

RIGA P.STRADINS UNIVERSITY

ZANE LAURINA

**EPIDEMIOLOGICAL AND MORPHOFUNCTIONAL
STUDY OF PERIODONTAL HEALTH**

Speciality - periodontology

Doctoral thesis

Supervisors:

**Dr. habil. Med.
Professor Mara Pilmane**

**Doctor of Medical Science
Professor Ruta Care**



Doctoral thesis carried with the support of the national ESF programme "Support to Doctor's studies and achievement of scientific degree at the P. Stradins University Agreement No. 2009/0147/1Dp/1.2.1.2/09/IPIA/VIAA/009"

Riga, 2010

Doctoral thesis was performed at:

The Riga P. Stradins University, Institute of Stomatology

The Riga P. Stradins University, Institute of Anatomy and Anthropology

Supervisors:

Dr. habil.med., Professor Māra Pilmane

Dr. med., Professor Rūta Care

Approved reviewers:

Dr. habil. med., professor Ilze Akota

Dr. habil. med., professor Alīna Pūriene (Lithuania)

Dr. med., assoc. professor Marina Aunapuu (Estonia)

Relevance of the doctoral thesis

Due to decreased birth rate world population, and also population of Latvia is ageing. With the increasing years highest proportion of population experiences changes of gum and bone tissues supporting teeth or so called periodontal disease.

The main cause of periodontal disease is bacterial plaque and toxins produced by it. Untreated periodontal disease affects quality of life due to bleeding of gingiva, development of periodontal pockets, clinical attachment loss, loosening and loss of teeth. If severity of periodontal disorder increases, the risk of infection's dissemination in the body occurs. Many studies showed unambiguous relation between periodontal disease and such general diseases as cardiovascular diseases, diabetes (Soskolne et al., 2001; Friedewald et al., 2009). Destruction of periodontal tissues caused by inflammation and immunological cascade reactions is controlled by genetic mechanisms. (Page et al., 1997) Cells of the gingival tissue are producing substances to combat bacterial irritant, thus providing local immunity. Various growth factors and they receptors are responsible for the cell migration, proliferation and synthesis of proteins and intercellular components. (Parkar et al., 2002)

Despite numerous *in vitro* and *in vivo* studies in oral, facial and mandibular region there is a comparatively small amount of data showing expression of genes related to craniofacial morphogenesis in periodontal tissues. Importance of transcription factors usually are described in context with other chronic inflammatory diseases such as rheumatoid arthritis, asthma, *lichen planus*. There are rather small number of studies regarding expression of growth factors and its receptors, findings of cytokines and cell adhesion molecules, and antibacterial proteins in the gingival and sulcular epithelium. There is also very limited amount of data about process of programmed cell death and findings in the gingival tissues.

To assess periodontal health situation in elderly, it was need for epidemiological study to be done due to the lack of data as last one was ICS-II study done in 1992. Therefore there was epidemiological study done in 2005. The results indicated poor periodontal health situation as healthy periodontium was in 13,9% and 4,1% in the age group of 35-44 and 65-74. These results indicate abundant inflammation caused periodontal pathology, and we decided to analyze condition of periodontal health situation morphologically. It should be mentioned that this study is the first study of this type in Latvia, where complex morphofunctional aspects of periodontal disorders were assessed.

Aim

To assess periodontal health status, attachment loss and number of lost teeth among population of Latvia aged 35-44 and 65-74 years in epidemiological study.

To determine genes, growth/transcription factors, antibacterial peptides, cell adhesion molecules and cytokines, and apoptosis and to perform immunohistochemical analysis of gingival tissue sample of patients with chronic periodontitis in morphological study.

Tasks

1. To assess periodontal status among 35-44 and 65-74 years old by means of Community Periodontal Index (CPI) and attachment loss.
2. To analyze hematoxylin and eosin stained gingival tissue samples of chronic periodontitis patients.
3. To determine relative gene (Shh, Barx1, Msx2) and nuclear transcription factor beta kappa expression in gingival tissue samples of chronic periodontitis patients by means of immunohistochemical methods.
4. To determine relative expression of growth factor and/or its receptors (NGF, NGFRp75, IGF-IR, FGF) in gingival tissue samples of chronic periodontitis patients by means of immunohistochemical methods.
5. To determine relative expression of cell adhesion molecule (ICAM-1), cytokine (IL-10), antibacterial peptide in gingival tissue samples of chronic periodontitis patients by means of immunohistochemical methods.
6. To determine distribution of apoptosis in gingival tissue samples of chronic periodontitis patients.
7. To assess the relationship between these findings in patients with chronic periodontitis by means of statistical analysis.

Hypotheses

1. Periodontal health status in age groups 35-44 and 65-74 reflects overall periodontal health of population in Latvia.
2. Chronic periodontitis is related to particular changes in relative expression of gene, growth/transcription factors, ICAM-1, IL-10, relative volume of β defensin and apoptosis in gingival and sulcular epithelium. Pathogenesis of chronic periodontitis is affected by severity of gingival tissue inflammation.

Novelty of the doctoral thesis

1. This is the first time when periodontal attachment loss and loss of teeth were determined in population of Latvia.
2. This is the first time when qualitative and quantitative changes of genes, growth and transcription factors, cytokines, cell adhesion molecules, antibacterial peptides and apoptosis were assessed in gingival and sulcular epithelium of patients with chronic periodontitis.

Structure and scope of the doctoral thesis

Doctoral theses are written in Latvian language. It consists of 12 chapters: Introduction, Literature review, Materials and methods, Results, Discussion, Conclusions and References. Introduction describes aim, tasks, novelty of the study and hypothesis for defense. Overall volume of thesis is 106 pages, including 32 tables and 10 figures. There are 53 microphoto images included in appendix. There are 227 published references.

Material and methods

Epidemiological study

In 2005 seven biggest towns of Latvia (Riga, Daugavpils, Jelgava, Jūrmala, Liepāja, Rezekne, Ventspils) and corresponding regions (Riga reg., Daugavpils reg., Gulbene reg., Jekabpils reg., Liepājas reg., Rezeknes reg., Valmieras reg.) were chosen for data collection. 849 participants of different age were examined during this study. In age group 35-44 years 361 subjects were examined, and in age group 65-74 – 246 subjects. Age and gender structure of study population was similar to those in corresponding geographic areas. Inclusion of particular person was random. Examination was performed by 7 calibrated professionals, including the author of this thesis, in health care institutions or medical offices of other institutions. This study was undertaken with the permission of the Ethics Committee of Riga Stradins University.

Community Periodontal Index (CPI) was used to assess periodontal status in three regions of mandibula and maxilla (sextants) - dd 17, 16, 11, 26, 27 in maxilla and dd 47, 46, 31, 36, 37 in mandibula. The highest measurement by special gradual probe was recorded for each sextant.

This was the first time when attachment loss indicating severity of periodontal disorder was assessed. It was also assessed in three mandibular and maxillary regions (sextants) by means of special gradual probe, and highest measurement was recorded. It was recorded as follows: attachment loss 0-3 mm; 4-5 mm; 6-8 mm; 9-11 mm; 12 and more mm. Mean attachment loss and proportion of attachment loss for each subject in each sextant was determined.

Patients examination data was recorded in clinical examination charts, where number of lost teeth also was recorded. Classification of the number of lost teeth was as follows: no lost teeth; up to 4 lost teeth, 5 to 8 lost teeth, 9 to 12 lost teeth, more than 12 lost teeth.

Statistical analysis of the epidemiological study data included mean number of sextants, distribution of CPI by sextants in age group 35-44 and 65-74 years, and mean CPI in each age group. Mean number of sextants by severity of attachment loss was calculated. Distribution of attachment loss by sextants in both age groups (35-44 y.o and 65-74 y.o.) also was calculated. Proportion of lost teeth and mean number of teeth in both age groups was calculated. Difference of mean values was assessed using t test. 5% level of statistical significance was chosen, i.e. probability of error does not exceed 5%. Standard software SPSS-PC 9.05 (SPSS Inc., USA) was used for statistical analysis.

Morphological study

Study samples were taken from 15 patients with chronic periodontitis and 10 controls. Healthy, non-smoking subject receiving treatment at the RSU Institute of Stomatology were included in the study. All subjects undergo periodontal tissue examination, which included assessment of plaque, bleeding after probing, depth of probing and gingival attachment level. Chronic periodontitis was diagnosed taking into account clinical and x-ray findings. Bleeding after probing, depth of probing ≥ 5 mm were observed in regions affected by disease. Case group included patients who failed initial periodontal treatment and there was need for surgical treatment to reduce periodontal pockets. Control group consisted of patients who had clinical crown-lengthening surgery to improve prosthetic aesthetics.

During periodontal flap surgery before detachment of mucoperiosteal flap paramarginal and intracrevicular incision around tooth was performed and this tissue

sample was taken for further morphological processing. Gingival tissue sample included gingival and sulcular epithelium.

Gingival tissue samples were used in this study with the permission of the Ethics Committee of Riga Stradin's University.

Samples were processed by routine histological method, hematoxylin and eosin stained (Лилли, 1969) and reviewed using Leica BME microscope.

Biotin and streptavidin immunohistochemical method was used to determine Shh, Barx1, Msx2, NF- κ B, NGF, NGFRp75, IGF-IR, FGF, IL-10, ICAM-1, β -defensin (Table 1).

Table 1. Data and antibodies applied in immunohistochemistry.

N₀	Factor	Obtained from	Code	Working dilution	Source
1	Shh	mouse	AF 464	1:60	<i>R&D Systems, Germany</i>
2	Barx1	rabbit	ab 26156	1:250	<i>Abcam, UK</i>
3	Msx2	mouse	ab 22601	1:400	<i>Abcam, UK</i>
4	NF-κB	rabbit	ab 7971	1:100	<i>Abcam, UK</i>
5	NGF	rabbit	ab 6199	1:500	<i>Abcam, UK</i>
6	NGFRp75	rabbit	M3507	1:150	<i>DakoCytomation, Denmark</i>
7	IGF-IR	goat	AF -305	1:100	<i>R&D Systems, Germany</i>
8	FGF	rabbit	ab 10646	1:100	<i>Abcam, UK</i>
9	IL10	rabbit	ab 34843	1: 400	<i>Abcam, UK</i>
10	ICAM- 1	goat	BBA17	1: 1000	<i>R&D Systems, Germany</i>
11	β-defensin	goat	AF 2758	1:100	<i>R&D Systems, Germany</i>

Semi-quantitative method described in scientific literature was used for recording relative frequency of values assessed by immunohistochemical method. Expression of factors was analyzed in three visual fields of vision of one section. Explanatory notes on the applied markings are given in Table 2.

Table 2 Semi-quantitative methods for marking relative frequency.

-	No positive structures seen in the visual field
0/+	Rare positive structures seen in the visual field
+	Few positive structures seen in the visual field
++	Moderate positive structures seen in the visual field
+++	Numerous positive structures seen in the visual field
++++	Abundance of positive structures seen in the visual field

TUNEL method (Negoescu et al., 1998) was used to assess relative frequency of apoptosis.

Descriptive and analytical statistical methods were used to analyze morphological data. Frequency tables were used to assess distribution of gene (Shh, Barx1, Msx2), transcription factor – kappa beta (NF- κ B), growth factor and its receptors (NGF, NGFRp75, IGF-IR, FGF), and ICAM-1, IL-10, β defensin and apoptosis in control group and group with chronic periodontitis. Statistical significance of differences was tested by means of non-parametric tests, i.e., Mann-Whitney test (Mann and Whitney, 1947). Apoptosis was described using central tendency measurement, mean and standard deviation, standard error. Both groups were compared by means of unpaired t test. Level of statistical significance was chosen as p value ≤ 0.05 . Two or more statistical comparison methods were used in order to compare different groups with different variables of interest (Altman, 1999; Rosner, 2000). Standard software SPSS-PC 9.05 (SPSS Inc., USA) was used for statistical analysis.

Results

Findings of the epidemiological study

Community Periodontal Index

Analysis of CPI showed that in 2005 in age group 35-44 years 13.9% were with healthy periodontium, 59.6% had periodontal pockets 4-5 mm, and 19.7% - periodontal pockets ≥ 6 mm. In age group 65-74 years 4.1% were with healthy periodontium, 36.2% had periodontal pockets 4-5 mm, and 13.4% - periodontal pockets ≥ 6 mm. Comparing both age groups, the statistical analysis showed significant results: healthy periodontium ($p=0,001$), periodontal pockets 4-5 mm ($p=0,000$) and periodontal pockets ≥ 6 mm ($p=0,000$).

In age group 35-44 years mean number of sextants with healthy periodont (CPI 0) was 0,2, gingival bleeding (CPI 1) – 0,3, dental calculus (CPI 2) – 1,9, periodontal pockets 4-5 mm (CPI 3) – 1,5, and periodontal pockets ≥ 6 mm (CPI 4) – 0,6. In the age group 65-74 years mean number of sextants with CPI 0 (healthy periodontium) was 0,05, CPI 1 (gingival bleeding) – 0,02, CPI 2 (dental calculus) – 0,8, CPI 3 (periodontal pockets 4-5 mm) – 0,5, CPI 4 (periodontal pockets ≥ 6 mm) – 0,1. The mean CPI index in the age group of 35-44 was $2,8 \pm 0,84$, whereas in age group 65-74 years – $3,5 \pm 2,29$.

Proportion of CPI distribution by sextants in age group 35-44 was as follows – 0,5% were healthy (CPI 0), 3,9% were with gingival bleeding (CPI 1), 27,73% were with dental calculus (CPI 2), 43,9% were with periodontal pockets 4-5 mm (CPI 3), and 23,8% were with periodontal pockets \geq 6 mm (CPI 4). In age group 65-74 years there were no healthy sextants, 1,8% were with gingival bleeding (CPI 1), 32,5% were with dental calculus (CPI 2), 41,1% were with periodontal pockets (CPI 3), and 10,4% were with periodontal pockets \geq 6 mm (CPI 4). 14,1% sextants were not assessed due to marked loss of teeth. This difference was statistically significant ($p=0,000$).

Attachment loss

Proportional distribution of gingival attachment loss by sextants in age group 35-44 years was as follows: 27,4% - 0-3 mm, 43,9% - 4-5 mm, 22,1% - 6-8 mm, 5,0% - 9-11 mm, and 1,4% - 12 mm and more. In age group 65-74 years 22,9% - 0-3 mm, 41,2% - 4-5 mm, 21,6% - 6-8 mm, 6,0% - 9-11 mm, and 3,3% - 12 mm and more. Comparing both age groups statistical analysis showed significant results ($p=0,001$). In age group 35-44 mean number of sextants with attachment loss 0-3 mm were 2,4, 4-5 mm – 1,6, 6-8 mm – 0,6, 9-11mm – 0,1, 12 mm and more – 0,01. In age group 65-74 years mean number of sextants with attachment loss 0-3 mm were 0,6; 4-5 mm – 0,6; 6-8 mm – 0,2; 9-11 mm – 0,04; 12 mm and more – 0,03.

Number of lost teeth

In the age group 35-44 years 6,9% had no lost teeth, 29,9% had up to 4 lost teeth, 29,6% - 5-8 lost teeth, 16,3% - 9-12 lost teeth, 17,1% had more than 12 lost teeth. In the age group 65-74 years 0,8% had no lost teeth, 6,1% had up to 4 lost teeth, 9,76% - 5-8 lost teeth, 12,2% - 9-12 lost teeth, 71,1% had more than 12 lost teeth. The number of lost teeth in the age group of 65-75 years was significantly higher and statistical results approves that ($p<0,01$). Mean number of teeth left in the age group of 35-44 years is $22,6\pm 7,53$ and $10,51\pm 9,74$ in the age group of 65-75 years.

Findings of the morphological study

Results of routine histology

Mild infiltration of connective tissues with inflammatory cells was observed in **control group**. Intraepithelial infiltration of lymphocytes was observed, more often in sulcular epithelium. There was one case of inflammatory infiltration away from basal

membrane. Gingival epithelium also produced marked proliferation of basal cells in connective tissues under epithelium. Occasionally extension of intercellular space and vacuolization of epithelocytes in upper layers was observed in sulcular epithelium. Based on this further attention was paid only to the patient's samples, and controls samples were included in normal group only conditionally, even described separately.

Moderate to marked infiltration of gingival connective tissues with inflammatory cells was observed in all samples of the **group of chronic periodontitis**. Most common cells in the chronic inflammatory infiltration were macrophages, neutrophils, lymphocytes and epitheliocytes. Sometimes gingival epithelium formed basal cell cores in lower connective tissues. Intraepithelial infiltration of inflammatory cells was observed also in epithelium, mainly in sulcular epithelium. Signs of basal cell hyperplasia was observed in both gingival and sulcular epithelium. Sometimes basal layer of sulcular epithelium was vacuolised and of irregular thickness.

Findings of immunohistochemical analysis and apoptosis

Control group

For **Shh** expression medium quantity of positive structures in the visual field and a lot of positive structures in the visual field was observed in samples of nine patients. Marked Shh expression was observed in five patients, in three patients – moderate and in one patient very marked expression was observed. This gene was expressed in cytoplasm of epithelial cells in perinuclear area.

Shh positive cells in sulcular epithelium were observed in four control group patients. Marked Shh expression in sulcular epithelium was observed in three control group patients, and in one case - rare positive structures seen in the visual field.

Barx1 was observed in gingival epithelium of five control group patients, and expression was observed in few cells in three patients, and in moderate amount in two patients. Expression was observed mainly in epithelocytes and only in separate macrophages. Expression of Barx1 in sulcular epithelium was observed only among two patients in few cells.

Expression of **Msx2** gene in gingival epithelium was observed only in four control group patients. Positive Msx2 gene expression was observed in cell cytoplasm, and it was focal in epithelium. Msx2 gene colored separate cells of three control group

patients and only in one patient marked expression was observed in gingival epithelium. In sulcular epithelium only for one control group patient some positive structures expressing Msx2 gene in the visual field was observed.

NF-κB in the gingival epithelium samples of control group patients was observed in rare to few positive cells in the visual field, in their nucleus. Expression was observed in nucleus of epithelocytes, and in some nucleus of macrophages. In general NF-κB was observed in occasional cells of three control group patients and in few cells of one control group patient. No NF-κB positive sulcular epithelium structures were observed in control group patients.

NGF positive cells were observed in five control group patients, and expression was observed not only in epithelocytes and inflammation cells, but also in one case in neuroendocrine cells. In three patients rare NGF positive cells, in one patient – moderate quantity of cells and in one patient – numerous positive cells in the visual field was observed. No NGF expression was observed in the sulcular epithelium samples.

NGFRp75 expression in gingival epithelium was observed in eight control group patients, and it was from few cells in the visual field up to very marked. NGFRp75 expression was observed only in basal layer cells, namely in cytolemms. It was very marked in three patients and moderate in two patients and marked in two patients.

NGFRp75 in sulcular epithelium was observed in four samples, and its expression varied from few positive cells to abundant seen in the visual field. In sulcular epithelium NGFRp75 was observed only in cytolemms of basal layer cells. In control group proportion of NGFRp75 found in cytolemms of few cells and NGFRp75 found in many structures and in a lot of structures in the visual field was similar.

Expression of **IGF-IR** was observed in the gingival epithelium samples of seven patients. Expression of IGF-IR was observed in cytolemms of epithelocytes, and inflammation cells in epithelium and connective tissues. Expression was observed from rare up to abundant positive structures found in the visual field. In three patients numerous positive cells were found, in two patients – abundant, in one patient – moderate quantity of positive cells seen in the visual field, and in one patient – only

occasionally. Only two patients had moderate IGF-IR expression in the samples of sulcular epithelium.

FGF was observed in gingival epithelium of six control group patients, and it was from rare positive cells seen in the visual field up to few cells. Expression was observed in cytoplasm of epithelocytes. Cell positivity to FGF antibodies was observed in separate cells of four patients, in few cells of one patient, and in medium quantity of cells of one patient. It was observed in few cells of sulcular epithelium of two patients and in very rare cells of one patient.

Expression of **ICAM-1** in gingival epithelium varied. It was marked in three patients, moderate in three patients, and in few cells of one patient. Only cytolemms of basal layer cells did not contain this molecule. In sulcular epithelium of control group this expression was marked in two patients, moderate in one patient and as a few colored cell cytolemms in one patient.

Expression of **IL-10** in gingival epithelium was marked in four patients, moderate in three patients and abundant in one patient material. This expression was observed in cytolemms of basal layer cells. Expression of IL-10 in sulcular epithelium was observed in four patients, and half of these patients had numerous positive structures found in visual field and half – abundant.

Expression of **β defensin** was observed in eight patients. Marked expression of β defensin was observed in three patients, moderate – in three patients and few structures in the visual field – in one patient, and occasional cells – in one patient. Expression was observed mainly in the upper layers of epithelium. Expression in sulcular epithelium was observed in four patients, and it was from rare positive structures seen in the visual field to abundant expression.

Apoptosis was observed in all samples of control group, and it was observed in occasional cells, in few cells and medium quantity of cells in the visual field both in gingival an sulcular epithelium, only in nucleus of epitheliocytes and some intraepithelially localized macrophages.

Chronic periodontitis group

Expression of **Shh** in gingival epithelium was observed for fourteen samples and it was from occasional to plenty of positive cells in the visual field. Expression of Shh gene in all samples was observed in perinuclear area of cell cytoplasm. Positive structures were separate and focal. We found expression of Shh in few cells of eight samples, in many cells of three samples, moderate positive cells in two samples and occasional structures in one sample. Relative expression of Shh gene in gingival epithelium of chronic periodontitis group was lower than that of control group, and this difference was statistically significant ($z=2,230$ $p=0,0257$).

Expression of gene Shh was observed in four sulcular epithelium samples. Two samples had expression of Shh gene in few cells, and two samples – in moderate quantity of positive structures in the visual field. Difference of Shh positive structures of sulcular epithelium in patient and control group was not statistically significant ($z=1,121$, $p=0,262$).

Expression of **Barx1** was observed in thirteen patients, and it was from occasional cells found in the visual field to numerous positive structures found in gingival epithelium. Expression was observed not only in cell cytoplasm, but also in macrophages between epithelocytes. Marked expression was observed in five samples, moderate – in two samples, in few cells – in four samples, and in separate cells seen in the visual field – in one sample. Mean relative volume of Barx1 containing structures was markedly higher than in the samples of control group, and there was statistically significant difference ($z=2,029$, $p=0,042$).

Expression of Barx1 in sulcular epithelium was observed in five patients. For two patients positivity of Barx1 was observed in a few cells, and for three patients – numerous positive structures in the visual field. Difference of Barx1 positive structures of sulcular epithelium in patient and control group was not statistically significant ($z=1,471$, $p=0,141$).

Expression of **Msx2** in gingival epithelium was observed in ten samples. This expression varied from separate cells to marked quantity of colored cell cytolemms in the field of vision. It was observed not only in cells of gingival epithelium, but in one case also in macrophages between cells. In general expression of Msx2 gene was observed in occasional cells for one patient with chronic periodontitis, in a lot of

structures in the visual field for three patients, in a few cells for one patient and in medium quantity of structures for one patient. There was no statistically significant difference in Msx2 expression of gingival epithelium between control group and group of patients with chronic periodontitis ($z=1,420, p=0,155$).

Expression of Msx2 was observed in sulcular epithelium of two samples, and it was observed in cytoplasm of epitheliocytes. For one patient expression of Msx2 gene was observed in occasional cells in the visual field, and for one patient in few positive structures in the visual field. There was no statistically significant difference in Msx2 expression of sulcular epithelium between control group and group of patients with chronic periodontitis ($z=0,112, p=0,911$).

Expression of **NF- κ B** in gingival epithelium was observed in six patient samples, in the nucleus of epitheliocytes and some macrophages. There were occasional expression in two, few positive structures in three and moderate amount of expression in one sample of chronic periodontitis patient material. There was no statistically significant difference in the expression of NF- κ B in gingival epithelium between control group and group of chronic periodontitis ($z=0,168, p=0,866$).

No NF- κ B positive sulcular epithelium structures were observed in patient group patients.

Expression of **NGF** in gingival epithelium was observed in samples of thirteen patients, and it varied from rare to numerous positive cells seen in the visual field. NGF positive structures were mainly observed in epitheliocytes, and in separate cases also in intraepithelial inflammation cells. Moderate expression of NGF was observed in seven patients, only separate cells colored in three patients, and in two patients expression was in few cells. In the sample of one patient expression was marked. Mean relative volume of NGF containing structures was markedly higher in the group of chronic periodontitis than in the samples of control group, and there was statistically significant difference ($z=2,244, p=0,024$).

Expression of NGF in sulcular epithelium was observed only in one sample, and occasional structures seen in the visual field were positive. There was no statistically significant difference in the expression of NGF between control and patients' group ($z=0,816, p=0,414$).

Marked expression of **NGFRp75** in gingival epithelium was observed in fifteen samples of chronic periodontitis patients. Positive NGFRp75 findings varied from rare cells seen in the visual field to abundant expression. NGFRp75 expression was observed only in basal layer cells, namely in cytolemons. Expression of NGFRp75 was marked in eight patients, very marked in three patients and moderately marked in other three patients. There was no statistically significant difference in the expression of NGFRp75 between control and patients' group ($z=0,220$, $p=0,825$).

Expression of NGFRp75 in sulcular epithelium, i.e in cytolemons of basal layer cells, was observed in the samples of four patients. NGFRp75 findings varied from few to medium quantity of cells seen in the visual field. For two patients in the group of chronic periodontitis expression was moderate, for one – marked and for one patient – separate positive cells seen in the visual field. There was no statistically significant difference in the expression of NGFRp75 in sulcular epithelium between control group and group of chronic periodontitis ($z=0,427$, $p=0,669$).

Expression of **IGF-IR** was observed in the gingival epithelium samples of seven patients. Positive findings varied from rare cells seen in the visual field to abundant expression in the gingival epithelium. Expression was observed mainly in membranes of epithelium, in the layer of basal and polymorphic cells. In one case expression was more marked in basal layer. Proportion of expression of IGF-IR found in separate cells, few cells and many cells was the same with two patients in each respective group. Very marked expression of IGF-IR was observed in gingival epithelium of one patient. There was no statistically significant difference in IGF-IR expression between control group and group of patients with chronic periodontitis ($z=1,702$, $p=0,088$).

Positive findings of IGF-IR were observed in sulcular epithelium of six patients, and expression was observed in separate structures and in medium quantity of structures seen in the visual field. Positivity was observed in membranes of sulcular epithelium. It was observed in rare cells of four patients and moderate quantity of structures of two patients. There was no statistically significant difference in IGF-IR expression of sulcular epithelium between control group and group of patients with chronic periodontitis ($z=0,649$, $p=0,516$).

Expression of **FGF** in gingival epithelium was observed in eleven patients, and it was from rare cells seen in the visual field to medium quantity of cells seen in the visual

field. Cytoplasm of epithelocytes was positive. Positive FGF was observed in few cells of six patients, medium quantity of four patients and in separate cells of one patient. There was no statistically significant difference in the expression of FGF in gingival epithelium between control group and group of chronic periodontitis ($z=1,661, p=0,096$).

Expression of FGF in the sulcular epithelium was observed only in one patient and it was moderate. There was no statistically significant difference in expression of FGF in sulcular epithelium between control group and group of patients with chronic periodontitis ($p=0,370, z=0,711$).

Expression of **ICAM-1** in gingival epithelium was observed in fourteen patients. ICAM-1 was observed in cytolemms of cells. Expression varied from few cells to marked quantity seen in the visual field. For ten patients very marked expression of ICAM-1 in gingival epithelium was observed, for one patient – marked expression, and for one patient - moderate quantity of positive cells was observed. One patient had a few positive cells. Difference of ICAM-1 positive structures of gingival epithelium in patient and control group was statistically significant ($z=3,248, p=0,0012$).

Expression of ICAM-1 in sulcular epithelium was moderate for five patients, marked for three patients, very marked for one patient and only in separate cytolemms of cells in one patient. There was no statistically significant difference in ICAM-1 expression of sulcular epithelium between control group and group of patients with chronic periodontitis ($z=0,151, p=0,880$).

Expression of **IL-10** in gingival epithelium was observed in fifteen patients. It was observed in cytolemms of basal layer cells, and it varied from moderate to very marked expression. Expression in cytolemms of cells was marked in nine patients, abundant in two patients and in one patient expression was moderate. Mean relative volume of immunohistochemical structures in group of patients with chronic periodontitis was slightly higher and this difference was statistically significant ($z=2,332, p=0,019$).

Marked expression of IL-10 in sulcular epithelium was observed in three patients, moderate expression – in one patient. Difference of IL-1 positive structures of

sulcular epithelium in patients with chronic periodontitis and control group was not statistically significant ($z=0,683$, $p=0,494$).

Expression of **β defensin** was observed in eleven samples of chronic periodontitis patients, and it varied from separate structures found in the visual field to very marked expression. Expression was observed in cytolemms of cells of four patients, and it was marked; in few cells of three patients and in separate cells of another three patients, and in one patient it was very marked. Difference of β defensin positive structures of gingival epithelium in patients with chronic periodontitis and control group was not statistically significant ($z=0,445$, $p=0,656$).

Expression of β defensin in sulcular epithelium was observed in five patients and it was observed in cytolemms of cells. In one patient cytolemms of epithelial cells colored abundantly, in one patient – moderate, in two patients – in few cells and in one patient – rarely. Mean relative volume of immunohistochemical structures in group of patients with chronic periodontitis was slightly higher and this difference was statistically significant ($z=2,017$, $p=0,043$).

In the samples of patients with chronic periodontitis **apoptosis** in gingival epithelium was both focal and dispersed. There was no apoptosis in one sample of chronic periodontitis. Mean number of cells with apoptosis in gingival epithelium in the group of patients with chronic periodontitis was higher than in control group and according to the results of unpaired t test this difference was statistically significant ($p=0,0002$, $t=4,950$).

We found occasional, few and moderate number of TUNEL positive cells in sulcular epithelium in patient group. Positive cells were in a small count thorough the sulcular epithelium. Mean number of cells with apoptosis in sulcular epithelium in the group of patients with chronic periodontitis was without statistical significance ($p=0,121$, $t=1,760$).

Correlations

We found abundant expression of ICAM-1, numerous Barx1 gene, IL-10, β defensin, NGFRp75 positive cells, moderate NGF and only few Shh and FGF positive cells in gingival epithelium, whereas in sulcular epithelium plenty of IL-10 positive, marked amount of β defensin, moderate quantity of NGFRp75 and ICAM-1, few Barx1 and IGF-IR cells in chronical periodontitis group (Table 3).

There were done statistical analysis of correlations in gingival and sulcular epithelium. Summarizing results, particular correlations were not found out, but we observe that low expression of NF- κ B in gingival and sulcular epithelium has a tendency to be connected with a very marked expression of antiinflammatory cytokine IL-10. Also marked expression of Barx1 gene in gingival epithelium in patient group has an impact from expression of FGF.

Table 5. Expression of genes, growth factors, transcription factor, cell adhesion molecule-1, intrleukin-10, β -defensin in patient matherial (- no positive structures in visual field; 0/+ -occasional positive structures in visual field; + - few positive structures in visual field; ++ - moderate positive structures in visual field; +++ - numerous positive structures in visual field; ++++ - abundance of positive structures in visual field). **Statistically significant findings.**

	Control group		Chronical periodontitis group	
	Gingival eithelium	Sulcular epithelium	Gingival epithelium	Sulcular epithelium
Shh	- / ++ / <u>+++</u> / ++++	- / 0/+ / <u>+++</u>	0/+ / <u>+</u> / ++ / +++ / ++++	- / + / ++
Barx1	- / + / ++	- / +	- / 0/+ / + / ++ / <u>+++</u>	- / + / +++
Msx2	- / 0/+ / +++	- / 0/+	- / 0/+ / + / ++ / +++	- / 0/+ / +
NF- κ B	- / 0/+ / + / ++	-	- / 0/+ / + / ++	-
ICAM	- / 0/+ / + / <u>+++</u> / ++++	+ / ++ / <u>+++</u>	+ / ++ / +++ / <u>++++</u>	+ / <u>+++</u> / ++++ / +++++
IL-10	- / <u>+++</u> / <u>++++</u> / +++++	+++ / +++++	++ / <u>+++</u> / +++++	+++ / <u>++++</u>
β defensin	- / 0/+ / + / <u>+++</u> / ++++	- / 0/+ / + / <u>+++</u> / ++++	- 0/+ / + / <u>+++</u> / +++++	- / 0/+ / + / <u>+++</u> / +++++
NGF	- / <u>+++</u> / ++++ / +++++	- / + / ++ / ++++ / +++++	+ / ++ / <u>+++</u> / +++++	- / + / <u>+++</u> / ++++
NGFR	- / 0/+ / ++ / +++	-	- / 0/+ / + / <u>+++</u> / ++++	- / 0/+
IGF-IR	- / 0/+ / ++ / <u>+++</u> / +++++	- / 0/+ / <u>++</u>	- / 0/+ / ++ / ++++	- / <u>0/+</u> / ++
FGF	- / <u>0/+</u> / + / ++	- / 0/+ / <u>+</u>	- / 0/+ / <u>+</u> / ++	- / ++

Conclusions

- 1.** In the age group 35-44 years only 13,9% had a healthy periodontium, which indicates poor periodontal health status. The main findings in this age group were attachment loss and periodontal pockets.
- 2.** In age group 65-74 years as compare to age group 35-44 years health of periodontium decreases, number of sextants with gingival bleeding, dental calculus and periodontal pockets and attachment loss decreases due to increased number of lost teeth.
- 3.** In the samples of tissues with chronical periodontitis all morphological signs of inflammation was observed, e.g. basal cell hyperplasia, tissue infiltration with inflammatory cells and remodelling of connective tissues (fibrosis). In the majority of Latvian population gingival inflammation is persistent. It is unusual, original and important finding. It is confirmed also by marked ICAM-1 results in gingival and sulcular epithelium of patients with chronic periodontitis, which indicates active response of body to bacterial irritant.
- 4.** In the group of chronical periodontitis marked expression of Barx1 in gingival epithelium and in few cells of sulcular epithelium was observed. Variable expression of FGF in both types of epithelium also was observed, which indicates possible stimulation of Barx1 expression by growth factor of fibroblasts, thus, providing regeneration of periodontal tissues and tissue homeostasis even in case of inflammation by participation in cell regeneration and cell proliferation.
- 5.** Variable expression of Shh in gingival and sulcular epithelium of patients with chronical periodontitis is related to ability of this gene to regulate cell cycle and possible function of Shh – to provide tissue regeneration.
- 6.** Surprisingly weak expression of NF- κ B indicates some unknown blocking mechanism, which needs to be studied more thoroughly and witch might be related to marked expression of IL-10.
- 7.** High number of apoptosis cell was observed in the gingival and sulcular epithelium of the patients with chronical periodontitis, which indicates good ability of both types of epithelium to respond to inflammation caused by bacterial plaque, thus, preventing increase of the severity of disorder. However, expression of growth factor in gingival epithelium of patients with chronical periodontitis indicates conditional blocking of apoptosis together with the importance of this growth factor in tissue healing process.

8. Expression of IL-10 in cell membranes of basal layer of gingival epithelium indicates active, inflammation limiting and immunosuppressing activity of this cytokine in periodontal tissues, thus, blocking migration of inflammatory cells towards basal layer.

9. Expression of IGF-IR on cell surface in the group of patients with chronic periodontitis was weak, which indicates that its antiapoptotic activity is limited by other factors, e.g. NGF and its receptors. Severity of chronic inflammation in the gingival tissues might be related to variable expression of IGF-IR.

10. Expression of β defensin mainly in upper layer of epithelium indicates marked stimulation of antibacterial peptide in patients with chronic periodontitis.

11. In the gingival epithelium of patients with chronic periodontitis abundant expression of ICAM-1, marked expression of Barx1, IL-10, β defensin, NGFRp75 and moderate quantity of NGF and Shh, FGF positive cells were observed. In general it confirms moderate gene expression, inflammation taking place with an upregulation of main cytokines, but suppression of NF- κ B, marked antimicrobial activity, expression of innervation stimulating growth factors, but suppression of connective tissues stimulating growth factors. Marked apoptosis in gingival epithelium confirms that quick reaction takes place to protect epithelium from development of inflammation. Main markers: Barx1, Shh, FGF, IGF-IR, NGF.

12. In the sulcular epithelium of patients with chronic periodontitis abundant expression of IL-10 in basal cell layer, marked findings of β defensin in upper layers of cell, moderate number of NGFRp75 positive cells in basal layer and moderate expression of ICAM-1 in whole epithelium, as well as few Barx1 and rare IGF-IR positive cells in whole epithelium were observed. The sulcular epithelium protects underlying connective tissue from irritation of bacterial plaque products by intense expression of antimicrobial peptides and antiinfective cytokines, limiting progression of inflammation beyond basal membrane and confirmation of this process is a reduced expression of growth factors. Apoptosis is weaker than in gingival epithelium, which indicates that sulcular epithelium is not so prone to fast programmed cell death. Main markers: Shh, ICAM-1, IL-10, β defensins.

Conference reports on thesis

1. RSU Scientific conference, Medical field, March 2-3, 2006, Riga (Latvia).
2. The 1st Baltic Scientific Conference of Dentistry, October 20-21, 2006, Pärnu (Estonia).
3. RSU Scientific conference, Medical field, March 29-30, 2007, Riga (Latvia).
4. 4. Baltic Morphology Scientific Conference, Riga (Latvia), November 19-20, 2007.
5. RSU Scientific conference, Medical field, March 13-14, 2008, Riga (Latvia).
6. The 6. Latvian Congress of Physicians, June 19-21, 2009, Riga (Latvia).
7. RSU Scientific conference, Medical field, March 18-19, 2010, Riga (Latvia).
8. The 7. Congress of Baltic Association for Maxillofacial and Plastic Surgery, May 20-22, 2010, Riga (Latvia).

Scientific publications

1. Care R., Brinkmane A., Krasone K., Arne G., Laurina Z., Stamere U. Izmaiņas kariesa intensitātē un periodonta patoloģijā Latvijā 2004. gadā. // RSU Medicīnas nozares Zinātniskā konference. – Rīga, 2006. gada 2.-3. marts. - 22. lpp
2. Care R., Brinkmane A., Krasone K., Arne G., Laurina Z., Stamere U. Caries experience and periodontal pathologies in 35-74 year old in Latvia in 2005. // The 1st Baltic Scientific Conference of Dentistry. Pärnu, Estonia, 20-21 October. - 2006. - P. 40.-41.
3. Care R., Brinkmane A., Krasone K., Ārne G., Lauriņa Z., Stāmere U. Izmaiņas kariesa intensitātē un periodonta patoloģijās Latvijā 2004./2005. gadā. // RSU Zinātniskie raksti 2007: 365-370.
4. Pugaca J., Urtane I., Liepa A., Laurina Z. The relationship between the severity of malposition of the frontal teeth and periodontal health in age 15-21 and 35-44. // Baltic Dental and Maxillofacial Journal, Stomatologija 2007;9(3):86-90.
5. Lauriņa Z., Care R. Periodonta stāvoklis Latvijā 35-44 un 65-74 gadu vecuma grupā 2005. gadā. // RSU Medicīnas nozares Zinātniskā konference. – Rīga, 2007. gada 29.-30. marts. - 30 lpp.
6. Laurina Z., Pilmane M., Care R. Genes/transcription and growth factors and periodontal pathologies. // Baltic Morphology 4th Scientific Conference. - Institute of Anatomy and Anthropology, Riga, Latvia, 19-20 November. – 2007. – P. 45.

7. Lauriņa Z., Pilmane M., Care R. Augšanas faktoru/iekaisuma marķieru/defensīnu un apoptozes saistība ar periodonta saslimšanām. // RSU Medicīnas nozares Zinātniskā konference. – Rīga, 2008. gada 13.-14. marts. – 193. lpp.
8. Lauriņa Z., Care R., Pugača J., Urtāne I. Periodonta stāvoklis 35-44 un 65-74 gadu vecu iedzīvotāju grupā 2005. gadā Latvijā. // RSU Zinātniskie raksti 2008: 345-350.
9. Laurina Z., Pilmane M., Care R. Genes, transcription factors and periodontal pathologies. // Acta Chirurgica Latviensis 2008; 8: 23-27.
10. Lauriņa Z., Pilmane M., Care R. Gēni, transkripcijas faktori un periodonta patoloģijas. // 6. Latvijas ārstu kongress. – Rīga, 2009. gada 19.-21. jūnijs. – 51. lpp.
11. Laurina Z., Pilmane M., Care R. Growth factors/cytokines/defensins and apoptosis in periodontal pathologies. // Baltic Dental and Maxillofacial Journal, Stomatologija 2009; 11(2): 48-54.
12. Lauriņa Z., Pilmane M., Care R. Intercelulārās adhēzijas molekulas - 1, tumornekrozes faktora- α , interleikīna - 10 ekspresija periodontālajos audos. // RSU Medicīnas nozares Zinātniskā konference. – Rīga, 2010. gada 18.-19. marts. - 299. lpp
13. Laurina Z., Pilmane M., Care R. Nerve growth factor, nerve growth factor receptor P75, apoptosis and periodontal pathologies. // 7th Congress of Baltic Association for Maxillofacial and Plastic Surgery, Riga, Latvia, 20-22 May. – 2010. - P. 72-73.

Acknowledgements

I would like to express my deep appreciation and sincere gratitude to both my supervisors Dr. habil. med., Dr. med. Professor Mara Pilmane and Dr. med., Professor Ruta Care, for guiding my work, offering valuable advice, support and encouragement from the beginning to the end in the course of my research.

I am thankful to Director of RSU Institute of Stomatology Dr. med., Professor Ilga Urtane for support and sincerity during course of this study. I also would like to thank Irena Rogovska for helping me with statistical analysis work.

I would like to thank laboratory assistants Natalija Moroza and Jelena Fjodorova at the RSU Institute of Anatomy and Anthropology for preparation of histological samples and offering valuable advices. I am thankful to technical assistant Janis Brede at the RSU Institute of Anatomy and Anthropology for helping me with microphotographies.

I would like to thank also Secretary of the Department of Doctoral studies for cooperation during my doctoral studies.

I also would like to express my appreciation to all my colleagues at the RSU Institute of Stomatology, Department of Periodontology for understanding and invaluable help during this study.

I am thankful to my beloved family for support and encouragement.