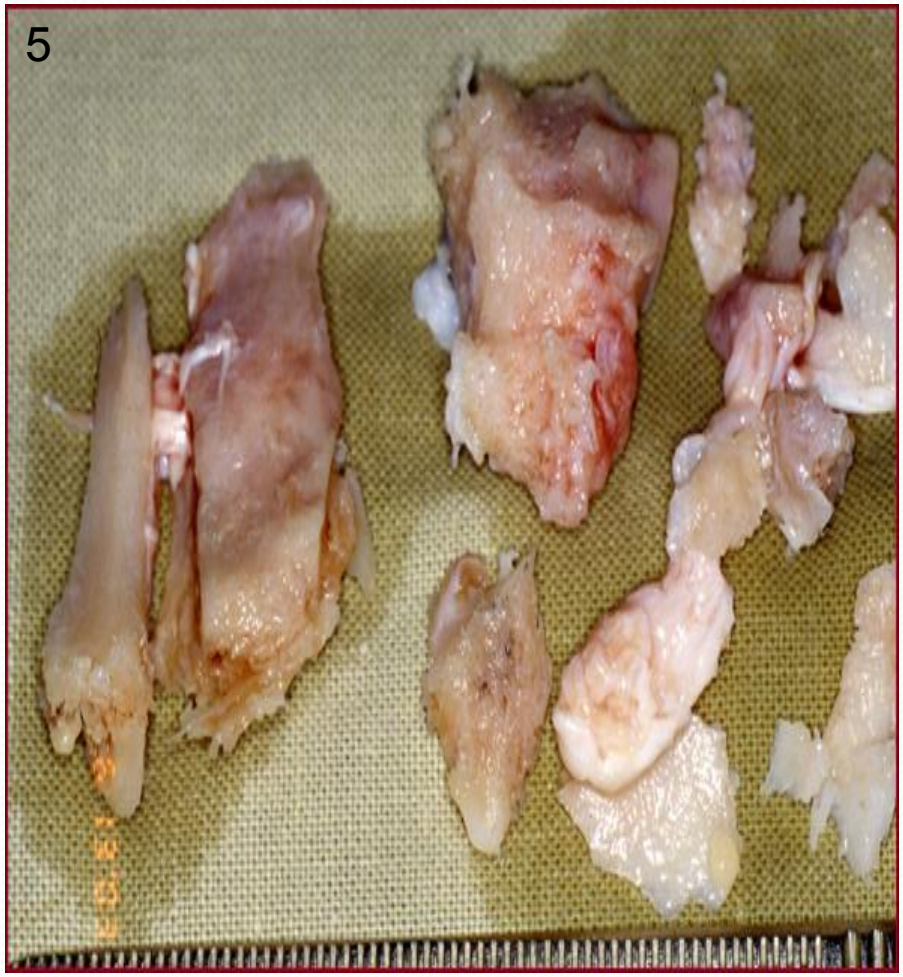
- **The ankylosis of TMJ** (hypomobility of temporomandibular joint is of multifactorial aetiology) **is a reason for decrease of functions in this region of face following by surgical treatment not always effective in long time period. Chondroid hyperplasia (Kim et al., 2009), transformation of the cartilage cells into osteocytes (Peltomäki et al., 2002), and also some growth factors like BMP and genes like Shox2 (Gu et al., 2008) are mentioned to play a role in morphopathogenesis of this disease.**

- **Our aim was complex detection of appearance and distribution of growth factors, facial bone growth stimulating genes and ground substance proteins in ankylotic tissues of TMJ.**

MATERIALS and METHODS

- Ankylotic tissue was obtained during the primary arthroplastic surgery from two 6 years old (one girl and one boy) old children with osseous type of disease. Despite the rib bone/cartilage autotransplantation in TMJ the girl underwent the repeated surgery due to the recurrence of disease in age of 12. Tissues were fixed in Stefanini's solution, dehidratated through a graded series of ethanol, embedded in paraffin, and sectioned into 5 μm thick slides.
- For each case routine staining with haematoxylin and eosin was performed.
- For immunohistochemistry (IMH) antibodies against:
- bone morphogenetic protein 2/4 (BMP 2/4, working dilution 1:100, R and D systems, UK),
- osteocalcin (working dilution 1:100, Abcam, UK),
- osteopontin (working dilution 1:100, Abcam, UK),
- transforming growth factor beta (TGFβ, working dilution 1:1000, Cambridge Science Park, UK);
- Msx2 (mouse, 1: 250, Abcam, UK) were performed IMH by using of standard Dako EnVision and RD Systems kits. IHC labelling was achieved using the standard streptavidin and biotin method (Guesdon et al., 1979; Hsu et al., 1981).
- To evaluate IHC reaction, semiquantitaive method was used. Scale was following: 0 – no positive structures found in visual field, 0/+ – occasional positive structures seen in visul field, + – few immunoreactive structures seen in visul field, ++ – moderate number of immunoreactive structures seen in visul field, +++ – numerous immunoreactive structures seen in visul field, and ++++ – abundance of immunoreactive structures seen in visul field.
- For apoptosis detection, terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate nick end-labelling (TUNEL) using *in situ* cell death detection kit (Roche Applied Science, Penzberg, Germany) was performed (Gavrieli et al., 1992).

RESULTS Bone fragments removed from site of ankylosis in 6 years old girl with osseous ankylosis of TMJ (Fig. 5). Bone demonstrated massive irregular compact bone formation mixed focally by neochondrogenesis (Fig. 6).



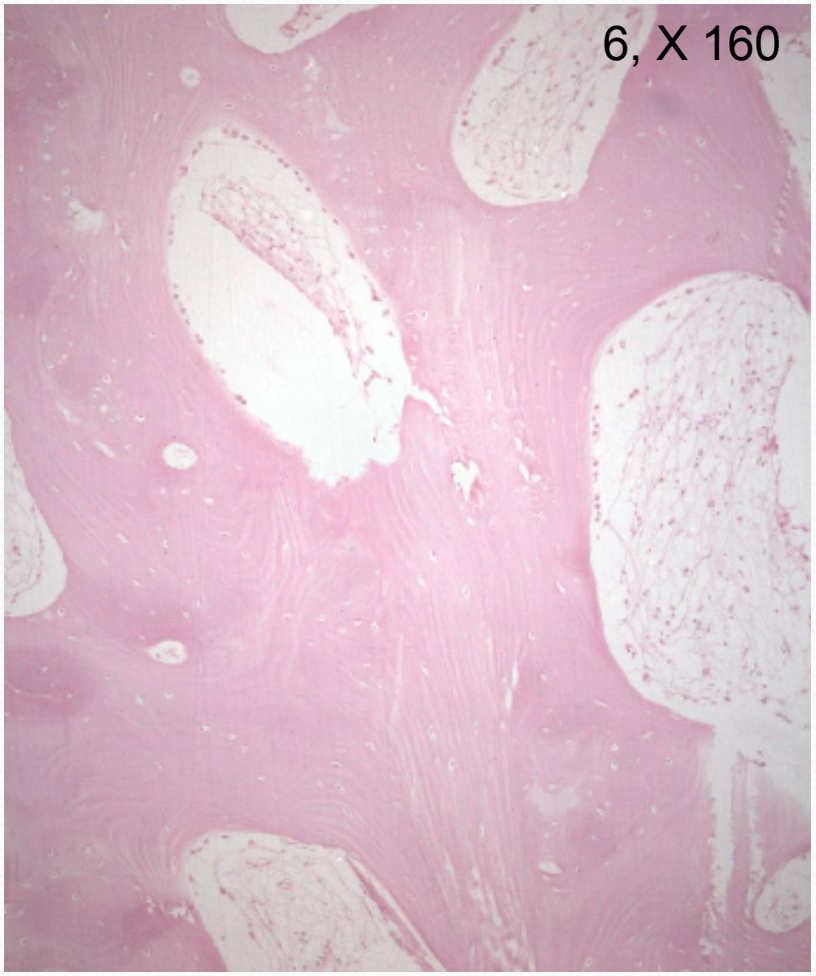


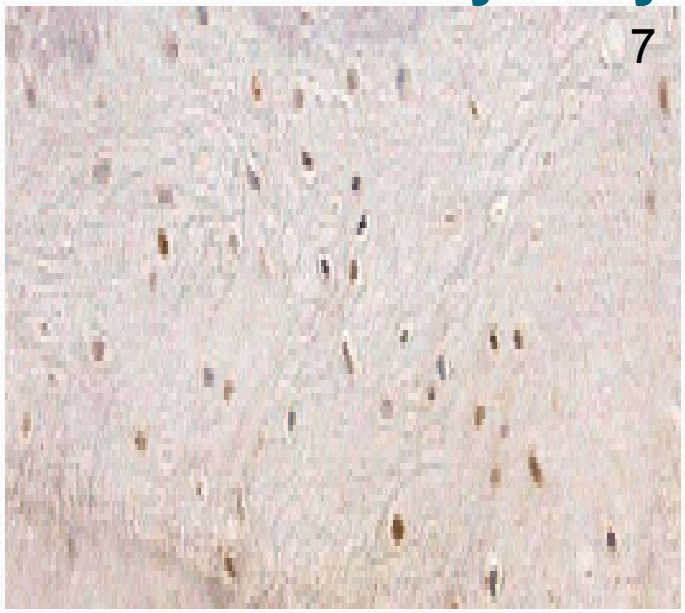
Table. 2. Semiquantitative evaluation of relative appearance in growth factors, genes, bone proteins and apoptosis in TMJ ankylotic bone before and after its reconstruction.

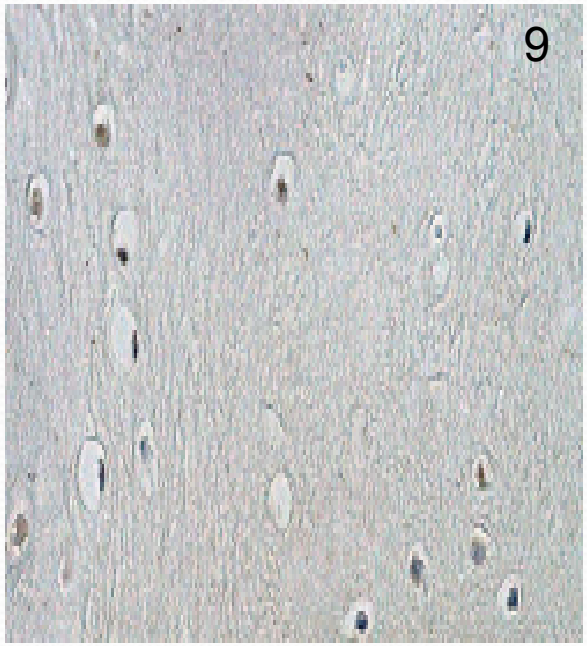
Factors/ / TMJ	TGFβ	BMP 2/4	Msx2	Osteopontin	Osteocalcin	TUNEL
Primary surgery cases n=2 (6 years old boy and 6 years old girl)	++++	-	0/+- +++	+++	+-+++	0/+
Repeated surgery case n=1 (12 years old girl, the same as above)	++++	-	+	+++	++++	++++

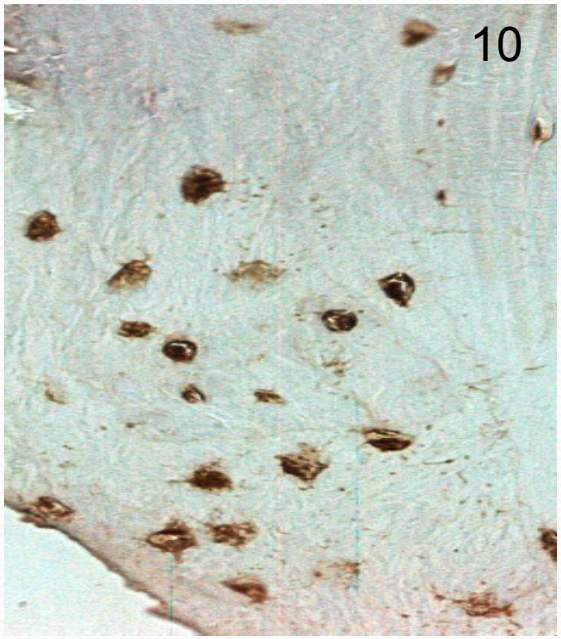
Abreviations:

0/+ – occasional immunoreactive structures seen in visul field, + – few immunoreactive structures seen in visul field, ++ – moderate number of immunoreactive structures seen in visul field, +++ – numerous immunoreactive structures seen in visul field, and ++++ – abundance of immunoreactive structures seen in visul field.

Bone regions with moderate number of Msx2-containing cells in TMJ of 6 years old child (7, X 240). Only few cells demonstrate Msx2 immunoreactivity in 12 years old girl TMJ affected by ankylosis recurrence (8, X 240).







Note occasional apoptotic cells in ankylotic bone of 6 years old child. 9, TUNEL, X 240. Total apoptosis in ankylotic bone in TMJ region 5 years after reconstruction of 12 years old girl. 10, TUNEL, X 400.

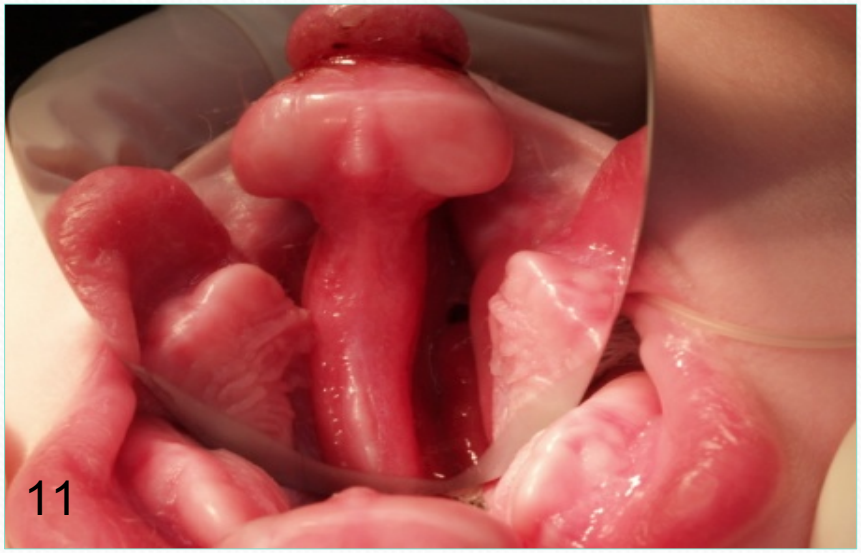
CONCLUSIONS

- **Persistent Msx2 expression is characteristic for the supportive tissue of recurrent ankylosis of TMJ and indicates the persistent stimulation of bone growth compensatory limited by massive increase of programmed cell death.**
- The lack of BMP2/4 expression in ankylotic bone proves the disorders in cellular (more likely osteoblasts) differentiation with simultaneous compensatory intensification of cellular proliferation and/or growth by rich expression of TGFβ leading to the remodelling of TMJ.
- Mainly rich distribution of osteocalcin and osteopontin indicate the intensive mineralization processes of ankylotic bone.

III The prevalence of orofacial clefts varies from 1/500 to 1/2500 births depending on geographic origin, racial and ethnic backgrounds and socioeconomic status (Figs. 11-12). Incidence of this disorder in Latvia population is on averange 1 per 1000 live births.

Unknown pathogenesis – multifactorial, ...

Growth factors are mentioned



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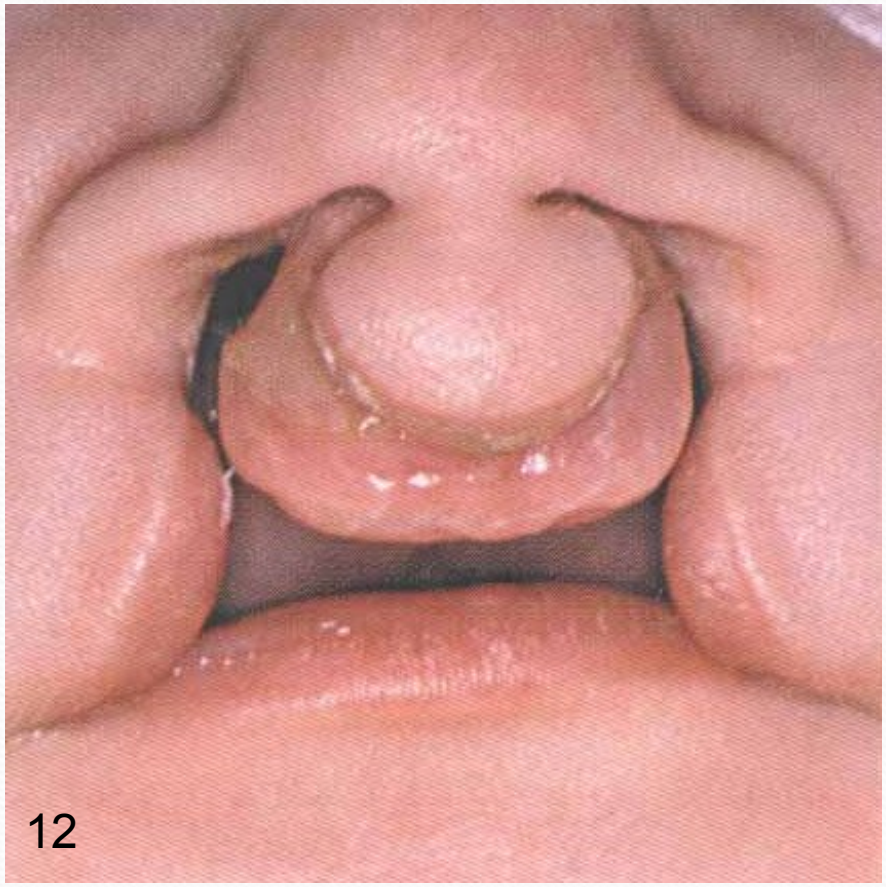

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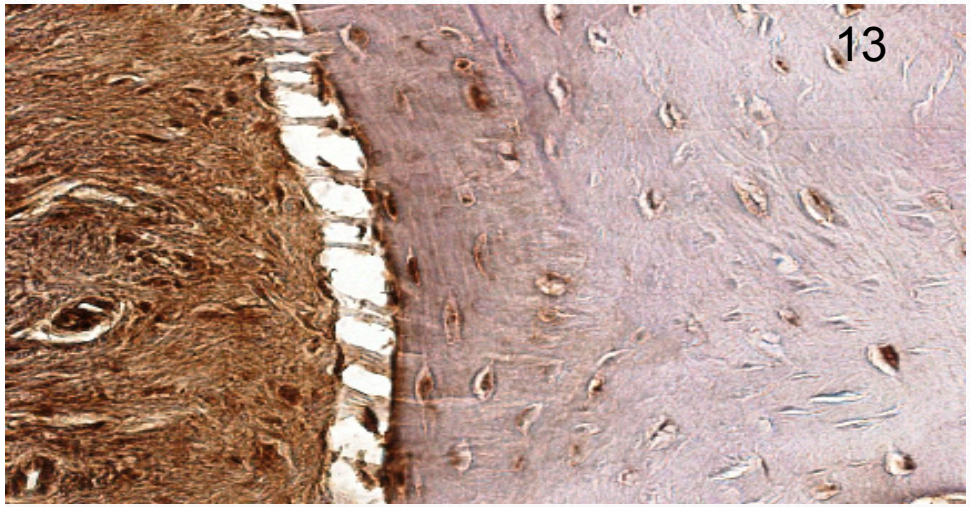
Bone and cartilage were obtained during first and following plastical surgery from 8 bilateral CLP patients. Time duration between the surgeries varied from 6 months up to 5 years (Table 3).

RESULTS

Table 3. Relative number of different factors-containing bone and cartilage cells in children with bilateral cleft.

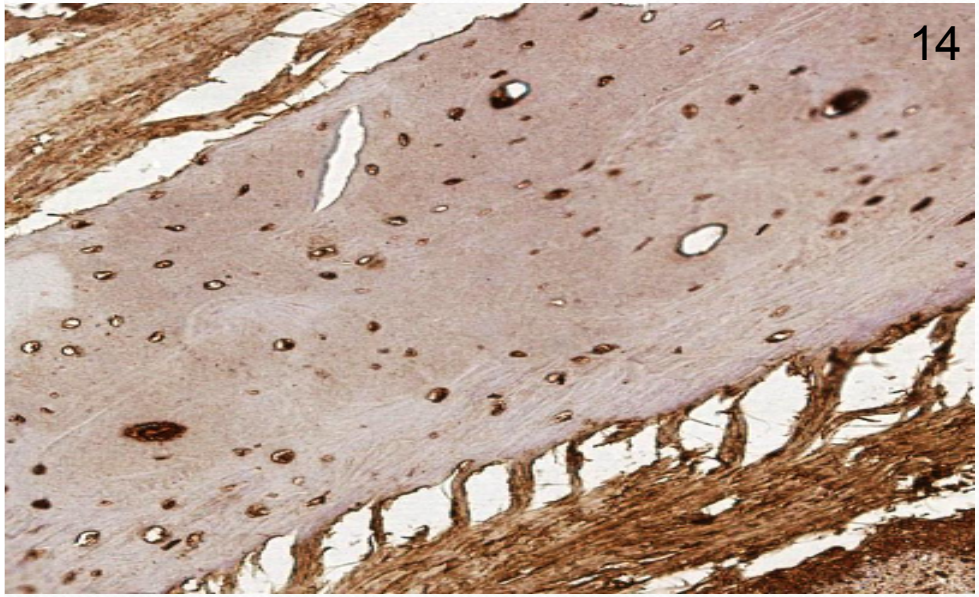
No	Period between surgeries	BMP2/4		TGFβ		TGFβ3		OPG	
		cartilage	bone	cartilage	bone	cartilage	bone	cartilage	bone
1	2 years 9 months	++++	++/+++	+	++/+++	-	+ +++o	+++	+++
1a		+++	++	-	-	-	-	-	+++
2	9 months	+++/++++	+/++	-	-		0/+	-	+
2a		++++	++	+	+++/+++ +	-	-	+++	+++
3	3 years 2 months	-	+	-	-	-	-	+++	+++
3a		-	+	-	-	0/+	+++o	-	0
4	4 years 11months	++		-	-	-	-	-	++
4a		++/+++	+	+	-	-	0/+ +++o	-	++
5	7 months	+/++	+	-	+++	-	-	-	-
5a		++	+	-	+++	-	-	-	-
6	6 months	+++	++	-	+++	-	-	+++	+++
6a		+++/++++	+++	+	+++/+++ +	-	+/++ +++o	+++	+++
7	6 months	+/++	+	-	+++	-	-	-	-
7a		++	+	-	++++	-	-	-	-
8	8 months	+++	+	+	++++	-	-	-	-
8a		+++	+	+	++++	-	-	-	-
Common		+++/++++	++ v	+	++++	0	+	+++	+++

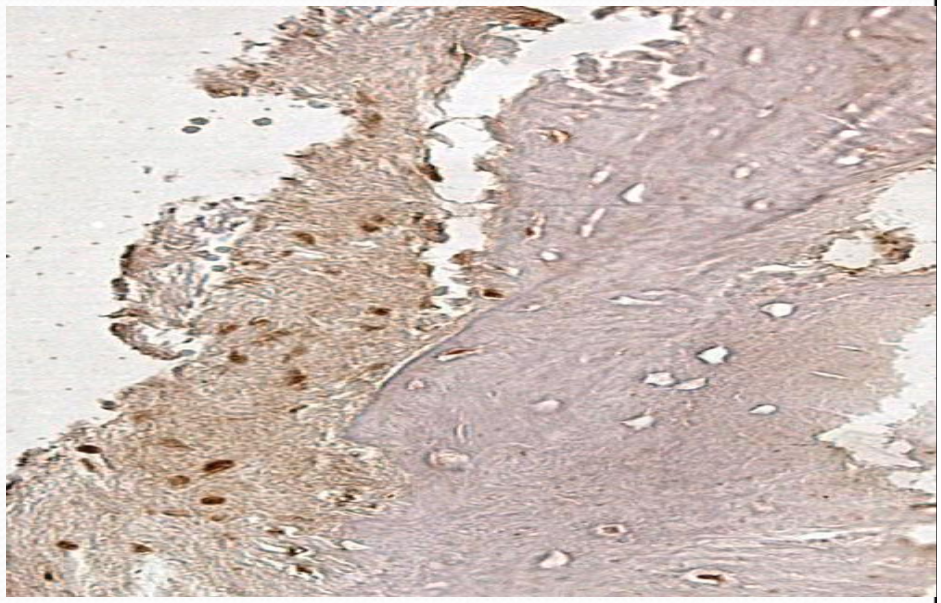
Moderate to numerous bone cells were OPG immunoreactive (13, X 250).



There were two patterns in appearance of TGFβ3 immunoreactive cells in bone. There were patients with abundance of these cells into the bone (14, X 250).

The other pattern were patients without any TGFβ3 expression (15, X 250). However, somewhere periostal undifferentiated cells were strongly TGFβ3 positive in these patients.





CONCLUSIONS

- **Bilateral cleft lip palate affected cartilage with numerous functionally active chondrocytes (abundance of OPG), but decreased TGFβ and TGFβ3 expression proves serious disturbances in the differentiation, but not of the principal growth.**
- **Relative frequency in distribution of TGFβ class factors of bilateral CLP affected supportive tissue do not correlate with age.**
- **Bilateral cleft lip palate pathogenesis enrolles affected bone with selective decrease/or absence of TGFβ3 despite the prominent expression of other TGFβ class growth factors.**

Dear Dr. Hamade ,

Your research and publications in stomatological studies and tissue engineering are extremely impressive. We are organizing the 7th Biennial World Cleft Lip and Palate Congress at Seychelles, coming May. It is a conference on Cleft and craniofacial deformities. www.cleft2012icpf.com

On behalf of the Congress President Prof. S.M. Balaji, we would like to invite you to present your works in your studies in tissue engineering.

Regards

Ms. Kathryn Lazaro

Scientific Committee

7th Biennial World Cleft Lip and Palate Congress...