patients. In these cases the mentioned receptors were mainly expressed in epithelial, connective tissue and vascular smooth muscle cells, endotheliocytes, cells of sebaceous glands and hair follicles, as well as new and mature cartilage and bone cells. We saw few to moderate number of immunoreactive structures in one, but few – only in two patients' tissue samples. In these cases the FGFR1 was expressed in epithelium, endotheliocytes, connective tissue and vascular smooth muscle cells.

The results from the **repeatedly taken children's cleft disordered tissues** did not significantly differ from the primary taken tissues. Comparing an average number of FGFR1 positive structures in the primary and repeatedly taken tissues, no statistically plausible differences were observed. Still, in the repeatedly taken material the number was statistically significantly bigger than in the control tissues ($z=2.492$; $p=0.013$).

The NGF - containing structures we observed in the primary taken tissues of six patients. In four cases they were few, in one - rare and in one - many. Quite frequently we used to see the expression of growth factor in epithelium, as well as in the nerve fibers, vascular smooth muscle cells, cells of sebaceous glands and hair follicles. In one case (N°2) we found many positive inflammatory cells and nerve fibers localized in subepithelium. It is interesting that the NGF finding in this case coincided with the

pronounced bFGF finding. In our study we stated that an average number of NGF positive structures in the primary taken tissues was just a bit less than in the material of the control group patients, still the difference can be evaluated as statistically significant (z=2.314; p=0.021).

In the repeatedly taken material we saw the NGF immune reactive structures in two cases. In one case we observed rare, but in the second case – few positive epithelial cells. An average number of the NGF - containing structures also in the repeatedly taken tissues was statistically significantly less than in the patients of the control group (z=2.131; p=0.033).

The expression of NGFR was found in the primary taken tissues of 12 patients. In the tissue of three patients we saw many, in one – moderate to many and in three – a moderate number of positive structures. In all mentioned cases – tissue of seven children, we observed the immunoreactive basal epithelial cells. It is interesting that epithelium in these places was often jut into the connective tissues in a form of deep outgrowth. In certain cases the NGFR was expressed in the nerve fibers and cells of hair follicle and in one case – in macrophages and plasmocytes. In one case we observed few to moderate number, in three – few and only in one case – rare cells containing the mentioned factor receptor. Also in these cases we observed positive reaction mainly in the basal epithelial cells and regionally, as well as in the nerve fibers and in the bundles of nerve fibers

In the repeatedly taken tissues we observed the expression of NGFR in all cases and mainly in the basal cells of epithelium. In certain prepared samples we saw positive reaction in the nerve fibers around blood vessels and in the walls of blood vessels, as well as around the salivary glands. In tissue of one patient (N°8) the number of the immunoreactive structures was moderate to many, in three – moderate, in one – few to moderate, and yet in one - rare. In the repeatedly taken tissues of the patient N°3 we observed few immunoreactive epithelial cells, but a moderate number of immunoreactive cells in the wall of blood vessels and the bundles of nerve fibers. The average fraction of the immunohistochemically determined structures was few to moderate, and significant or statistically plausible differences between the primary taken and control group patients' tissues were not stated.

The expression of TGFß was observed in the primary taken tissues of all patients. We saw many positive structures in five, a moderate to many – in two and a moderate number - in three cases. In these cases the mentioned factor was expressed abundantly in epithelial cells, osteocytes, cells of connective tissues, vascular walls, sebaceous glands and hair follicles. In tissue of three children the number of the TGFß containing structures was comparatively small, and still in such tissues we could also see positive reaction in epithelium, subepithelium and vascular wall. In two cases (N°7, N°12) we observed a different number of immunoreactive structures in various tissues, but the most extensive expression was found exactly in the bone.

In the repeatedly taken tissue material we did not find the TGFß expression in one patient. It is interesting that in the primary taken material there were many positive structures. We observed the abundant expression of the factor in epithelium of two, but a moderate – of one patient, while in one patient we saw the moderate number to many immunoreactive epithelial cells, vascular endothelocytes and smooth muscle cells, as well as connective tissue cells. In the tissue of patient N°3 we observed many immunoreactive chondrocytes, but a moderate number of regionally positioned immunoreactive epithelial cells. In general, the relative number of the TGFß - containing structures was moderate and we did not find considerable differences in the primary and repeatedly taken tissues, as well as in tissues of the control group patients.

The expression of Barx1 we observed in the primary taken tissues of ten children. We saw many positive structures and moderate to many in every single case. We found a moderate number of structures containing the mentioned gene in two, few – in two, but rare in four cases. In total, in seven tissue samples we saw the Barx1 - containing epithelial cells in the comparatively different quantities. In two cases Barx1 was expressed in the cells of sebaceous and in sweat glands and in the inflammatory cells. In some children the positive reaction was found also in the cells of vascular wall, hair follicle and connective tissue. Similar to the tissue material of the control group also in the cleft-affected tissues the expression of the mentioned gene prevailed in the epithelium. Although an average number of positive structures in the cleft disordered tissues was a bit greater, the difference cannot be evaluated as statistically significant.

In the repeatedly taken tissue samples the Barx1 expression was not found in any case. Comparing an average number of positive structures in the repeatedly taken tissues with finding in control ($z=3.086$; $p=0.002$) and the primary taken tissues ($z=2.705$; $p=0.007$), we observed statistically significant differences.

PGP 9.5 stained the structures of diffuse neuroendocrine system (DNES) practically in tissues of all patients. We found moderate number, moderate to many and many positive structures on the whole in the primary taken material of five patients. PGP 9.5 in the mentioned tissues abundantly stained certain nerve fibers in the walls of blood vessels, around sebaceous glands and hair follicles, in epithelium, and in the bundles of nerve fibers as well. In certain cases it marked the neuroendocrine cell in the basal epithelial layer and in the hair follicles. In the material acquired from the rest of the patients we saw comparatively many positive and fine nerve fibers in subepithelium, in the walls of blood vessels and around them, as well as in the salivary glands.

In the repeatedly taken tissue samples of six patients we found few, but in one – a moderate number of neuropeptide - containing structures. Their localization does not differ from the above-described. In general, in the primary taken tissues we observed a slightly greater relative number of positive DNES structures if compared to the repeatedly taken and control group patients' tissues, however, this difference was not evaluated as statistically plausible.

The TUNEL method demonstrated apoptosis practically in the primary taken tissues of all patients. We observed plenty apoptotic cells in the tissue of two children, many – in three, moderate to many – in two, moderate - in three, few to moderate – in one and rare – in two children. In one case (N°6) the relative number of positive cells was dramatically different in epithelium and connective tissues. We saw the cell death most frequently in epithelium and in the connective tissues. In epithelium it often happened regionally. Apoptosis could be observed less often in the sebaceous glands, hair follicles and in the cells of vascular wall. In one tissue sample with a cartilage fragment we saw a total apoptosis through the cartilage, apart from the edge zone. In the tissue of other patient we found rare positive bone cells. During the study we stated that the average relative number of positive structures in the primary taken

tissues of patients with unilateral clefts was statistically significantly greater than in tissues of the control group patients (z=2.049; p=0.040).

In repeatedly taken tissue we observed apoptotic cells in material of all seven patients. In one case we saw moderate to many, in two – a moderate number, in two – few to moderate and yet in two – few TUNEL - positive cells in epithelium and connective tissue. An average number of positive structures was similar to the control group and a bit less than the quantity defined in the primary taken tissues.

Findings in the group of children with unilateral cleft lip and palate in mixed dentition age

The group of older patients with unilateral cleft lip and palate was formed from 22 children with mixed occlusion. In nine cases the material was taken repeatedly.

The bFGF positive structures were found in the primary taken tissues of 17 patients (Table 5). In eleven cases they were rare and few. Such expression was observed in the cells of epithelium and sebaceous glands, inflammatory and vascular smooth muscle cells. Yet in one patient (N°2) we observed a relatively different bFGF expression in epithelium and connective tissue and in the cells of sebaceous and salivary glands. In tissue of other patient we saw few to moderate number of immunoreactive vascular smooth muscle cells. We found the abundant bFGF expression in four cases and only in hard tissues – in cartilage and in bone.

In tissues repeatedly taken during surgical correction in two cases we observed rare and in two – few positive inflammatory cells. In tissue of one patient we saw few immunoreactive epithelial cells. It is interesting that we again observed the abundant bFGF expression in cartilage (N°9) and in bone (N°20). When compared the relative number of positive structures of said growth factor in the primary and repeatedly taken tissues and tissues of the control group patients, we did not observe considerable and statistically significant differences.

An abundant **FGFR1 expression** was observed in the primary taken tissues of 13 patients. Therein we saw many immunoreactive epithelial and connective tissue cells, cells in the walls of blood vessels, as well as bone and cartilage cells. It is interesting that exactly in hard tissues the expression of both basic FGF and their receptors was

Table 5. Semiquantitative distribution of immunoreactive structures in the material of children with unilateral cleft lip and palate in mixed dentition age.

N°	Code	bFGF	FGFR1	NGF	NGFR	TGFb	Barx1	PGP 9,5	TUNEL
1	26	0/+	++	-	0/+	+ - ++	-	+	+++
	95	0/+	+++	-	+	++	-	0/+	++
2	6	+ - ++	+++	++	++	+++	+++	++	+++
	92	+	+++	0/+ - +	0/+	+++	+	+	0/+
3	93	-	+++	-	0/+	+++	-	0/+	++/+++
	146	0/+	+++	-	-	+/++	-	+	0/+
4	127	+++	+	0/+	-	+	-	+	++ - +++
5	20	+	+++	++/+++ - +++	+/++	++/+++	-	++	+++
	84	+	+ + +	-	+/++	++	-	-	+++
6	176	+	+	-	+/++	++/+++	-	0/+	+++
7	49	0/+	++	0/+	-	++/+++	+	+	+
8	1A	0/+	++	-	+/++	++	-	+	+++
9	40	0/+ - +	+++	+ - ++	+	++	++	+/++	+++
	97	+++	+++	+	-	+++	+++	-	+
10	50	-	+++	-	-	+++	-	-	+/++
	123	-	+++	0/+	-	++	+	+	++ - +++
11	171	0/+	+++	0/+	+/++	+++	-	0/+	++
12	112	+	++	+	+	++	++	+	0/+
13	10	-	+++	-	+	+++	-	+	+
14	47	-	++ - ++/+++	-	-	+	-	-	+
15	21	+/++	+++	+/++	++	++ - +++	++	+++	+ - +++
16	183	0/+	+++	++	+	++/+++	0/+	+	+++
17	83	+++	+++	-	-	++ - +++	+	-	+
18	8	+	+++	-	-	++	+	+	++
	71	-	+	-	-	+ - +++	-	-	+

19	19	+	-	-	-	++	-	-	-
20	155	-	+	-	-	++	-	-	+++
	188	+++	+++	0/+	-	+++	-	+	++
21	74	+++	+++	0/+	-	++	0/+	+/++	++
22	64	+++	+++	-	0/+	+/++ - +++	++	+/++	+
	113	+	++	0/+	-	++	+	-	-

-- no positive structure seen in the visual field; 0/+ rare positive structures seen in the visual field; + few positive structures seen in the visual field; +/++ few to moderate number of positive structures seen in the visual field; ++ moderate number of positive structures seen in the visual field; ++/+++ moderate to numerous positive structures seen in the visual field; +++ numerous positive structures seen in the visual field; +++/++++ plenty of positive structures seen in the visual field

relatively very similar. FGFR1 was less often stated also in the epithelium of the ducts of salivary gland and in the connective tissue fibers. In tissue of one patient we observed a moderate number of immunoreactive chondrocytes and moderate to many - osteocytes. Moderate number of the said growth factor receptors was expressed in epithelium, connective tissue and cells of walls of blood vessels in tissues of four patients. Only in three cases we saw few immunoreactive structures. In two cases there were regionally localized connective tissue cells, but in one – bone cells. It is interesting that in the primary taken bone material of this child (N°20) the bFGF expression was not observed.

In the repeatedly taken tissues we observed the FGFR1 immunoreactive structures in tissue samples of all patients. In five cases we saw many positive structures in epithelium and connective tissue, where they were expressed by epithelial cells, fibroblasts, lymphocytes and cells in the vascular wall. In tissue of two patients we found an abundant expression of the said receptors in cartilage cells. In one case we saw a moderate number of immunoreactive epithelial and connective tissue cells, while in one – few positive connective tissue cells.

As regards an average relative number of positive structures, no statistically plausible differences appeared in the primary and repeatedly taken cleft-affected tissues. Although in general a relatively greater number of immunoreactive structures was observed in the cleft disordered tissues than in tissues of the control group, these differences were not statistically significant.

The expression of NGF was observed in the primary taken tissue samples of nine patients. Only in one case we saw many positive cells in the cartilage growth zone, as well as moderate to many immunoreactive cells of sebaceous gland, vascular smooth muscle cells and basal epithelial cells in hair follicle. In two cases we observed a moderate number, in one – few to moderate and in one – few positive structures. The mentioned growth factor was expressed by the inflammatory cells, epithelial cells, vascular smooth myocytes and epithelial cells in the ducts of salivary glands. In tissue material of one patient we saw a moderate number of positive basal cells of atrophic epithelium, but few immunoreactive epitheliocytes in the ducts of salivary gland. We observed few positive structures in tissues of four patients. In two cases they referred to chondrocytes, while in two – vascular smooth muscle cells.

In the repeatedly taken tissue material the expression of NGF we saw in tissue of five patients. Mainly we observed rare positive structures in epithelium, connective tissue and cartilage. In one case we found few immunoreactive chondrocytes, and yet in one – relatively different positive structures deposit in the connective tissue and in epithelium. An average number of immunohistochemically determined structures both in the primary and in repeatedly taken tissues was similar, but a bit different from the deposit in patients of the control group. Still, when compared the number of positive structures in the repeatedly taken and control patients' tissue, we stated a statistically significant difference ($z=2.107$; $p=0.035$).

NGFR containing structures we found in the primary taken tissue material of 13 children. In three cases we observed rare, in four – few, in four – few to moderate, but in two – moderate number of positive structures. The mentioned growth factor receptors were expressed mainly in basal epithelial cells and in the nerve fibers of blood vessel walls and around them. In the tissue of one patient we saw positive reaction in epithelial cells of the ducts of salivary gland.

In the repeatedly taken tissue material we observed the expression of NGFR in three cases. In one case we saw rare, in one – few and in one – few to moderate number of positive structures, which in all mentioned cases were regionally localized basal epithelial cells. An average number of immunohistochemically determined structures both in the primary and in the repeatedly taken tissues was different, as well as comparatively less than in the patients of the control group. In our study we stated statistically significantly less NGFR positive structures ($z=3.241$; $p=0.001$), also in the repeatedly taken tissues ($z=3.297$; $p=0.001$), if compared to the control group's material.

The expression of TGFß was observed in the primary taken tissue of all patients. We saw many positive structures in five, moderate to many – in four, and a moderate number – in material of seven children. TGFß in these cases was expressed in new and mature cartilage cells, osteocytes, epithelial and connective tissue cells, as well as in vascular endotheliocytes and smooth myocytes. In tissue of three patients we found a relatively different number of immunoreactive structures in various tissues. In the said cases we observed an abundant expression of the growth factor in bone cells, but relatively less – in cartilage and connective tissue cells. We found few positive

structures in tissues of two patients, and we observed immunoreactivity in chondrogenic cells and in osteocytes. In one case we saw a moderate number of positive bone cells, but few positive vascular endotheliocytes and smooth muscle cells.

In the repeatedly taken tissue samples we observed the TGFß expression in all cases. In tissue of three patients we saw many, in four – a moderate number, in one – few to moderate number of positive structures. We found an abundant expression of the said growth factor in epithelial, connective tissue and cartilage cells, but averagely pronounced – in epitheliocytes, fibroblasts, macrophages, plasmocytes and endotheliocytes. In tissue of one patient (N°18) we saw many immunoreactive bone cells, but few positive connective tissue cells. In general, an average number of the TGFß – containing structures in the primary and repeatedly taken tissues was moderate to many. Expression of the said growth factor in unilateral cleft disordered tissues of children in mixed dentition age was relatively more pronounced than in tissues of the control group patients, still this difference was not evaluated as statistically plausible.

The expression of Barx1 we observed in the primary taken prepared samples of tissue of nine patients. We saw many positive structures in one, a moderate number – in four and few – in two cases. We observed an abundant expression of the said gene in epithelium, we found a moderate number of immunoreactive cells in cartilage, epithelium and connective tissue, as well as in vascular walls and in the salivary gland. Few positive cells were seen in epithelial tissue and connective tissue of two patients.

In the repeatedly taken tissues we observed expression of the **Barx1** gene in material of four patients. In the material of one child we saw many positive cartilage cells, but in three cases – few positive and oftener regionally localized epithelial cells. In general, the relative number of the Barx1 immuoreactive structures in the primary and repeatedly taken, as well as in tissues of the control group patients was similar.

PGP 9.5 stained the structures of DNES in the tissue material of 16 patients. In one case we observed many neuropeptide-containing nerve fibers in the vascular walls and in the bundles of nerve fibers. In tissue of two patients we saw a moderate number and in three – few to moderate number of positive nerve fibers in the walls of blood

vessels and around them, and in one of the mentioned cases (N°2) also in epithelium, hair follicles and in the bundles of nerve fibers. In the material of the rest of patients we observed mainly few, as well as in three rare neuropeptide-containing structures. In these cases the PGP 9.5 stained rare nerve fibers in epithelium, connective tissue below epithelium and in the vascular walls, as well as in fine bundles of nerve fibers. **In the repeatedly taken tissue samples** we found the structures of DNES in five cases. Their fraction was mainly small. In the mentioned tissues of all children we observed rare nerve fibers in the walls of blood vessels and around them. In general, the relative number of the PGP 9.5-containing structures in the repeatedly taken tissues was smaller than in the primary taken and control patients' tissue, but we did not find any significant differences in their localization.

The TUNEL discovered apoptosis in the primary taken tissue samples of 21 patients. The relative number of positive structures was very variable. We observed many positive cells in tissue of eight patients – in epithelium, connective tissue, bone and cartilage. In material of one patient (N°4) we found a pronounced apoptosis in the zone of cartilage mature cells, but we observed a moderate number of apoptotic cells in the proliferation zone. In tissue of other patient (N°15) we saw many positive cells in cartilage, but few – in connective tissues. We saw moderate to many apoptotic cells in cartilage of one patient. In samples of three patients we stated a moderate number, in one – few to moderate, an in four – few positive cells. Also in these cases we found apoptotic cells in epithelium, connective tissue and in the walls of blood vessels, in bone and cartilage, and often their localization was regional. Only in one case (N°12) we observed rare apoptotic osteocytes.

In the repeatedly taken tissues TUNEL demonstrated apoptosis in eight cases. Also in these tissues the relative number of apoptotic cells varied from rare to many positive cells in the visual field. We observed a pronounced apoptosis in the connective tissue of one patient. In material of child N°10 we found many TUNEL-positive cells in the connective tissue and in the vascular walls, but a moderate number – in epithelium. We saw a moderate number of apoptotic osteocytes in two cases, but few apoptotic cartilage and connective tissue cells in every single case. In the tissue of patient N°2 we saw apoptosis in rare osteocytes, but in material of child N°3 – in rare connective tissue cells. The relative number of positive structures in the

repeatedly taken tissue material was few to moderate. In our study we encountered the apoptotic cells relatively more often in the primary taken cleft disordered tissues than in the repeatedly taken or control samples, still these differences were not evaluated as statistically significant.

Findings in the group of bilateral cleft lip and palate patients –children in age before and primary dentition

The younger group of patients with bilateral cleft lip and palate was formed from seven babies and children with milk occlusion. In two patients the tissues were taken repeatedly during surgical correction.

Expression of basic FGF was observed in the primary taken prepared tissue samples of six children (Table 6). Said growth factor was abundantly expressed in epithelial (N°6) and inflammatory cells (N°5). In material of two patients we saw a moderate number of positive structures – cells of epithelium and sebaceous gland. Yet in tissue of two patients the expression of bFGF varied in various tissues from few to moderate number of positive structures in visual field. In tissue of the patient N°2 we saw a very light expression of the said factor in the cells of sebaceous gland, but it was relatively distinct in vacuolisated epithelial cells, whereas in child N°7 we found few immunoreactive structures in the vascular walls, but a moderate number – in connective tissue. On the average the relative number of immunohistochemically determined structures was moderate (++), and it was considerably higher than in the patients of the control group. This difference can be evaluated as statistically significant ($z=2.640$; $p=0.008$).

In the repeatedly taken tissues of one patient we observed an abundant bFGF expression in vascular smooth muscle cells, but in the tissue of the second patient we found only rare positive connective tissue cells.

The expression of FGFR1 was observed in the primary taken tissue samples of all children. In two cases the mentioned receptor was abundantly expressed in the basal epithelial cells and in connective tissue cells. In tissue of five patients we found relatively different number of immunoreactive structures in various tissues. It is interesting that we again observed manu positive structures in epithelium.

Table 6. Semiquantitative distribution of immunoreactive structures in the material of children with bilateral cleft lip and palate in age before and primary dentition.

N°	Code	bFGF	FGFR1	NGF	NGFR	TGFβ	Barx1	PGP 9,5	TUNEL
1.	177	++	++/+++ - +++	++	+++	-	+/++	++	++/+++
2.	168	+/++	++ - +++	++	++/+++	-	+/++	++	++
	187	0/+	+++	0/+	++	-	++	0/+	++ - +++
3.	24	++	+++	-	++	-	+/++ - ++	++	++/+++ - +++
	180	+++	++	0/+	++	-	-	+	++ - +++
4.	150	-	+/++ - +++	-	++	-	-	+	++/+++ - +++
5.	134	+++	++ - +++	-	++/+++	-	+	+	+++
6.	81	+++	++ - +++	-	+++	-	++	+	+++
7.	79	+ - ++	+++	+	++	-	++	+/++	++ - +++

- no positive structure seen in the visual field; 0/+ rare positive structures seen in the visual field; + few positive structures seen in the visual field; +/++ few to moderate number of positive structures seen in the visual field; ++ moderate number of positive structures seen in the visual field; ++/+++ moderate to numerous positive structures seen in the visual field; +++ numerous positive structures seen in the visual field; +++/++++ plenty of positive structures seen in the visual field

The expression of FGFR1 mainly was found in all epithelial layers, however in the tissue of patient N°2 it was more prominent in basal epithelial layer, but in tissue of patient N°3 – in upper layers. Moderate to many or mostly moderate number of immunoreactive structures were observed in the connective tissue and in the walls of blood vessels, and FGFR1 were expressed in the cells of connective tissue and smooth muscle cells, as well as in one case in the cells of hair follicle and sebaceous gland. An average number of immunohistochemically determined structures was large (+++), still, if compared to the control group's tissue, the differences were not statistically significant ($z=1.936$; $p=0.053$).

In the tissues repeatedly taken during surgical correction FGFR1 immunoreactive cells were observed in the epithelium and connective tissue of both patients. In one case we found moderate, but in other – many positive structures.

Expression of NGF was observed in the primary taken tissue samples of three patients. In two cases we found a moderate number of mentioned growth factor-containing regional epithelial cells. In tissue of one patient NGF was expressed in few cells of connective tissue and salivary gland.

We saw **in the repeatedly taken tissue** rare immunoreactive cells in connective tissue and in the walls of blood vessels. On the average the relative number of immunohistochemically determined structures was 0/+ (rare positive structures in the visual field).

Expression of NGFR was observed in the primary taken tissue samples of all children. In two cases it was abundantly expressed in the basal epithelial cells and in the nerve fibers in the walls of blood vessels and around them, as well as in the cells of sebaceous glands. In tissue of two patients we observed moderate to many and in three – a moderate number of positive structures. Besides, in these cases the said growth factor receptors were expressed in the basal epithelial cells, the nerve fibers in the walls of blood vessels and around them, as well as in one case in basal epithelial cells of hair follicle. It is interesting that the NGFR expression was pronounced exactly in the epithelial overgrowth. On the average the relative number of immunohistochemically determined structures was moderate to many, and it was statistically significant different from the findings in tissues of the control patients ($z=2.787$; $p=0.005$).

We observed **in the repeatedly taken tissues** a moderate number of the NGFR – containing basal epithelial cells and nerve fibers in the walls of blood vessels and around them. It is interesting that we saw the expression of the said receptors in the patient N°2 exactly at the place of the inflammation.

We did not find **TGFβ** tissue of in any patients' primary and repeatedly taken tissue. If compared to the control group's tissue, the differences were statistically significant (z=3.612; p=0.001; z=2.205; p=0.027).

Expression of the Barx1 gene was observed in the primary taken tissue of six patients. In two cases we found a moderate number of positive basal epithelial cells, and in one of the mentioned cases Barx1 was expressed also in the inflammatory cells. In the tissue of patient N°3 we saw a moderate number of the Barx1-containing inflammatory cells and few to moderate number epithelial cells. We found few to moderate number of immunoreactive cells in tissue of two children, but few – in one child's tissues. Also in these cases the Barx1 was expressed in epitheliocytes, as well as in the cells of sebaceous gland. The relative number of the Barx1-containing structures was few to moderate. Although it was larger than in tissues of the control group patients, we did not observe the statistically significant difference (z=1.479; p=0.139).
In the repeatedly taken tissues the said gene was expressed by a moderate number of epithelial cells of one patient.

The PGP 9.5-containing structures were found in the primary taken tissue of all patient. We saw a moderate number of the immunoreactive nerve fibers in three cases. They were localized mainly in the walls of blood vessels and around them, in hair follicles, as well as in epithelium, beneath them or in the bundles of nerve fibers. We observed few to moderate number of positive structures in tissues of one child, which in this case were not only the nerve fibers, but also the cells localized in the basal epithelial layer. We found few fine PGP 9.5-containing nerve fibers in three cases. An average relative number of DNES-containing structures in the primary taken tissues was statistically significantly greater than in the control group (z=2.064; p=0.039).

We saw **in the repeatedly taken tissue** in one case few fine PGP 9.5-containing nerve fibers in the walls of blood vessels and around of the salivary glands, but in the second case – rare structures in epithelium.

We observed **apoptotic cells** in the primary taken tissues of all patients. The TUNEL method demonstrated many apoptotic epithelial and connective tissue cells in the tissue of two children. Also in other cases the relative number of apoptotic cells was big, and in epithelial and connective tissue of one patient varied from moderate to many. Often apoptosis was regional and it could be observed also in the vascular walls and in the glandular cells.

We obtained similar results when analyzed **the repeatedly taken tissue samples**. In both cases we saw a moderate number of apoptotic cells in epithelium and many - in the connective tissues, but in tissue of one patient also in the minor salivary gland. We found a relatively moderate number up to a great number of apoptotic cells both in the primary and repeatedly taken tissue cells. In our study we stated that the number of apoptotic cells was larger both in the primary ($z=3.251$; $p=0.001$) and repeatedly taken tissues ($z=2.019$; $p=0.044$) than in the material of the control group, and these differences can be evaluated as statistically significant.

Findings in the group of bilateral cleft lip and palate patients –children in mixed dentition age

The group of patients with bilateral cleft lip and cleft palate comprised eight children with mixed occlusion. Tissues of two patients (N°1, N°2) were taken repeatedly during surgical correction, but in the patient N°4 – from the right and left side of cleft-affected area.

The basic FGF expression was found in the primary taken tissue samples of all patients (Table 7). The number of the said growth factor - containing structures distinctly varied in children's various tissue groups. We saw an abundant bFGF expression in one patients' (N°2) vascular smooth muscle cells, but in one patient (N°5) - in sebaceous gland cells. In tissue of child N°2 we observed a moderate number (++) positive inflammatory cells – lymphocytes and segmentated neutrophils,

Table 7. Semiquantitative distribution of immunoreactive structures in the material of children with bilateral cleft lip and palate in mixed dentition age.

N°	Šifrs	bFGF	FGFR1	NGF	NGFR	TGFβ	Barx1	PGP 9,5	TUNEL
1.	145	++	+++	0/+	+++	-	-	0/+	+++
	172	-	+++	-	++	-	-	-	-
2.	3	++ - +++	+++	+	+++	-	-	++/+++	++++
	141	++	+++	-	0/+	-	-	-	+++
	186	++	+++	+++	++	+	0/+ - +	+++	++/+++
3.	132	+ - ++	+++	-	+++/++++	-	0/+	+	++ - ++++
4.	55L	+	+++/++++	-	++	0/+	0/+	++/+++	+++
	55R	0/+ - ++	+++/++++	-	+++	0/+	0/+	++/+++	+++
5.	185	+ - +++	++	+	-	-	-	-	-
6.	100	+ - ++/+++	++/+++	-	++	-	0/+	++	+++
7.	35	++	+++	-	-	-	-	-	+++
8.	125	++/+++	++ - +++/++++	++	+++/++++	+	++	+/++	+++

- no positive structure seen in the visual field; 0/+ rare positive structures seen in the visual field; + few positive structures seen in the visual field; +/++ few to moderate number of positive structures seen in the visual field; ++ moderate number of positive structures seen in the visual field; ++/+++ moderate to numerous positive structures seen in the visual field; +++ numerous positive structures seen in the visual field; +++/++++ plenty of positive structures seen in the visual field

as well as other connective tissue cells, but in tissue of the patient N°5 the relative number of immunoreactive cells was, however, smaller (+). We observed a moderate number to much referred growth factor – containing cells in two patients' (N°6, N°8) cartilage, and in tissue of one of them – the patient N°6 we found few positive smooth muscle cells in the vascular wall. We observed a moderate number of immunoreactive cells in epithelium of two patients, and in one of them (N°4) we saw rare and few positive inflammatory cells and cells of sebaceous gland. In one case there was found a moderate number of positive salivary gland cells and few immunoreactive inflammatory cells and yet in one – a moderate number of positive osteocytes. We saw a moderately great number of positive structures in the visual field in the primary taken tissues in this patients' group. In our study we stated that the relative number of bFGF immunoreactive structures was considerably bigger in the primary taken bilateral cleft disordered tissues than in the material of the patients of the control group, and this difference is statistically significant ($z=3.750$; $p=0.001$).

When analyzing the tissue samples taken repeatedly, we observed the bFGF expression in tissue of one patient, where we saw a moderate number of positive regionally localized epithelial cells. If compared to the control tissues, we did not find statistically significant differences.

The expression of FGFR1 was observed in the primary taken tissue material of all patients. In one case we saw plenty of immunoreactive epithelial cells, cells of sebaceous glands, smooth muscle cells in the walls of blood vessels, as well as muscle fibers, cells of endoneurium in the bundles of nerve fibers and inflammatory cells. In the tissue of patient N°8 we observed plenty of FGFR1-containing cells in the zone of mature cartilage, but a moderate number – in the zone of proliferation. In the tissue samples of three children we found many FGFR1 – containing structures – epitheliocytes, connective tissue cells and fibers, vascular smooth muscle cells. We saw a different relative number of osteocytes expressing the said growth factor receptors in three cases (N°5, N°6, N°7). In the study we stated that the number of the FGFR1 positive structures in the primary taken bilateral cleft disordered tissues was statistically significantly larger than in the material of the patients of the control group ($z=2.602$; $p=0.009$).

In the tissues repeatedly taken during surgical correction many immunoreactive cells were observed in epithelium and connective tissue, as well as in these cases we

observed the positive structures in the cleft disordered tissue in a statistically plausible greater number than in the control tissues (z=2.175; p=0.030).

We observed **the expression of NGF** in the primary taken tissue of four patients. Localization of the positive structures was mainly regional. A moderate number of the said growth factor was expressed in chondrocytes of one patient, few – in basal epithelial and connective tissue cells of two patients, but in tissue of one patient we stated rare positive cells in the basal epithelial layer.

In the repeatedly taken tissue samples we saw an abundant expression of NGF in one patient's epithelium, but few positive cells in the connective tissue. Both in the primary and repeatedly taken tissue material the relative number of the NGF positive structures was a bit different than in the material of the control group patients, still this difference cannot be evaluated as statistically significant.

Expression of NGFR we found in the tissue material of six patients. In two cases we saw plenty and yet in two cases – many positive structures. In these tissues the said growth factor receptors were expressed by basal epithelial cells and the nerve fibers, which in one case were localized in the walls of blood vessels or around them. In the tissue samples of patient N°4 the relative number of the immunoreactive structures was a bit different and the NGFR expression was observed in the basal cells of epithelium and hair follicles, in epithelium of the ducts of salivary glands, in the nerve fibers in the walls of blood vessels and around them, as well as in the bundles of nerve fibers. We stated a moderate number of immunoreactive nerve fibers in one case.

In the repeatedly taken tissue material the relative number of the NGFR – containing structures varied from rare to moderate number. In these cases we saw positive and mainly regionally localized epithelial cells, as well as nerve fibers in the walls of blood vessels and around them and bundles of nerve fibers. Although we found more positive structures in the primary and repeatedly taken cleft disordered tissues than in the control material, these differences could not be evaluated as statistically significant.

We observed **TGFβ** in the primary taken tissues of two patients. In one case rare positive cells of connective tissue and hair follicle were found, but in the second – few positive chondrocytes.

In the tissues repeatedly taken during surgical correction we observed the said factor in one case, which were regionally located epithelial cells. In our study we stated that the TGF-β positive structures both in the primary (z=3.684; p=0.001) and repeatedly taken cleft disordered tissues (z=2.601; p=0.009) were statistically significantly less than in the control patients' material.

The expression of Barx1 gene was observed in tissue of four patients. We mostly saw rare positive epithelial, connective tissue and sebaceous gland cells. In one case there was a moderate number of the Barx1 – containing cartilage cells.
In the repeatedly taken tissues we observed rare positive epithelial cells and few positive connective tissue cells in one case. In our study we did not state any considerable differences between the average number of the Barx1 positive structures in the primary and repeatedly taken cleft disordered tissues, as well as in the tissues of the control group patients.

The PGP 9.5 – containing DNES structures were found in the primary taken tissues of six patients. In two cases we saw moderate to many, in one – moderate, in one – few to moderate, in one - few and in one – rare positive structures in the visual field. PGP 9.5 stained the nerve fibers, which were mainly localized in the walls of blood vessels and around them, in subepithelium or in the bundles of nerve fibers. Less often we saw the neuropeptide – containing nerve fibers in epithelium or in the connective tissues around the salivary glands.
In the repeatedly taken tissues of one patient we saw many positive fine nerve fibers. In our study we did not observe statistically significant differences comparing the average number of DNES structures and the primary and repeatedly taken and the control group patients' tissues.

We observed **the cell apoptosis** in the primary taken tissue of seven patients. The TUNEL method showed plenty of the apoptotic connective tissue cells and smooth muscle cells in the vascular walls in the tissue samples of the patient N°2. In material of child N°3 the number of apoptotic cells greatly varied: plenty of them were in the connective tissues, in epithelium – a moderate number, in the salivary gland – few positive cells. In other five cases we saw many apoptotic cells in epithelium, connective tissues, cartilage and bone, as well as in the walls of blood vessels, in

salivary and sebaceous glands. In our study we stated that the relative number of apoptotic cells was statistically significantly greater in the primary taken tissues than in the material of the control group patients (z=3.018; p=0.003).

In the repeatedly taken tissues we observed cell apoptosis in one patients' epithelium, connective tissue and in the vascular tunica media.

Cross – correlation of growth factors, Barx1 gene and apoptosis

The expression of basic FGF was observed in all groups of cleft patients (Table 8). In general, an average relative number of the bFGF – containing structures in children with unilateral cleft lip and palate in age before and primary dentition was not big (rare to few positive structures). In the group of older patients with unilateral cleft lip and palate we observed on the average relatively few positive structures, besides, abundant expression of the said growth factor was characteristic exactly for this patients' group only in hard tissues – in bone and cartilage. As regards to the children with bilateral cleft lip and palate in both age groups the expression of bFGF was more pronounced, besides, an average relative number of the bFGF – containing structures in the primary taken tissues was statistically significantly larger than in the control material. During data processing using the Kruskal Wallis test, we stated that the variation of bFGF in the patients' groups was statistically significant (χ^2=15.907; df=4; p=0.003).

We stated the FGFR1 expression in all cleft patients' groups. As regards to the children with unilateral clefts in both age groups, the relative number of the FGFR1 – containing structures was similar. It was a bit larger in the repeatedly taken tissues of the said patients. It is interesting that exactly in hard tissues of children in mixed dentition age the expression of both basic FGF and receptors thereof was comparatively abundant and very similar. As regards the bilateral cleft lip and palate patients in both age groups, the FGFR1 expression was even more pronounced, and the relative number of FGFR1 immunoreactive structures was mainly large (+++). Although the statistically significantly larger expression in comparison to the control was observed only in the bilateral cleft older patients' primary and repeatedly taken

Table 8. Semiquantitative distribution of immunoreactive structures in the patients with unilateral and bilateral cleft lip and palate and in control group.

Patient group /factor		bFGF	FGFR1	NGF	NGFR	TGFb	Barx1	PGP 9.5	TUNEL
children with unilateral cleft lip and palate in age before and primary dentition	primary	**0**/+ - +	++/+++	**0**/+	+/++	++	+	+/++	++
	repeatedly	+	+++	- - **0**/+	+/++	++	-	+	+/++
children with unilateral cleft lip and palate in mixed dentition age	primary	+	++/+++	**0**/+	**0**/+ - +	++/+++	**0**/+	+	++
	repeatedly	+	+++	**0**/+	- - **0**/+	++/+++	**0**/+	**0**/+	+/++
children with bilateral cleft lip and palate in age before and primary dentition	primary	++	+++	**0**/+	++/+++	-	+/++	+/++	++/+++
	repeatedly	+/++	++/+++	**0**/+	++	-	+	**0**/+	++/+++
children with bilateral cleft lip and palate in mixed dentition age	primary	++	+++	**0**/+	++	**0**/+	**0**/+	+/++	+++
	repeatedly	+	+++	**0**/+	++	**0**/+	**0**/+	**0**/+	++
control		**0**/+	++	+	+/++	++	**0**/+	+	+/++

□ factor, who variations in the patients' groups was statistically plausible, and its relative number

-- no positive structure seen in the visual field; 0/+ rare positive structures seen in the visual field; + few positive structures seen in the visual field; +/++ few to moderate number of positive structures seen in the visual field; ++ moderate number of positive structures seen in the visual field; ++/+++ moderate to numerous positive structures seen in the visual field; +++ numerous positive structures seen in the visual field; +++/++++ plenty of positive structures seen in the visual field

tissues, still the Kruskal Wallis test evidenced that the variation of FGFR1 in the patients' groups is statistically significant (χ^2=9.873; df=4; p=0.043). In the patients' material we observed also plausibly positive weak correlation between the bFGF and FGFR1 relative number (p=0.032; r=0.233).

An average relative number of the NGF – containing structures in all cleft patients' groups was not large (rare positive structures), and it was relatively smaller than in the control material. We found positive plausibly weak correlation between the average relative number of NGF and bFGF in the patients' cleft disordered tissues (p=0.028; r=0.242).

We observed the expression of NGFR in all cleft patients' groups. An average relative number of the NGFR – containing structures varied, and in the younger patients with unilateral cleft lip and palate it was, similar to the control, few to moderate. As regards the older unilateral cleft patients, an average relative number of the NGFR – containing structures was statistically significantly smaller than in the control patients. But in the bilateral cleft patients' groups the expression of NGFR, if compared to the control, was larger, besides, the relative number of the NGFR – containing structures in exactly primary taken tissues of children in age before and primary dentition was statistically significantly larger. The Kruskal Wallis test demonstrated that the NGFR in the patients' groups varied statistically significant (χ^2=33.324; df=4; p=0.001).

The expression of TGFβ in the cleft disordered tissues was variable. An average relative number of positive structures in the group of younger patients with unilateral cleft lip and palate, similar to the control tissues, was moderate (++), but in the group of children in mixed dentition age - a bit larger. In tissues of younger patients with the bilateral clefts the expression of the said growth factor was not observed, but in children in mixed dentition age an average relative number of the TGFβ – containing structures was negligible (0/+). Both age groups of children with bilateral cleft lip and palate in comparison to the control the differences can be evaluated as statistically significant. Processing the data, using the Kruskal Wallis test, we stated that the variation of TGFβ in the patients' groups was statistically significant (χ^2=44.791; df=4; p=0.001).

The relative number of Barx1 gene containing structures lightly varied in the groups of patients. An average relative number of Barx1 immunoreactive structures in the groups of children in age before and primary dentition was a bit larger than in control group, however in the repeatedly taken tissue of children with unilateral cleft lip and

palate expression of mentioned gene was not observed. Exactly that difference in comparison to the control can be evaluated as statistically significant. Kruskal Wallis test showed, that the variation of Barx1 in patients' groups is not statistically significant.

An average relative number of PGP 9.5 containing structures in the patients' groups was variable, and did not significantly differ from the result of control group. The variation of mentioned factor cann't be evaluated as statistically significant.

We observed the cell apoptosis in the largest part of the tissue material. An average relative number of the positive structures in the primary taken tissues of children with unilateral cleft lip and palate in both age groups was similar, slightly larger if compared to the control. We did not find any considerable differences in the repeatedly taken cleft disordered tissue samples, if compared to the control. In the bilateral cleft lip and palate patients' material we observed the increase of average relative number of apoptotic cells. Besides, in the patients' groups it statistically plausibly weak correlates with NGFR ($p=0.007$; $r=0.291$). The Kruskal Wallis test evidenced that the variation of the average relative number of the apoptotic cells in the patients' groups is statistically significant ($\chi^2=13.779$; $df=4$; $p=0.000$).

Conclusions

1. The main morphological changes in unilateral and bilateral cleft disordered tissues are very thin atrophic or very thick epithelium, sclerotic blood vessels between the bundles of collagen fibers in the connective tissue, infiltration of inflammation cells, wide zone of hypertrophic chondrocytes in cartilage and irregular localization of osteons in the bone.
2. The affected tissue of unilateral cleft lip and palate show increased bFGF and FGFR1 local expression with a tendency to increase in mixed dentition age and in repeatedly taked tissues.

   The difference in relative quantity of FGFR1 positive structures in repeatedly taked tissues of children in age before and primary dentition and in controls was statistically significant.

   Results demonstrate a stimulating effect of the mentioned growth factor and growth factor receptors on cells proliferation in cleft disordered tissue and successful remodelation.

3. Bilateral cleft lip and palate affected tissue show increased bFGF and FGFR1 local expression in primary taken tissue with indistinct trend to decrease in repeatedly taken material.

   Relative quantity of bFGF positive structures was significantly higher in primary taken tissue of both dentition ages than in controls.

   Relative quantity of FGFR1 positive structures was significantly higher in primary and repeatedly taken tissue of children in mixed dentition age than in controls.

   These results probably indicate greater tissue compensatory facility in bilateral facial clefts.

4. Unilateral cleft lip and palate affected tissue show indistinct NGF and variable NGFR expression.

   Significantly lower amount of NGF immunoreactive structures than in control were in the primary taken tissue of children before and at primary dentition age and in repeatedly taken tissue in mixed dentition period.

   Significantly lower amount NGFR immunoreactive structures was characteristic for primary and repeatedly taken tissue in mixed dentition age.

   Our findings probably show weak potential of autocrine survival system of epithelium, as a result**,** there is a rather slow reepitelization and delayed wound closing.

5. The bilateral cleft lip and palate tissues are characterized by still weak NGF expression, but stabilization of the NGFR expression.

   Statistically significant were mostly NGFR positive structures, which were mainly the basal epithelial cells, and were stated in the primary taken tissues of children in age before and primary dentition.

   This is indicative for stabilization of the autocrine regulation system of epithelial cells rather than of qualitative regeneration of epithelial tissues.

6. The unilateral cleft lip and palate affected tissue, similar to the control, are characterized by a moderately pronounced TGFβ expression, which is slightly increasing in mixed dentition age and it is relatively more prominent in hard tissues.

   Our results indicate insignificant role of TGF β in the regulation of unilateral cleft tissue homeostasis.

7. Bilateral cleft lip and palate affected tissue show the absence of local TGFβ expression in tissue of children in age before and primary dentition and weak expression in mixed dentition period, giving evidence to the involvement of this growth factor in the cleft severity morpfopathogenesis.

   Relative quantity of TGFβ positive structures was significantly lower in primary and repeatedly taken tissue of children in mixed dentition age than in controls.
8. Unilateral and bilateral cleft lip and palate affected tissue show variable and similar to control quantity of DNES structures and a tendency to decrease in tissues, obtained during repeated surgery.

   Our findings suggest the decrease of neuropeptide-containing innervation in several cleft tissues, affected by surgical correction.
9. Unilateral and bilateral cleft affected tissue shows more prominent Barx1 expression in the primary taken material of younger patients of both cleft types and stabilization of Barx1 expression in mixed dentition age.

   The expression of Barx1 in tissue of youngest patients probably is delayed, but is still cell proliferation and differentiation contributing signal.
10. Unilateral and bilateral cleft lip and palate affected tissue show more prominent apoptosis, which tends to increase in the case of bilateral cleft, and thereby conversely to proliferation stimulating factors provides homeostasis of cell population.
11. In general, cleft tissue show increased bFGF and FGFR1, but decreased NGF - local expression, prominent apoptosis, variable NGFR, TGFβ and Barx1 expression and various neuropeptide-containing innervation.

Approbation of the study

1. RSU Scientific medical conference, Riga (Latvia), March 5, 2004.
2. RSU Scientific medical conference, Riga (Latvia), March 3, 2006.
3. The 8th Joint Symposium Riga – Rostock, Jaunmokas (Latvia), May 11 – 14, 2006.
4. The 1st Baltic Scientific Conference in Dentistry, Parnu (Estonia), October 19 – 21, 2006.
5. COST B23 Symposium, Paris (France), December 7 – 8, 2006.

6. RSU Scientific medical conference, Riga (Latvia), March 29 - 30, 2007.
7. The 2nd Baltic Scientific Conference of Dentistry, Riga (Latvia), November 8 – 10, 2007.
8. Baltic Morpfology 4th Scientific Conference, Riga (Latvia), November 19 – 20, 2007.
9. RSU Scientific medical conference, Riga (Latvia), March 13 - 14, 2008.
10. The 5th Tissue Engineering Symposium, Tampere (Finland), April 22 – 25, 2008.
11. The 6th Congress of Latvian Physicians, Riga (Latvia), June 19 – 21, 2009.
12. Baltic Morpfology 5th Scientific Conference, Kaunas (Lithuania), August 27 -28, 2009.
13. RSU Scientific medical conference, Riga (Latvia), March 18 - 19, 2010.
14. Meeting of Association of Clinically Integrated Morphology (KIMA), Riga, (Latvia), April 21, 2010.

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Summary

Cleft lip and palate is among most common birth defects worldwide. In Latvia the cleft lip and palate occur on the average in one child in 700 - 800 newborn infants.

The development of the face and the oral cavity includes cell growth, differentiation, cell-to-cell and cell-extracellular matrix interactions, and the programmed cell death. These processes are caused and regulated by various growth factors and genes.

Nonetheless, irrespective of many studies, there is no research regarding the distribution and appearance of various growth factors and genes in cleft disordered tissue or in its role in the healing of wounds.

Aim of the research was to make an immunohistochemical study of the growth factors, the growth factor receptors and the gene relative abundance and location, determination of cell death and data correlation in the cleft-affected tissues in children with unilateral and bilateral cleft lip and cleft palate in the ontogenetic aspect with the development of a diagnostic /prognostic algorithm.

Applying the immunohistochemical and TUNEL methods, there were studied the tissues from the cleft correction area of 52 patients with unilateral and bilateral cleft lip and palate. There has been proved and documented in pictures the bFGF, FGFR1, NGF, NGFR, TGFβ, PGP 9.5 and Barx1 gene expression and the relative abundance of immunoreactive cells in the tissue material taken not only during the first, but also during the repeated surgical operation.

Unilateral cleft lip and palate disordered tisue show increased bFGF and FGFR1 local expression with a tendency to increase in mixed dentition age and in repeatedly taked tissues. Bilateral cleft lip and palate affected tissue show increased bFGF and FGFR1 local expression in primary taken tissue with indistinct trend to decrease in repeatedly taken material.

Unilateral cleft lip and palate affected tissue show indistinct NGF and variable NGFR expression. The bilateral cleft lip and palate tissues are characterized by still weak NGF expression, but stabilization of the NGFR expression.

The unilateral cleft lip and palate affected tissue are characterized by a moderately pronounced TGFβ expression, which is slightly increasing in mixed dentition age and it is relatively more prominent in hard tissues. Bilateral cleft lip and palate affected tissue show the absence of local TGFβ expression in tissue of children in age before

and primary dentition and weak expression in mixed dentition period, giving evidence to the involvement of this growth factor in the cleft severity morpfopathogenesis.

Unilateral and bilateral cleft lip and palate affected tissue show variable quantity of DNES structures and a tendency to decrease in tissues, obtained during repeated surgery.

Unilateral and bilateral cleft affected tissue shows more prominent Barx1 expression in the primary taken material of younger patients of both cleft types and stabilization of Barx1 expression in mixed dentition age.

Unilateral and bilateral cleft lip and palate affected tissue show more prominent apoptosis, which tends to increase in the case of bilateral cleft.

Резюме

Врожденные расщелины губы и неба («заячья губа» и «волчья пасть») находятся среди самых распространенных врожденных пороков в мире. В Латвии врожденная расщелина губы и неба встречается в среднем у одного младенца из 700-800 новорожденных.

Развитие лица и ротовой полости включают рост клеток, дифференциацию, взаимодействия типа клетка-клетка и клетка-внеклеточная матрица, а также запланированную смерть клеток. Эти процессы вызываются и регулируются различными факторами роста и генами.

Тем не менее, несмотря на множество исследований, нет данных о распределении и появлении факторов роста и генов в тканях, пораженных расщелинами или о их роли в лечении ран.

Целью исследования является проведение иммуногистохимического исследования факторов роста, рецепторов факторов роста и относительного избытка и положения генов, определение смерти клеток и сопоставление данных в тканях, пораженных расщелинами у детей с односторонней и двусторонней врожденной расщелиной губы и неба в онтогенетическом аспекте с разработкой диагностического/прогностического алгоритма.

Ткани из области коррекции дефектов расщелин 52 пациентов с односторонней и двусторонней врожденной расщелиной губы и неба были изучены с применением иммуногистохимического метода и метода TUNEL (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling, терминальное дезоксиуридиновое мечение концов). Была доказана и документирована на снимках экспрессия генов bFGF, FGFR1, NGF, NGFR, TGFβ, PGP 9.5 и Barx1, а также относительный избыток иммунореактивных клеток в клеточном материале, взятом не только при первом, но и при повторном хирургическом вмешательстве.

Пораженные ткани односторонней врожденной расщелины губы и неба демонстрируют увеличенную местную экспрессию bFGF и FGFR1 с тенденцией к возрастанию в возрасте сменного прикуса и в тканях, взятых повторно. Пораженные ткани двусторонней врожденной расщелины губы и неба демонстрируют увеличенную местную экспрессию bFGF и FGFR1 в первоначально взятых тканях с неявной тенденцией к уменьшению в тканях, взятых повторно.

Пораженные ткани односторонней врожденной расщелины губы и неба демонстрируют неявную экспрессию NGF и переменную экспрессию NGFR. Пораженные ткани двусторонней врожденной расщелины губы и неба характеризуются также слабой экспрессией NGF, но стабильной экспрессией NGFR.

Пораженные ткани односторонней врожденной расщелины губы и неба характеризуются средне выраженной экспрессией TGFβ, которая немного возрастает в возрасте сменного прикуса и является относительно более значительной в твердых тканях. Пораженные ткани двусторонней врожденной расщелины губы и неба демонстрируют отсутствие местной экспрессии TGFβ в тканях детей в возрасте перед и во время молочного прикуса и слабую экспрессию в период сменного прикуса, что свидетельствует о вовлеченности этого фактора роста в тяжесть морфопатогенеза расщелины.

Пораженные ткани односторонней и двусторонней врожденной расщелины губы и неба демонстрируют переменное количество структур DNES (diffuse neuroendocrine system, рассеянная нейроэндокринная система) и тенденцию к уменьшению в тканях, полученных при повторном хирургическом вмешательстве.

Пораженные ткани односторонней и двусторонней расщелины демонстрируют более выраженную экспрессию Barx1 в первоначально взятых тканях более юных пациентов обоих типов расщелины и стабилизацию экспрессии Barx1 в возрасте сменного прикуса.

В целом, пораженные ткани односторонней и двусторонней врожденной расщелины губы и неба демонстрируют более значительный апоптоз, который имеет тенденцию к увеличению в случае двусторонней расщелины.