GENETIC INVESTIGATIONS IN CHRIST-SIEMENS-TOURAINE AND PAPILLON-LEFÈVRE SYNDROME IN THE EYES OF THE DENTIST

Summary of the Ph.D. thesis

Péter Vályi D.M.D.

[1]Department of Medical Genetics, Faculty of Medicine
[2]Department of Oral Surgery, Faculty of Dentistry
University of Szeged, HUNGARY

Supervisors:
Nikoletta Nagy M.D., Ph.D.[1]
Katalin Nagy D.M.D., Ph.D.[2]

SZEGED
2014

List of publications

Publications directly related to the subject of the dissertation

I. Ágnes Kinyó, **Péter Vályi**, Katalin Farkas, Nikoletta Nagy, Brigitta Gergely, Kornélia Tripolszki, Dóra Török, Zsuzsanna Bata-Csörgő, Lajos Kemény, Márta Széll. A newly identified missense mutation of the EDA1 gene in a Hungarian patient with Christ-Siemens-Touraine syndrome. Arch Dermatol Res. 2014 Jan;306(1):97-100. IPF: 2.708.

II. Katalin Farkas, Ekaterine Paschali, Ferenc Papp, **Péter Vályi**, Márta Széll, Lajos Kemény, Nikoletta Nagy, Zsanett Csoma. A novel seven-base deletion of the CTSC gene identified in a Hungarian family with Papillon-Lefèvre syndrome. Arch Dermatol Res. 2013 Jul;305(5):453-5. IF: 2.708

III. Nikoletta Nagy, **Péter Vályi**, Zsanett Csoma, Adrienn Sulák, Kornélia Tripolszki, Katalin Farkas, Ekaterine Paschali, Ferenc Papp, Lola Tóth, Beáta Fabos, Lajos Kemény, Katalin Nagy, Márta Széll. *CTSC* and Papillon-Lefevre syndrome: detection of recurrent mutations in hungarian patients, a review of published variants and database update. Molecular Genetics & Genomic Medicine. 2014 May:2(3):217-228.

IV. **Péter Vályi,** Katalin Farkas, Adrienn Sulák, Kornélia Tripolszki, Lajos Kemény, Katalin Nagy, Nikoletta Nagy, Márta Széll. European recurrent missense mutation in a Hungarian pedigree with Papillon-Lefevre syndrome. Fogorv Sz. 2014 Sept.: 107(3): 87-92

Publications indirectly related to the subject of the dissertation

I. **Péter Vályi**, István Gorzó, Tiina Varella, Liisi Sewón, Pekka Vallittu:. Effect of occlusal therapy with FRC splint on periodontal parameters in maintenance phase. Fogorv Szle.: 2005 Aug; 98(4):159-63. Hungarian.

II. **Péter Vályi**, István Gorzó. Periodontal abscess: etiology, diagnosis and treatment. Fogorv Sz. 2004 Aug; 97(4):151-5. Review. Hungarian.

III. **Péter Vályi**, István Gorzó, András Kocsis, Endre Kiss, Attila Tóth.. Direct application of fiber-reinforced composites in splinting in a case of periodontitis. II. Fogorv Sz. 2003 Feb; 96(1):29-32. Hungarian.

IV. **Péter Vályi**, István Gorzó. Current splinting methods in dentistry. I Fogorv Sz. 2003 Feb; 96(1):25-8. Review. Hungarian.

V. **Péter Vályi**, István Gorzó, Albert Mari: Hygiene in dentistry. I. Contamination of handpieces and dental units. Fogorv. Sz. 1999 Jun; 92(6):167-74.

VI. **Péter Vályi**, István Gorzó, Albert Mari: hygiene in dentistry II: Disinfection of dental handpieces. Fogorv. Sz. 1999 Jul; 92(7):213-8

INTRODUCTION

Rare diseases are defined as disorders with smaller incidence than 1:2000. However a rare disease affects nationwide only a few patients, but - due to their thousands of different types – altogether they affect a significant portion of the population. In opposite with common diseases, which usually show multifactorial etiology including environmental, life style and genetic factors in their development, rare diseases are usually monogenic ones. In this study, I have investigated four rare diseases – Christ-Siemens-Touraine syndrome, Papillon-Lefèvre syndrome, Haim-Munk syndrome and aggressive periodontitis type 1 – with overlapping dental symptoms and different skin ones.

Christ-Siemens-Touraine syndrome

Christ-Siemens-Touraine syndrome (OMIM 305100) is a rare ectodermal dysplasia characterized by a triad of signs comprising sparse hair (hypotrichosis), abnormal or missing teeth (anodontia or hypodontia), and inability to sweat (anhidrosis or hypohidrosis). The prevalence is estimated between 1:100000 and 1:1000000 individuals.
The majority of individuals with hypohidrotic ectodermal dysplasia show the X-linked form. Ectodysplasin A (EDA1; GenBank accession number NM_001399.4) is the only gene, in which pathogenic variants are known to cause X-linked hypohidrotic ectodermal dysplasia. Pathogenic variants in EDAR, EDARADD and WNT10A genes are known to be associated with both autosomal dominant and autosomal recessive forms of hypohidrotic ectodermal dysplasia.

Papillon-Lefèvre syndrome

Papillon-Lefèvre syndrome (OMIM 245000) is a rare ectodermal dysplasia characterized by early-onset periodontitis associated with palmoplantar keratoderma. The periodontal inflammation result in loss of both the primary and permanent teeth. Keratoderma in Papillon-Lefèvre syndrome can present in the first three months of life, although palmoplantar hyperkeratosis generally first appears in years 1–4. However, several late-onset variants of Papillon-Lefèvre syndrome have also been reported. In addition to these symptoms, recurrent skin infections and liver abscesses are frequently reported Moreover mild mental retardation,

intracranial calcifications and hyperhidrosis can also occur. Japanese patients might have an increased risk of developing melanomas at the sites of hyperkeratosis than other ethnic groups. Papillon-Lefèvre syndrome is transmitted as an autosomal recessive condition affecting males and females equally. Mutations of the cathepsin C gene are responsible for the development of the disease.

Haim-Munk syndrome

Haim-Munk syndrome (OMIM 245010) is a rare ectodermal dysplasia characterized by early-onset severe periodontitis associated with palmoplantar keratoderma, onychogryposis, pes planus, arachnodactyly and acroosteolysis. The prevalence is estimated to be 1:1000000 individuals. Haim-Munk syndrome is very rare with less than 100 cases reported in the literature so far.

Haim-Munk syndrome is transmitted as an autosomal recessive condition affecting males and females equally. Similarly to Papillon-Lefèvre syndrome, Haim-Munk syndrome is also caused by mutations of the CTSC gene.

Aggressive periodontitis type 1

Aggressive periodontitis type 1 (OMIM 170650) is a rare ectodermal dysplasia characterized by severe periodontal inflammation leading to tooth loss. Since other organs are not affected, aggressive periodontitis type 1 also belongs to the family of non-syndromic aggressive periodontitis. There are only a few cases in the literature, which are genetically confirmed and diagnosed as aggressive periodontitis type 1. Regarding these patients, the male to female ratio is 1:1. The prevalence is estimated to be less than 1:1000000 individuals.

Aggressive periodontitis type 1 is transmitted as an autosomal recessive condition affecting males and females equally. Similarly to Papillon-Lefèvre and Haim-Munk syndromes, aggressive periodontitis type 1 is also caused by mutations of the CTSC gene.

Papillon-Lefèvre syndrome, Haim-Munk syndrome and aggressive periodontitis type 1 have some overlapping and some distinctive clinical features, therefore earlier they were considered as different entities. In 1999, with the identification of their causative gene, the CTSC gene, their common genetic background was identified. First it was suggested that different mutations of the CTSC gene might lead to the development of different disease, but genotyping patients

with Papillon-Lefèvre syndrome, Haim-Munk syndrome or aggressive periodontitis type 1 did not confirm this hypothesis.

AIMS

In this study, I have investigated the Christ-Siemens-Touraine syndrome and the allelic variants of Papillon-Lefèvre syndrome, which include Haim-Munk syndrome and aggressive periodontitis type 1. These variants show overlapping dental symptoms and different other ones. I aimed to investigate Hungarian pedigrees and sporadic cases with Papillon-Lefèvre syndrome. After performing the relevant, adequate dental care for these patients, I have initiated dermatological investigations to perform complete workup for these patients.

Besides the teamwork of the clinical care, I have also initiated genetic screening for these patients to help them in family planning and also to elucidate the genotype-phenotype correlations of the disease. Therefore I aimed to compare the clinical symptoms and the identified mutations in all investigated patients. After comparing Hungarian patients to each other, I have also compared their data with the so far reported ones in the literature. To do this, I have performed literature search (http://www.ncbi.nlm.nih.gov/pubmed) to identify the reported patients Papillon-Lefèvre syndrome and all the known CTSC mutations.

PATIENTS AND METHODS

However Christ-Siemens-Touraine and Papillon-Lefèvre syndrome are rare diseases, there are several patients under my dental care. In this study, I will describe three families and two sporadic cases in details, in whom the genetic investigations identified the causative abnormalities.

In Hungary, mutation screening for the EDA1 and CTSC genes have been available since 2011. Screening is performed with direct sequencing of all coding regions and flanking introns of the EDA1 and CTSC genes. Once a putative causative variant was identified in a patient, the available, clinically symptom-free family members and unrelated, healthy control individuals were also investigated.

PATIENTS

Pedigree I

I have recently identified a 35-year-old Hungarian patient with Christ-Siemens-Touraine syndrome and with characteristic dysmorphic facial features, sparse hair, reduced sweating and missing teeth. On investigation the classic triad of Christ-Siemens-Touraine syndrome was present. Dermatological symptoms included sparse hair and reduced sweating and dental symptoms highlighted hypodontia.

The investigated patient is the only affected family member who exhibits the complete triad with sparse hair, missing teeth and reduced sweating. His sister and his daughter also have some conical-shaped teeth, but otherwise they are healthy. The older brother of the patient died at the age of 4 months due to hyperpyrexia. The symptomless parents reported on a 16-year-old sister of the affected daughters, who has no symptoms as well as the parents.

Pedigree II

I have recently identified a Hungarian family with two sisters affected with severe periodontitis leading to the loss of all primary teeth. On dermatological investigation an 11-year-old Hungarian girl (Patient I) was referred with the typical skin symptoms of Papillon-Lefèvre syndrome, complicated with palmoplantar eruption. On referral sharply circumscribed erythema with minimal hyperkeratosis and shedding was seen on both palms. The erythema on the plantar surfaces was minimal, however hyperkeratosis with deep fissures dominated. These abnormalities first appeared at her age of 19 months.

The other patient (Patient II) was a 2-year-old Hungarian girl, the younger sister of Patient I. She was also referred with having similar symptoms as her older sibling. Palmoplantar eruptions started at her age of 10 months. On referral, minimal erythema was seen on the distal fingertips and erythema with minimal hyperkeratosis was present on the soles of the feet.

The symptomless parents reported on a 16-year-old sister of the affected daughters, who has no symptoms as well as the parents.

Pedigree III

There is another pair of affected siblings, who are also under my regular dental care. In this Hungarian family the affected two sisters were referred to our out-patient dental clinic years ago with severe tooth loss due to severe periodontitis.
Regarding their dermatological symptoms, there is a significant contrast between the severity of the palmar and the plantar hyperkeratosis. The sisters show very mild palmar symptoms on both hands, it looks like hand dryness, but the hyperkeratosis on their soles is very severe.
The symptomless parents reported on having no other affected or symptomless child. Since these siblings are now young adults, Patient I is 24 and Patient II is 28-yeard-old. I have performed dental investigations and complete clinical and genetic workup for their symptomless partners as well.

Sporadic cases

A 39-year-old Hungarian woman was referred from the Mór Kaposi Teaching Hospital (Kaposvár) with a common phenotype of Papillon-Lefèvre syndrome. The patient was presented with the typical skin symptoms and she has lost all her permanent teeth and wear permanent prosthesis. Hyperkeratotic plaques were seen on both palms and soles. Besides these symptoms, arachnodactily was also present on the right hand raising the possibility of the allelic variant of the Papillon-Lefèvre syndrome, the Haim-Munk syndrome.
Another patient, a 25-year-old Hungarian man unrelated to the previous 39-year-old Hungarian woman. He was also referred from the Mór Kaposi Teaching Hospital (Kaposvár) with complete teeth loss and hyperkeratotic skin symptoms on his palms and soles. He does not show arachnodactily.

METHODS

All patients are in complete clinical care including dental and dermatological care. Written informed consents were obtained from all investigated individuals during pre-test genetic counselling before genetic investigations were carried out. The investigated individuals were informed about the results of the investigations during post-test genetic counselling. The study was conducted according to the Principles of the Declaration of Helsinki.

DNA isolation

Blood samples were taken from the patients and from the clinically unaffected family members. Genomic DNA was isolated from whole blood samples using a QIAamp DNA Blood Mini Kit (QIAGEN; Hilden, Germany). During the isolation, after proteinase K digestion, washings with alcohol were done following the instructions. Genomic DNA was dissolved in 100 µl distilled water.

Polymerase chain reaction

During polymerase chain reaction (PCR) amplification, 4 µl genomic DNA was used as template. In addition, the reaction mix contains 9 µl Dream Taq Green PCR Master Mix (Fermentas), 4 µl distilled water, 1.5 µl forward and 1.5 µl reverse primers.
During PCR reaction, the 2^{nd} (denaturation), 3^{rd} (annealing) and 4^{th} (synthesis) steps were repeated 40 times. The annealing temperature and the number of the cycles were depended on the primers, the synthesis reaction time was determined according to the length of the reaction product.

Gel electrophoresis

The PCR products were checked on 2% agarose gel (SeaKem LE agaróz, Lonza) using TBE buffer (Lonza) and visualized by 2.5 µl GelRed (Biotium) staining. The gel was analyzed by BioRad Molecular Imager® GelDoc™ XR gel documentation system with QuantityOne software.

Sequencing

The sequencing was performed after the suitable purifying of the PCR reaction products using Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems) with ABI Prism 7000 (Applied Biosystems) sequencing machine. The service of the sequencing was offered by Delta Bio 2000 Kft.

RESULTS

Patient in Pedigree I carried hemizygous missense mutation

Direct sequencing of the coding regions and the flanking introns of the EDA1 gene revealed a novel missense mutation in the eight exon (c.971T/A, p.Val324Glu;). The investigated healthy controls carried only wild-type sequences. The identified p.Val324Glu missense mutation is located in the TNF domain of the ectodysplasin protein. Therefore this genetic variant may affect the ectodysplasin/NFκB signaling pathway.

Patients in Pedigree II carried homozygous deletion

Direct sequencing of the coding regions and the flanking introns of the CTSC gene revealed a seven-base deletion in the fourth exon (c.566delCATACAT, p.T189fsX199). This deletion causes frameshift and leads to the development of a premature termination codon (TGA) 32 bases downstream of the mutation.

The patients carried the mutation in homozygous form, while the unaffected family members – the parents and the symptomfree sister – carried the same mutation in heterozygous form. The unrelated controls carried the wild type sequence. The family was not aware of consanguinity. This frameshift mutation has also been previously published for two Moroccan patients with Papillon-Lefèvre syndrome.

Patients in Pedigree III carried homozygous missense mutation

Direct sequencing of the coding regions and the flanking introns of the CTSC gene revealed a missense mutation in the seventh exon (c.901G/A, p.G301S). This missense mutation causes amino acid change in the cathepsin C protein.

The patients carried the mutation in homozygous form (Figure 26.), while the symptomfree partners of the patients carried the wild type sequence. The unrelated controls carried the wild type sequence as well. The family was not aware of consanguinity. This mutation has also been previously published for a German patient with typical Papillon-Lefèvre syndrome.

The unrelated cases carried the same nonsense mutation

In a pair of unrelated Hungarian patients with Papillon-Lefèvre syndrome, we have identified a nonsense mutation in the fifth exon (c.748C/T, p.R250X) using direct sequencing of the coding regions and the flanking introns of the CTSC gene. This missense mutation causes truncation of the cathepsin C protein.
Unfortunately, both of these patients were grown up in state care and not aware of any known relatives; therefore, investigation of the family was not possible. The fact that both individuals carry the same mutation raises the possibility that these patients somehow are relatives. This mutation has also been previously published in the literature in a Turkish family with Papillon-Lefèvre syndrome.

DISCUSSION

Significance of the investigations in the EDA1 gene

My investigations have identified a novel hemizygous missense mutation (c.971T/A, p.Val324Glu) of the EDA1 gene in a Hungarian patient shows full expression of the Christ-Siemens-Touraine phenotype with severe dental abnormalities. The daughter and sister of the investigated male patients are heterozygous carriers of the identified mutation have some conical-shaped teeth, but exhibit otherwise normal phenotype. These findings supporting previous results of investigation of EDA1 mutations in non-syndromic tooth agenesis, suggesting that dental tissues are particularly sensitive to ectodysplasin protein abnormalities.
Since the novel hemizygous missense mutation (c.971T/A, p.Val324Glu) is located in a highly conserved region within the TNF domain of the ectodysplasin protein, it is hypothesized that this mutation affects the NFκB signaling pathway.

Variants in the CTSC gene

Papillon-Lefèvre syndrome is a rare ectodermal dysplasia characterized by early-onset periodontitis associated with palmoplantar keratoderma. Clinical diagnosis of Haim-Munk syndrome, an allelic variant of Papillon-Lefèvre syndrome, is based on the presence of arachnodactly, acroosteolysis, pesplanus and onychogryposis in addition to palmoplantar hyperkeratosis and periodontal inflammation. Aggressive periodontitis type 1, which can be

also considered a variable expression of the Papillon-Lefèvre syndrome phenotype, is characterized by periodontal inflammation and the lack of other symptoms. All the three entities develop as a consequence of CTSC mutations. Identification of a CTSC mutation gives a definite diagnosis of Papillon-Lefèvre syndrome, Haim-Munk syndrome or aggressive periodontitis type 1 depending on the presented clinical symptoms. In contrast, the absence of CTSC mutation suggests a diagnosis of another palmoplantar keratoderma or non-syndromic tooth abnormality.

Cathepsin C is a lysosomal cysteine protease that was first characterized as an activator of serine proteases from immune and inflammatory cells. The encoded cathepsin C precursor contains 463 amino acids and includes a signal peptide (24 amino acids), an exclusion domain (110 amino acids), a propeptide (96 amino acids), as well as heavy (164 amino acids) and light (69 amino acids) chain regions. The most highly conserved regions are the heavy chain, the light chain and the C-terminal portion of the exclusion domain, which is thought to be important for enzyme activity.

Half (53%, n=40) of all CTSC gene mutations affect the heavy chain domain and result in different positioning of its N-terminus. Since the N-terminal region is involved in oligomer contacts with the N-terminal region of the light chain, the mutation may interfere with tetramer formation. This finding indicates that tetramerization of the cathepsin C enzyme is crucial for its function. The majority of the two most common types of CTSC mutations (missense and nonsense) affect this domain. Sixteen percent (n=12) of all CTSC mutations affect the exclusion domain, which blocks access to the active site and prevents substrates from binding any part except their N-termini. Thirteen percent (n=10) of all CTSC gene mutations affect the propeptide fragment, which plays a pivotal role in the activation of the cathepsin C precursor. The majority of frameshift mutations are located in this domain. Twelve percent (n=9) of all mutations affect the light chain domain, which is important for tetramerization of the mature enzyme: four are missense mutations, two are nonsense variants and one is an in-frame deletion. Three percent (n=3) of all mutations are located in the signal peptide region, presumably affecting the translocation or secretion of the protein: one nonsense mutation and one frameshift variant.

Analysis of data reported worldwide and the findings of the Hungarian patients with Papillon-Lefèvre syndrome revealed 75 CTSC gene mutations: 53% are missense (n=40), 23% are nonsense (n=17) and 17% are frameshift (n=13) variants. There are two in-frame deletions, one intronic splice-site variant and one point mutation in the 5' untranslated region of the CTSC gene. Previous study reported the ratio of the homozygous and heterozygous forms in the 56

investigated patients with CTSC gene mutations is 2:1. Recurrent mutations (25% of all mutations, n=19) occurred both in homozygous and in compound heterozygous forms and were detected in geographically distant, unrelated families, suggesting mutational clustering on the CTSC gene. However, there are also examples in the literature describing that the same initial founder effect and the subsequent migration of carriers can lead to the presence of the same mutation in geographically distant and unrelated families.

The known mutations, that have been sequenced, are unequally distributed on the CTSC gene: 53% are located within exons 5–7, encoding amino acids 231 to 394 in the heavy chain region, 16% (n=12) are located within exons 1–3 encoding amino acids 25 to 134 in the exclusion domain, 12% (n=9) are located within the second half of exon 7 encoding amino acids 395 to 463 in the light chain region, 13% (n=10) are located within exon 4 and the first half of exon 5 encoding amino acids 135 to 230 in the propeptide region, 3% (n=2) are located in the 5' end of exon 1 encoding amino acids 1 to 24 in the signal peptide region and 3% (n=2) are located within untranslated regions. In addition, the majority of missense, nonsense and frameshift mutations occur in exons 5–7. These findings indicate that exons 5–7, encoding the heavy chain region of the cathepsin C protein, is the most important region for genetic screening of patients with Papillon-Lefèvre syndrome.

Papillon-Lefèvre syndrome has been reported in a diverse range of ethnic groups from all over the world. A quarter of the mutations have been reported twice or more in different ethnic groups.

In general, no strict genotype–phenotype correlations have been identified for Papillon-Lefèvre syndrome. Mutations in the CTSC gene can lead to the development of Haim-Munk syndrome or aggressive periodontitis type 1 as well as Papillon-Lefèvre syndrome. The common characteristic of these three entities is periodontal inflammation. Variable expression of the phenotype associated with the CTSC mutation may reflect the influence of other genetic and/or environmental factors.

Future efforts might provide insight into these correlations and elucidate the mechanism of the different phenotypic variants of Papillon-Lefèvre syndrome. The availability of the extended clinical findings from CTSC mutation carriers is critical for furthering both our understanding of the disease and the development of causative therapies that will be more specific and effective than the symptomatic treatments currently available for patients with Papillon-Lefèvre syndrome and its allelic variants.

SUMMARY

In this study, my aim was to investigate Christ-Siemens-Touraine syndrome, Papillon-Lefèvre syndrome and its allelic variants: Haim-Munk syndrome and aggressive periodontitis type 1. The common phenotypic feature of these rare entities is the presence of severe periodontitis. In aggressive periodontitis type 1, there is no further associated symptom, while in Papillon-Lefèvre syndrome and in Haim-Munk syndrome there are some further skin symptoms.

Before the huge advances in the development of the sequencing methods these entities based on their clinical symptoms were separated as different diseases. With decoding the human genome in the former decade a lot of disease-causing genetic variations have been discovered. These findings elucidated the genetic background of Christ-Siemens-Touraine syndrome, Papillon-Lefèvre syndrome, Haim-Munk syndrome and aggressive periodontitis type 1 and it turned out that they are the results of the mutations in the EDA1 and CTSC genes. Since the same mutation can lead to the development of different phenotypes, it was concluded that Papillon-Lefèvre syndrome, Haim-Munk syndrome and aggressive periodontitis type 1 are not different entities, but the allelic variation of the same disease.

In this study, I have investigated a Hungarian pedigree with Christ-Siemens-Touraine syndrome, two Hungarian pedigrees and two sporadic cases with Papillon-Lefèvre syndrome. Besides complete clinical care including dental and dermatological examinations and interventions, I have initiated the genetic screening and the identification of the underlying causative abnormalities on the EDA1 and CTSC gene. These investigations had great significance for the patients because with the identification of the causative abnormalities family planning can be helped with prenatal diagnostic interventions. In long term, my aim is also to raise the awareness of dentists and other physicians for rare diseases and to create an efficient multidisciplinary team to the clinical workup for these patients.

ACKNOWLEDGEMENTS

I would like to thank to Dr. Nikoletta Nagy her great supervising activity and co-operation in the treatment and supporting of patients with rare genetic diseases. I am greatly indebted to my supervisor, Professor Katalin Nagy providing me excellent working environment at the Faculty of Dentistry, University of Szeged.

Special thanks to all my colleagues for their kind help in the dental workup of the patients.

I am grateful to Dr. Nikoletta Nagy and to Dr. Zsanett Csoma for the dermatological examinations of the patients.

Special thanks to Dr. Beáta Fábos and her colleagues at the Mór Kaposi teaching Hospital (Kaposvár) for enrolling two pateints into the study.

I would like to thank to Prof. Dr. Márta Széll for the opportunity to perform the genetic investigations of this study in the molecular laboratory of the Department of Medical Genetics Institute, University of Szeged.

I am grateful to all the patients, symptomless family members and control individuals for participated in this study.

Finally, I owe a great debt of gratitude to my teacher, Professor István Gorzó. He supported me in taking the first steps in my scientific work and made me possible to become the employees of the Department of Periodonology.