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DOCTORAL THESIS ABSTRACT

BIOCHEMICAL AND HISTOLOGICAL RESEARCH — ORAL REHABILITATION METHODS AND TECHNIQUES

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THESIS OBJECTIVES AND PURPOSE

After the age of 25, the most common cause of tooth loss is gum disease, also known as **periodontal** disease or **periodontitis**. Periodontal disease is characterised by inflamed and bleeding gums, abscesses, receding gums, teeth that become longer and longer, all of which ultimately lead to tooth loss. Periodontal disease starts with poor oral hygiene, but can be easily prevented by proper brushing, flossing and interdental brushes. The disease progresses painlessly, and by the time the first abscess appears, some teeth can no longer be saved.

Periodontal disease is a chronic, progressive and complex condition that affects the tooth's supporting structures: gums, ligaments and bone. The complexity of this condition is determined by a number of characteristics both in terms of etiology and diversity of clinical forms or evolutionary and therapeutic features.

Worldwide, the World Health Organization (WHO) states that the prevalence of periodontal disease is 19%, affecting over 1 billion adults. In 2019, Simona Ioana Hategan, Angela Ruth Kamer and their collaborators have highlighted in their paper entitled "Periodontal disease in a young Romanian convenience sample: radiographic assessment" that the incidence of periodontal disease in Romania is 65.8%, a percentage 3 times higher than the international figures.

Gum disease is arguably the most common dental problem in the world, but the most common is periodontal disease, affecting up to 90% of the global population. This inflammatory condition is mainly found in adults over 30-40 years old and occurs when toxins produced by periodontal plaque bacteria accumulate, causing damage to the soft tissue of the gums, alveolar bone and periodontal ligaments. Periodontitis is a hidden, silent and very dangerous condition that needs to be detected and treated early, because without proper treatment, patients suffering from periodontitis can end up losing their natural teeth. The main causes of periodontitis are poor oral hygiene, namely the bacteria *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* which are highly pathogenic, unhealthy diet, smoking, chronic diseases, certain drug therapies, genetic predisposition and even pregnancy. It is very important to note that these bacteria not only affect the oral field, but can also cause distant diseases such as heart disease, digestive diseases etc.

During my career as a specialist periodontist I have successfully treated more than 10,000 patients for whom each time I have developed personalized therapy plans specific to their needs.

Minimally invasive dentistry is the fastest growing trend in modern dentistry. The main premise is the control of disease and then the use of minimally invasive procedures to restore the aesthetic and functional balance of the entire oral cavity.

The use of the Dental Microscope and magnification allows Superdent '92 dental clinic specialists to remove only as much of the tooth, bone or soft tissue structure as needed without damaging healthy tissue.

The use of the Surgical Dental Microscope brings a major breakthrough in modern dentistry. Nowadays, thanks to the dental microscope, we can work with maximum precision and safety obtaining aesthetic results and more predictable therapies in:

- Restorative Dentistry;
- Endodontics;
- Dental Aesthetics;
- Dental Prosthetics;
- Periodontology.

For current dentistry, one of the keys to success is the minimally invasive approach. This is only possible if the position of the teeth on the arch is appropriate and does not require soft or hard tissue adjustments. This is where the importance of an interdisciplinary (rather than multidisciplinary) team comes in. The major difference between interdisciplinary and multidisciplinary is that in an interdisciplinary approach, the therapy plan is drawn up by a group, whose members are all present, complement and support each other's efforts. In the case of a multidisciplinary approach, each of the disciplines or dental areas involved acts independently, non-unitarily.

Another important aspect of the therapeutic plan is occlusion. In order to achieve a long-lasting restoration, a perfect and balanced occlusion registration is mandatory. The next step and the objective of this presentation is to present a new concept, which involves creating a customized personal image that expresses the patient's identity. This concept helps the dentist to create restorations that are aesthetic, but at the same time manage to reflect the psychosocial traits that make up the patient's image, including emotions, sense of identity, behaviour and self-esteem. These factors, in turn, affect how the patient will be perceived by others after therapy.

To achieve such results, an interdisciplinary team is needed. Every member of the team (including specialists, technicians and the patient) must know the therapy plan from the start and anticipate the final outcome. Once this is done,

the major condition for success remains communication. The key to excellence in aesthetics is communication with the patient on the one hand, and communication with the laboratory and specialists on the other, throughout the therapy.

Given all the opportunities today, we have many digital tools at our disposal to keep communication on social and professional networks. Together with the innovations of CAD/CAM technology and combining all these digital tools, there should be no problem, whether the technician, specialist or any other person with whom the doctor collaborates is in the same clinic or in another country, as digital communication makes it easier for everyone to work together as a synergistic team.

The aim of the paper is to demonstrate that in the evolution of periodontal disease, the microbial factor is decisive and that the success of the therapy is to destroy the bacterial biofilm. It is not sufficient to use only mechanical methods to remove bacterial plaque, tartar and granulation tissue, but also adjuvant methods. The most important thing is deposits removal from the periodontal pocket, 90% of which is caused by bacteria. Anaerobic bacteria are also present in people with healthy periodontium, but only people with favourable conditions develop BP. Non-surgical therapies are important because they allow us to reduce the pocket by 60-70%, improve the quality of the gum tissue, and those that do not reduce, can undergo surgical therapy if the condition of the gums allows, pockets up to 5-6 mm deep. The advantage is favourable and it is why you have to perform the periodontal therapy first before any other operation.

In early cases a quick cure can be achieved. The aim is to make patients aware of the presence of this condition and its seriousness and consequences.

In addition to mechanical therapy, it is necessary to use melatonin, vitamin D and patient hygiene.

The main purpose of these studies is to highlight the importance of non-surgical methods of treatment of periodontal disease and to demonstrate that the promoting factors are co-dependent on the determining ones. In 80% of the patients suffering from periodontal disease, it has been shown that the evolution of the disease is not only determined by the microbial factor, but is also directly influenced by different types of deficiencies in the body, by the presence of a enabling environment for the development of gram-negative (genetic) bacterial flora, as well as by the patient's willingness to attend regular check-ups and to maintain oral hygiene at home. One of the adjuvant methods of SRP treatment is the local application of autologous plasma injections, the aim being to:

- Identify growth factors

- Understand mechanism of action

- Possibilities of using them to improve wound healing.

The transition from one stage to another was marked by the discovery that the influence of oxygen on macrophages and tissue oxygenation in general is directly mediated by angiogenesis and other growth factors. The use of platelet plasma is one of the few valid methods of improvement due to tissue regeneration, growth factors (GF).

Tissue regeneration using growth factors is a revolutionary method in all medical fields but it is still new, there is much more to discover in this method. The exact mechanism of action of the different factors is not yet fully understood.

Another aim is to investigate the anti-inflammatory and antioxidant properties of melatonin supplements by measuring the clinical status of selected periodontitis patients, as well as improvement of inflammatory and antioxidant parameters.

Another aim is to address the controversy around the relationship between periodontal disease and changes in periodontal tissues and to assess the type of changes in periodontal disease using nutrients by measuring the clinical status of selected patients affected by periodontitis.

This paper highlights the interaction of *Porphyromonas gingivalis* with other bacteria in determining periodontal disease and valid treatments (PRP, systemic and local antibiotics, antiseptic solutions and antimicrobial plant derivatives). In our research we proposed biochemical and histological study on the use of new therapeutic methods in some oral and facial pathologies. Thus, in our previous work (Acatrinei et al., 2019) we aimed to demonstrate that tissue regeneration using growth factors is a revolutionary method in all medical fields: skin attachments, juvenile/vulnerable acne, alopecia, androgenetic alopecia, dentistry, traumatology, rehabilitation medicine, gynecology. At the basis of the use of platelets themselves is the discovery that platelets contain protein factors (PRP FACTORS) that initiate cell regeneration processes.

Thus, we tried to demonstrate the regenerative capacity of the organism under the influence of tissue growth factors found in autologous plasma. Although the results are also encouraging in patients undergoing only SRP and laser therapy, in the long term it was observed that those who also received injectable autologous plasma sessions had more stable results over time and a lower risk of relapse.

The experimental work carried out in these studies was mainly aimed at highlighting the correlations between different factors favoring periodontal disease and the evolution of this disease over time.

In the context of this approach, we proposed customized experiments such as:

- **Biochemical and histological research on the use of new therapeutic methods in some oral and facial pathologies**

The main purpose of these studies is to highlight the importance of non-surgical methods of treatment in periodontal disease correlated with and demonstrate that the promoting factors are co-dependent on the determining ones. In 80% of the patients suffering from periodontal disease it has been shown that the evolution of the disease is not only determined by the microbial factor, but is also directly influenced by different types of deficiencies in the body, by the presence of an enabling environment for the development of gram-negative (genetic) bacterial flora, as well as by the patient's willingness to attend regular check-ups and to maintain oral hygiene at home. One of the adjuvant methods of SRP treatment is the local application of autologous plasma injections, the aim being to:

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- **Antioxidant and anti-inflammatory properties of melatonin and micronutrients in patients with periodontal disease**

Another aimis to investigate the anti-inflammatory and antioxidant properties of melatonin supplements by measuring the clinical status of selected periodontitis patients, as well as improvement of inflammatory and antioxidant parameters.

- **The action of micronutrient and bioactive compound treatments in halting the progression of periodontal disease**

Another aim is to address the controversy surrounding the relationship between periodontal disease and changes in periodontal tissues and to assess the type of changes in periodontal disease with nutrients by measuring the clinical status of selected patients affected by periodontitis.

- ***Porphyromonas gingivalis* bacteria interaction with other bacteria in diagnosis of periodontal disease and valid treatments**

The aim of the paper is to demonstrate that in the evolution of periodontal disease, the microbial factor is decisive and that the success of the treatment is to destroy the bacterial biofilm. It is not sufficient to use only mechanical methods to remove bacterial plaque, tartar and granulation tissue, but also adjuvant methods. The most important is deposits removal from the periodontal pulp, 90% of which is caused by bacteria. Anaerobic bacteria are also present in people with healthy periodontium, but only people with favourable conditions develop BP. Non-surgical treatments are important because they allow us to reduce the pockets by 60-70%, improve the quality of the gum tissue, and those that do not reduce, can undergo surgical treatment if the condition of the gums allows, pockets up to 5-6 mm deep. The advantage is favourable and it is why you have to perform the periodontal treatment first before any other operation.

In early cases a quick cure can be achieved. The aim is to make patients aware of the presence of this condition and its seriousness and consequences.

- **Comparative research in the non-surgical treatment of periodontal disease: SRP, laser therapy *vs.* plasma**

Thus, we tried to demonstrate the regenerative capacity of the organism under the influence of tissue growth factors found in autologous plasma. Although the results are also encouraging in patients undergoing only SRP and laser therapy, on the long term it was observed that those who also received injectable autologous plasma sessions had more stable results over time and a lower risk of relapse.

The experimental work carried out in the framework of this paper was mainly aimed at highlighting the correlations between different factors favoring periodontal disease and the evolution of this disease over time.

PART II. PERSONAL CONTRIBUTIONS

INTRODUCTION

Biochemical and histological research on the use of new therapeutic methods in some oral and facial pathologies and tissue regeneration therapies.

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Tissue regeneration using growth factors is a revolutionary method in all medical fields but it is still new, there is much more to discover in this method. The exact mechanism of action of the different factors is not yet fully understood.

Antioxidant and anti-inflammatory properties of melatonin and micronutrients in patients with periodontal disease

Another aim is to investigate the anti-inflammatory and antioxidant properties of melatonin supplements by measuring the clinical status of selected periodontitis patients, as well as improvement of inflammatory and antioxidant parameters. The study was conducted on 9 patients (4 men and 5 women) with gingivitis and moderate periodontitis, resistant to treatment. Age ranges between 30 and 70 years old. In this study biochemical investigations were performed, i.e. nutrigenetic test was performed in "Synevo" Medical Analysis Laboratory. A simple saliva sample was collected, after which a report was generated by the laboratory, which included information about the patients deficiencies.

The action of micronutrient and bioactive compound treatments in halting the progression of periodontal disease

The aim of the research is to address the controversy around the relationship between periodontal disease and changes in periodontal tissues and to assess the type of changes in nutrient-mediated periodontal disease by measuring the clinical status of selected patients affected by periodontitis. In total, there were 9 patients (4 males and 5 females) with moderate/severe chronic periodontitis aged 36-71 years old. The patients underwent laboratory tests of biochemistry, bacteriology and were performed nutrigenetic test for vitamins A, B1, B2, B3, B6, B9, B12, C, D, E, copper, zinc, selenium, calcium in "Synevo" Medical Analysis Center.

Porphyromonas gingivalis bacteria interaction with other bacteria in diagnosis of periodontal disease and valid therapies

The study was conducted on a total of 6 patients (2 men and 4 women) with moderate/severe chronic periodontitis, aged 27-51 years old. They were subjected to biochemical and biological bacteriological laboratory tests in the Military Hospital Constanta. This report highlights *Porphyromonas gingivalis* interaction with other bacteria in diagnosis of periodontal disease and valid treatments (PRP, systemic and local antibiotics, antiseptic solutions and antimicrobial plant derivatives). In our research we proposed biochemical and histological study on the use of new therapeutic methods in some oral and facial pathologies. Thus, in our previous work (Acatrinei et al., 2019), we aimed to demonstrate that tissue regeneration using growth factors is a revolutionary method in all medical fields: skin attachments, juvenile/vulnerable acne, alopecia, androgenetic alopecia, dentistry, traumatology, rehabilitation medicine, gynecology. At the basis of the use of platelets themselves is the discovery that platelets contain protein factors (PRP FACTORS) that initiate cell regeneration processes.

Comparative research in the non-surgical treatment of periodontal disease: SRP, laser therapy *vs.* plasma

The study involved 35 patients, 19 men and 16 women. The method of obtaining the results is done by a multiplex polymerase chain reaction (PCR) with colorimetric detection which is called Micro-Dent A Test in "Synevo" Medical Analysis Center. It enables to establish the five important periodontitis markers: *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedio, Bocteroides forsythus* and *Treponema denticola*. In some cases of periodontitis, the diagnostic spectrum can be extended by determining 6 other germs *Peptostreptococcus micros, Fusobacterium nucleatum/periodonticum, Eikenella corrodens, Campylobacter rectus, Eubacterium nodatum and Capnocytophago spp* (micro-IDent B test). Thus, we tried to demonstrate the regenerative capacity of the organism under the influence of tissue growth factors found in autologous plasma. Although the results are also encouraging in patients undergoing only SRP and laser therapy, in the long term it was observed that those who also received injectable autologous plasma sessions had more stable results over time and a lower risk of relapse.

The experimental work carried out in these studies was mainly aimed at highlighting the correlations between different factors favouring periodontal disease and the evolution of this disease over time.

CHAPTER 6 MATERIAL AND METHODS, TECHNIQUES, EQUIPMENT, THERAPIES

The experiments were carried out in the SuperDent 92 Clinic, equipped with the latest equipment and products for oral rehabilitation and periodontal pathology treatments. In the SuperDent 92 Clinic, about 2700 patients were treated.

The personal experiments described in the PhD thesis were conducted on 59 patients.

Microbiological and histological determinations were carried out at the "Synevo" Medical Analysis Centre and the "Provita 2000 Medical Centre" Clinic.

Biochemical determinations were performed at the Laboratory of Analysis of the Emergency Clinical County Hospital "Sf. Andrei" in Constanta.

Materials and Methods, Equipment, Techniques

- Paraclinical radiological investigations

Rayscan ALPHAPLUS 160 is a highly efficient sensor technology requiring much lower radiation for investigations, which offers much greater patient safety.

All radiological investigations necessary to confirm the clinical diagnosis were performed in the Clinic. Paraclinical investigations such as panoramic radiography and computed tomography were performed. With their help, there is a possibility of confirming the diagnosis of periodontal disease. Radiographs show bone defects, widening of the lamina dura, the presence of infectious intraosseous granuloma/cyst formations or lateral periodontal abscesses.

CT - Computer tomography - A computed tomography (CT) scanner is a machine used to produce images using X-rays. These images are used to establish the diagnosis that leads to the most effective treatment. Compared to orthopantomography, we can see all the structures concerned in 3D format. The images obtained can be viewed in real time in electronic format.

OPG - orthopantomogram - is a panoramic radiological representation of the oral cavity. It gives the doctor a 2D overview of the patient's clinical situation. The OPG includes both dental arches and shows quite precisely the relationship of the teeth to the neighbouring anatomical formations: maxillary sinuses, mandibular canals. It is usually performed at the beginning of treatment and is very important for establishing a correct general diagnosis. Often, retroalveolar radiographs are needed, which are smaller in size but provide more detailed information.

- DOCTOR SMILE Laser

One of the most effective devices in dental technology.

The DoctorSmile laser is a diode laser with very good results in dental practice. It has multiple effects on tissues: decontaminating, biostimulating, photo-ablative, surgical, haemostatic with the following applications:

- periodontology: decontamination of periodontal pockets

- surgery: frenectomies, gingival eviction, haemostatics

- cosmetic dentistry, ablation, gingivoplasty, hemangioma treatment

- therapeutic: desensitization, biostimulation, herpes simplex, thrush

- endodontic: pulp capping, decontamination

It operates at the following parameters: wavelength 810-980 nanometres and a power of 5 Watts, and it is classified as medical class IIB. For the decontamination function, the fibre tip is inserted into the periodontal pocket in contact with the mucosal wall and 20 vertical movements are performed so as to minimise the amount of necrotic tissue. Avoid contact with the root surface of the teeth to avoid thermal necrosis of the pulp tissue. For a superior antimicrobial effect, it is recommended to irrigate the working surfaces with 3% hydrogen peroxide aqueous solution or 0.12% chlorhexidine gluconate.

Advantages: minimally-invasive treatment, fast post-operative recovery, no post-operative bleeding or swelling.

Hydrogen peroxide: Hydrogen peroxide has antibacterial properties and it is most often used in wound disinfection. Hydrogen peroxide not only removes stains from teeth, but also prevents stains and bacteria.

Chlorhexidine: chlorhexidine gluconate 0.12% is the most effective substance against dental bacteria. The antiseptic inhibits the multiplication of bacteria in the mouth and destroys them, fighting effectively against dental plaque.

Drugs with specific action on bacteria involved in periodontitis

- The following drugs were used on each of the two groups of patients:
- Parodium gel, gingival gel with rhubarb extracts with anti-inflammatory action
- Hyben-X-substance with decontaminating role on oral tissues. It is applied to the lesions for 20 seconds, then washed with plenty of water.

Metronidazole

- Metronidazole 10mg/0.5mg/g, gingival gel with the active substance metronidazole as metronidazole benzoate 10 mg and chlorhexidine digluconate solution 20%, 0.5 mg/1g gel

It is a compound that combines metronidazole antibacterial and antiprotozoal agents chlorhexidine antibacterial, antifungal and antiviral agents.

Metronidazole has a bactericidal effect on protozoa and anaerobic bacteria that cause periodontal disease.

Therapeutic indications:

Infectious-inflammatory diseases of the periodontium and mucosa of the oral cavity, including:

- acute and chronic gingivitis;

- Vensan acute ulcero-necrotic gingivitis;

- acute and chronic periodontitis;

- juvenile periodontitis;

- periodontitis complicated with gingivitis;

- aphthous stomatitis;

- baldness;

- inflammation of the oral cavity lining in denture wearers;

- post-extraction alveolitis (inflammation of the alveolus after tooth extraction);

- periodontitis, periodontal abscess (in complex therapy).

COE-PACK periodontal cement

COE-PACK periodontal cement

This cement is a bicomponent material used for covering post-operative suture wounds and for the immobilization of periodontal teeth. It has various features and benefits including: availability in cartridges or hand-mixed versions; speeds up the wound healing process by preventing food debris from penetrating; acts as a conformer. It is easy to shape with the help of a liquid substance that facilitates shaping.

- **Perioflow teeth hygiene device**

This device is very powerful and extremely useful in the prophylactic and curative treatment of gum disease because it allows clinical detection of bacterial plaque deposits using a plaque indicator. This indicator can be in various forms: pellets, chewable tablets, liquid, etc. The one used in the study is marketed by EMS and is distributed in the form of blue pellets. On contact with plaque, it is coloured in pink (recent plaque) or blue (old plaque).

- **Dental scaler**

For scaling, the EMS No Pain system loops are attached to the ultrasonic piece of the Perioflow unit. The set consists of 10 thin and flexible tips able to adapt to the topography of periodontal or peri-implant pockets.

The PS Piezon No Pain tool is thin and smooth like a probe. It is also minimally invasive, maximally preventative and gives the doctor an excellent tactile sensation when working subgingivally. The biggest advantage of its use is that it preserves the epithelium due to its absolute linear movements and painless as long as the recommended protocol is complied with.

- **Periodontal Bacteria Test Kit**

The use of this test allows microbiological analysis of bacteria present in the patient's oral cavity. For collection, a paper cone from the packet is inserted into each representative area or areas with deep periodontal pockets.

The micro-Dent A test makes it possible to determine the five important periodontitis markers *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedio, Bocteroides forsythus* and *Treponema denticola*. In some cases of periodontitis, the diagnostic spectrum can be extended by testing for 6 other germs *Peptostreptococcus micros Fusobacterium nucleatum/periodonticum. Eikenella corrodens, Campylobacter rectus, Eubacterium nodatum* and Capnocytophago spp (micro-IDent B test).

As a conclusion, micro-IDent tests are useful for:

- risk areas identification and relapses early recognition;

- choosing the appropriate antibiotic and documenting therapeutic success,

- risk of implant failure assessment before treatment.

Moreover, knowledge of the microbiological situation allows early therapy to prevent disease progression.

Sampling is done by the dentist using the paper cones contained in the collection kit, necessarily before antibiotic treatment. A sterile forceps is used to grasp a sterile cone in the periodontal pouch up to its base (the depth of the periodontal pouch must be at least 4 mm). The cone remains in place for 10 seconds, after which it is removed and inserted into a transfer tube. A maximum of 4 samples can be taken from different periodontal bags. All cones from the same patient will be placed in one transfer tube. The tube is placed in the blue collection kit together with the patient data sheet.

The method of obtaining the results is a multiplex polymerase chain reaction (PCR) with colorimetric detection.

- **Centrifuge**

The centrifuge is a device that can be used to obtain various blood preparations, such as autologous plasma, autologous membrane, etc.

The steps to be able to use the centrifuge for the purpose of obtaining various products for use in the dental field are as follows:

- Fit the tourniquet to the hand of interest, disinfect the elbow crease

- Fit the needle to the vacutainer and insert it into the vein

- Fit the vial and collect the desired amount of blood

- Spin at the following parameters: 1200 revolutions 14 minutes

The resulting plasma is injected into the gingiva at the level of the mobile mucosa and into the periodontal pockets.

- **SIRONA CEREC CAD-CAM Scanner**

The PRIMESCAN CAD/CAM system is a new technology developed to facilitate the design, modelling and manufacturing processes of various computer-aided digital projects. In dentistry, this system makes it easy to design dental prosthesis and print them in zirconium, metal, acrylic or ceramic cubes. One of its advantages is the scanner's intra-oral camera which records the patient's prosthetic field with very high fidelity, making it a much more comfortable scanning process for the patient than conventional methods of imprinting with conventional materials. Its use also guarantees a very good result of the future dental prosthesis, as it minimizes the risks and failures that can occur due to inaccurate calculation and design in the dental laboratory.

CAD, short for Computer-aided Design, is the 3D design of future work designed on a computer. CAM, short for Computer-aided Manufacture, is the computer-aided manufacture of the dental prosthesis based on the three-dimensional model.

Indications of CAD/CAM technology

– structures for different types of prostheses;

– fixed crowns and bridges made of ceramic, zirconium dioxide, metal, acrylate;

– inlays;

– specially shaped posts for a more reliable and ergonomic connection of the implant to a crown or denture;

– veneers for aesthetic restoration.

The technology extends the range of materials used in prosthetics and allows the inclusion of:

– special types of ceramics developed according to the requirements of biocompatibility, strength, density, optical properties, similar to those of tooth enamel;

– polymer composite materials;

– metals (including titanium, cobalt-chromium);

– special composites etc.

Benefits of CAD/CAM technology

High dimensional accuracy. Error when using CAD/CAM technology does not exceed 25 microns. This allows for maximum ergonomics of dentures, bridges, crowns, dental implant abutments, inlays, etc. It also allows accurate prediction of dental occlusion and the distribution of forces developing in the bite, and complete reproduction of the anatomical shape of the tooth when making a crown or veneer.

There are no restrictions on design complexity, including at the production stage. The technology makes it possible to manufacture denture frameworks that precisely take into account the patient's jaw structure or create dental prosthesis with an optimal shape and design for transferring the masticatory load.

Treatment time is reduced. Design and manufacture are faster and more accurate. The fabrication of prosthetic structures in a dental laboratory can take only 2-3 days, thanks to 3D CADCAM technology, veneers, inlays, onlays, ceramic prosthetics can be fabricated in the dental office in only 45 minutes.

Results accurate forecasting. Highly accurate design of dental prosthesis allows for the results - both aesthetic and biomechanical properties - to be assessed in advance. Finite element analysis allows changes to be made in the design of dentures, inlays, prosthetic abutments for dental implants, calculation of their characteristics in advance, etc. After completion of the CAD stage, the patient can assess how the future teeth will look even before they are produced.

Rapid adaptation of the patient with the new prosthetic work. This is due to the high accuracy of the computer model. The prosthesis reproduces the destroyed or lost tooth so accurately that the patient cannot feel it.

Cerec CAD CAM technology is a forward-looking technology, it allows aesthetic dental prosthesis execution, but also in terms of the very short time needed to perform a restoration. The CAD CAM system consists of a scanner, a computer with 3D software and a milling unit that transforms the processed data into a finished product.

Steps:

1. Tooth preparation according to the desired operation

2. Optical impression - three-dimensional photographs of the prepared tooth

3. The milling unit uses diamond tools that mills a block of the chosen material with micron precision in a very short time according to the information received from the computer.

4. Fixing - the kits offer the possibility of glazing and pigmenting the object so that the final look is as natural as possible.

Working method:

1. Register the patient in the scanner database.

2. Choose the teeth, material and type of dental prosthesis to be done.

3. The actual patient case is created.

4. The patient is seated in a supine position so that the patient maintains the same position throughout the scan.

5. The dental vaults are scanned by recording the 3D image with the scanner and any dental and gum details.

6. After scanning the two vaults, the inter-maxillary relationship is recorded.

7. Create the digital models resulting from the scanning.

8. Process the final 3D image and export it in STL format and/or in the office, transfer the project to the CAD/CAM milling unit and execute the actual work.

Aesthetics.

Possibility to use different materials.

- **Nikon camera**

Due to the fact that the field of dentistry has evolved a lot, it is necessary that the patient's portfolio is also improved with up-to-date techniques for archiving the clinical situation. The camera allows us to record the patient's dental situation in an up-to-date manner that does not change its characteristics over time, as they are saved on a digital medium. For each patient a complete set of intraoral frontal and lateral normal photos is taken with the help of the retractors, mirrors and contrast and extraoral portrait photos taken in the studio.

- **ZEBRIS**

The Zebris system is easy to use, as a digital facebow, but also for analysis, measurement and laboratory transfer of jaw movements. The high-precision JMA (Jaw Motion Analyzer) optical system is useful for any prosthetic treatment, from single restorations to complex oral rehabilitations or guards, using a full digital protocol, with intraoral scans, or a mixed protocol, with impressions and classic articulator-mounted models.

Data obtained can be automatically transferred into Exocad or Ceramill Mind CAD software either by individual programming of the virtual articulator or by importing the patient's real movement for the full digital protocol. Data can also be used for model transfer and individual programming of the Artex physical articulator, i.e. condylar slopes (SCI), Bennett angles or incisal mass inclination. The system represents the missing link in the digital protocol, simplifies treatment, reduces subsequent manual adjustment of restorations and ultimately increases daily profitability.

It has the following characteristics:

— Accurate mandible-to-maxilla ratio registration for any prosthetic indication - from single restorations to complex oral rehabilitations

— Simple and intuitive predictive working protocol that can be profitably integrated into everyday practice

— Accurate digitization of functional information allows for accurate restorations, reduces post-fitting time and streamlines communication with the dental laboratory

FACIAL ARCH - AMANN GIRRBACH - Artex

The facebow is a device that allows precise recording of relative position of maxillary ridge and teeth on the patient and makes it possible to transfer it to the articulator, still obtaining a relative position, which facilitates the interpretation, in general, of mandible-maxillary ratios.

So, with the help of facial arches, a relative position of the models in the articulator is established, which is as close as possible, if not identical to the existing ratios between the maxilla and mandible. This common framework gives us the possibility to obtain restorations that are in perfect harmony with the other TMJ structures.

Instruments

- **Periodontal probe**

The CPITN (Community Periodontal Index of Treatment Need) probe was designed by Professors George S. Beagrie and Jukka Ainamo in 1978 and it is a first generation probe. It is recommended for use in screening and monitoring patients using the CPITN index. The index and related probes were first described in the "Epidemiology, etiology and prevention of periodontal diseases" report by the World Health Organisation which recommended that manufacturers separate the instruments as follows: CPITN-E (epidemiological), those with 3.5 and 5.5 mm markings; CPITN-C (clinical), those with 3.5; 5.5; 8.5 and 11.5 mm markings. CPITN probes have a thin handle, they are lightweight (5g), the tip is 0.5mm in diameter, and have a black band between the 3.5 and 5.5 mm markings. They are very useful in dental practice, being used to measure the depth of periodontal pockets, gingival retraction, etc.

- **Consultation kit**

It is indispensable in any dental operation, consisting of a mirror, probe, brush and oral spatula. Each of these aids the clinical examination by inspection, palpation and percussion, examinations through which the initial diagnosis is established.

- **Gracey curettes**

Gracey curettes are a category of instruments dedicated mainly to those working in the periodontal field. They are indicated in supragingival and subgingival scaling situations, both by surgical (open) and non-surgical (closed) approach.

There are universal and area specific curettes. The latter category includes both Gracey and Vision curettes, modifications of the Gracey design (after five, mini five) and the Langer and Rigida curette furcation series.

The reason why Gracey curettes are so widely used is due to their shape with two sharp, curved edges that adapt perfectly to different tooth surfaces and act simultaneously on both the root surface and the gingival wall from which they remove necrotic tissue.

Gracey no. 1/2, 3/4 and 5/6 are used for scaling all teeth in the anterior sextants. Pairs 7/8 and 9/10 are used for the buccal and oral/lungual sides of the teeth in the posterior sextants. 11/12 and 15/16 for the mesial faces of posterior teeth, and 13/14 and 17/18 for the distal faces of the same teeth.

Supplementary means of oral hygiene

- Dental floss

The best way to protect against gum disease is to remove plaque. In addition to traditional brushing techniques, it is recommended to use additional means such as floss and mouthwash. Flossing comes in different forms: tube, bracket, etc. and it is the main way to remove plaque from the interdental spaces, thus reducing the risk of periodontal inflammation and cavities.

It is recommended for use after the age of 12, once a day before brushing.

- Mouthwash

Another adjuvant method of maintaining oral health is the use of mouthwash. In periodontal diseases it is recommended that this product also contains an antimicrobial component such as chlorhexidine gluconate 0.12%. This can be used twice a day for a limited period of time (maximum two weeks) with the disadvantage that it causes a yellow staining of the mucous membrane and teeth. Its effect is achieved by mechanically removing the remaining food debris following brushing and flossing.

- Interdental brushes

Due to their size, they help to significantly reduce interdental cavities by cleaning these surfaces of food debris, sugars and thus reducing local acidity. They also produce increased breath quality and reduced halitosis, often caused by such spaces not being properly cleaned. By using them, patients will acquire better overall control over the hygiene of the oral cavity and places that are difficult to reach with a traditional toothbrush or even flossing.

- Melatonin, Vitamin D3

Researchers studied the relationship between salivary melatonin levels and the Current Periodontal Index (CPI), a score used for periodontal disease status. Salivary melatonin plays an important role in maintaining periodontal health, according to a new study published in the Journal of Periodontology. This is one of the first attempts to examine the influence of salivary melatonin on periodontal disease, inflammation that destroys both the tissues and bones that support

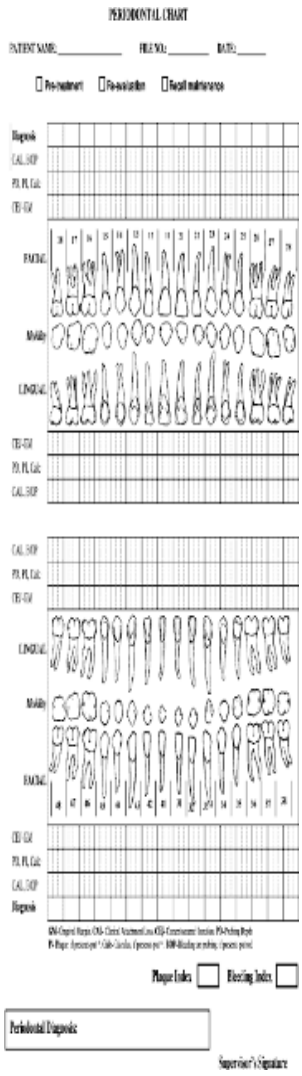
teeth. Researchers have found that melatonin, a hormone secreted by the pituitary gland, has the ability to protect the oral cavity against free radicals produced by inflammatory diseases (Acatrinei et al., 2021, Dietrich et al., 2004).

Melatonin has powerful antioxidant effects that can protect cells from the action of inflammatory processes and destructive oxidative effects. Supplements containing melatonin are known for their sleep accelerating effects and airplane sickness inhibition. "Patients with high salivary melatonin levels have a low Community Periodontal Treatment Need Index (CPI). CPI is the score used to assess periodontal status," says Pablo Galindo, from the Department of Oral Surgery, Faculty of Dentistry, University of Granada, Spain. "This finding suggests that melatonin may help the body in the fight against infection and inflammation, making this possible due to its antioxidant, immune boosting and age prevention effects."

A study found that lower serum levels of Vitamin 1.25-(OH)2-D3 are associated with greater attachment loss, which can be explained by the anti-inflammatory effects of vitamin D_3. Krall (Krall et al, 2021, 2021) conducted two studies. One showed no association between vitamin D intake from food and supplements and the number of teeth with progressive periodontal bone loss. The second stated that although the number of studies on the effects of calcium and vitamin D intake on oral outcomes is limited, they suggest that high intake levels are associated with reduced prevalence of clinical attachment loss and lower risk of tooth loss. Data from a prospective oral hygiene study in men demonstrated a similar association between increased calcium intake and reduced alveolar bone loss (Miley et al., 2009, Jabbar et al. 2011, Hildebolt, 2005)

Means of periodontal immobilization

- Metal bar retention is one of the periodontal immobilisation methods used by dentists to reduce the mobility index of affected teeth. A special retainer bar is used, which is adhered by a fluid composite adhesive technique to the supracingular level of the affected teeth.
- Containment by prosthetic dental bridgework that includes both movable teeth and immovable teeth that support them.



Periodontal chart

The periodontal chart allows recording of tooth mobility, furcation detection, gingival margin retraction (mm), depth of periodontal pockets on probing (mm). The level of epithelial attachment (point of maximum periodontal pocket depth) is marked in blue and the level of the free gingival margin in red. The depth of the periodontal pocket is derived from the difference between the two.

The following symbols are used to indicate the degree of furcation:

— uncoloured circle — grade I

— semicircle- — grade II

— coloured circle — grade III

CHAPTER 7 OWN EXPERIMENTS

7.1. Biochemical and histological research on the use of new therapeutic methods in some oral and facial pathologies

(Acatrinei D. et al., 2019)

Introduction

The first reports of self-blood injection in the treatment of wounds were made in 1876 (Schede, Germany).

The founders of the method of injecting one's own blood into the body are considered to be Graffstrom and Elfstrom.

In 1898, in the United States, they used autologous blood injections in saline for the first time in the world to treat pneumonia and tuberculosis.

In 1905, August Bier, a German surgeon, used autologous blood infiltration injections to stimulate healing of fractured bones. The German surgeon's most important observation was that the healing rate of various pathologies increased by 30% compared to conventionally performed treatments. The same doctor injected autologous blood in thighs to boost immunity.

In 1934, Russian surgeon V.F VOINO YASENETSKY publishes the paper "ESSAY ON SEPTIC SURGERY" in which he described the methods of autologous blood therapy in the form of infiltration of blood into the foci of inflammatory processes of soft tissues.

The Russian surgeon evaluated the therapeutic effect of autologous blood injections (e.g. treatment of boils) as positive. Due to positive side effects and minimal side effects, autohemotherapy and autoserotherapy were used as adjuvant methods of treatment until the beginning of the antibiotic era.

The next stage in the development of injection methods based on the patient's own blood became the administration of platelet-rich blood, but not red blood cells. The basis for the use of platelet-derived plasma was the discovery that platelets contain protein factors (PRP FACTORS) that initiate cell regeneration processes.

In late 1980, a group of researchers led by R.E. Marx were among the first to start using plasma in gel form (Marx, 1994, Marx et Carlos, 1998, Marx et Garg, 1998).

Autologous plasma gel technology has been proposed for use in dentistry (Harvest Company). Thrombocyte plasma in gel form has been used for the correction of lower jaw defects after tumour resection (5cm), but also after extractions in the alveoli.

The group led by Marx conducted a study. The study was divided into two groups of patients, the first group was given autologous plasma gel which was mixed with autologous bone, and the control group was given only autologous bone. The study demonstrated faster and harder bone formation in patients with autologous bone. Also in this study, it was shown that receptors that bind to continuous growth factors in platelets exist in autologous bone.

Other research has shown that bone and epithelial regeneration occurred more rapidly in the alveoli of extracted teeth in which thrombocyte plasma gel was introduced (Akhmerov, 2014, Anita, 1999, Forum et al., 2002).

A new milestone in the development of regenerative medicine was the creation of autologous injectable platelet plasma developed in 2003 by Russian scientists Renat Rashitovich Akhmerov, PhD, and Roman F Zarudi. This method has been called 'PLASMOLIFTING' (2003).

The main advantage of the autologous platelet-rich plasma form of injection is the convenience of use, not only in surgical but also in therapeutic practice.

It is important to note that this technology represented by "PLASMOLIFTING" does not require the use of calcium chloride and a double centrifugation, maintaining its efficiency.

1. Tissue regeneration is one of the most up-to-date methods of contemporary medicine. In the 1980s of the 20th century, in order to stimulate regeneration processes, the main attention was paid to tissue oxygenation. This is a fundamental factor in improving the phagocytic and bactericidal capacity of immune cells, contributing to protein and collagen synthesis.

2. The main purpose of research into regeneration processes is:

- Identifying growth factors
- Understanding the mechanism of action
- Possibilities of using them to improve wound healing.

The transition from one stage to another was marked by the discovery that the influence of oxygen on macrophages and tissue oxygenation in general is directly mediated by angioneogenesis and other growth factors. The use of platelet plasma is one of the few valid methods of improvement due to tissue regeneration, growth factors (GF).

2. Obtaining autologous plasma involves separating platelet plasma from red blood cells. Thrombocyte plasma is neither toxic nor immunoreactive, accelerating natural regeneration processes due to the presence of growth factors. Autologous platelet plasma modulates and regulates the function of primary, secondary and tertiary growth factors,

influencing all stages of regeneration simultaneously.

Platelets contain the following growth factors (GF):

-I.G.F (insulin growth factor)

-P.D.G.F. (Platelet-Derived Growth Factor)

-E.G.F. (epidermal growth factor)

-F.G.F. (Fibroblast Growth Factor)

-T.G.F. (Transforming Growth Factor)

-P.D.E.G. F (platelet-derived epidermal growth factor)

-P.L.G.F. (platelet growth factor)

P.D.G.F. - activates osteogenic mesenchymal cell proliferation and migration and induces angiogenesis.

I.G.F. - insulin factor stimulates the differentiation of young cells, increases bone tissue formation and collagen synthesis.

Growth factors are introduced into tissues using plasma as the injectable form and the increased concentration is achieved by injecting a large volume of plasma. This stimulates the formation of fibroblasts (connective tissue cells) and, the fibroblasts also produce collagen, hyaluronic acid and elastin, producing young connective tissue and capillary blood vessels.

- Growth factors block the activity of osteoclasts, stimulate the activity of osteoblasts, stopping the degeneration processes of bone tissue and contributing to its regeneration.

3. The advantage of the method is to increase the volume of platelets in the tissue by injecting a large amount of plasma. (In practice this means injecting 1-2 ml). This accelerates the regeneration process. This method is widespread in various medical fields nowadays, it is mainly used for the treatment of chronic inflammatory pathologies: chronic acne, localized and generalized periodontitis, gingivitis, periimplantitis, endocervicitis.

It accelerates the regeneration process of connective, epithelial and cartilaginous tissues.

It also stimulates the processes of collagenogenesis, angiogenesis and osteogenesis.

It activates metabolism in tissues and normalizes tissue respiration.

It alsoboosts immunity.

4. Equipment

- Centrifuges (centrifugal force 3200 rpm)

- Sterile, pyrogen free, medical glass vacuum 9 ml tubes, coated inside, top and middle with a microdispersion of sodium heparin and a separating gel at the bottom (thixotropic).

- Peripheral venous catheters of at least 1.1 mm
- 2-3 ml sterile luer lock syringes
- Needles for injections

PRP method:

The tubes are arranged in the centrifuge, the centrifuge is set according to the manufacturer's instructions. 3500 rpm - 5min

During centrifugation, the blood is separated into two main compounds:

1. Leukocyte concentrate, erythrocyte
2. Platelet-rich plasma

Using a 2-3 ml syringe, collect platelet-rich plasma that is at the top of the tube above the separation gel

Directions

1. Skin and cellular tissue conditions
2. Senile atrophy, skin thinning
3. Dermatology
4. Skin disorders: juvenile/vulgar acne, alopecia, androgenic alopecia
5. Dentistry
6. Traumatology, rehabilitation medicine
7. Gynaecology

Contraindications

1. Malignant neo-formation
2. Systemic blood diseases
3. Mental illness
4. Allergic reactions to anticoagulant (heparin)

Method of usage

In Senile Atrophy (withered skin)
Skin cleansing with water-based antiseptics
Injection site: subcutaneous
Quantity: 0-4 ml
Surface: 2 cm
Duration: 30-40 min
Treatment consists of 8 sessions, from 1 to 7 days

Fig 12. Platelet-rich platelet injections to the face and neck

2. Juvenile acne
Skin cleansing with water-based antiseptics
Location: Intradermal
Quantity: 0.1ml
Surface: 1 cm
Treatment consists of 4-7 sessions every 7-14 days

3. Alopecia
Skin cleansing with water-based antiseptics
Location: In the scalp
Quantity: 1 ml
Surface: 1 cm
Treatment consists of 2-10 sessions every 7 days

4. In dentistry

PRP method as injections makes it possible to stimulate self-renewal processes. The method can be combined with any other treatment techniques. Simple technique that excludes allergic reactions, which does not produce dependence, performed in the necessary areas (Oproiu. 2017).

Directions:

Gingivitis. Periodontal disease prophylaxis. Localized marginal periodontitis. Localized marginal periodontitis.
Apical periodontitis. Peri-implantitis extractions.
Insertion of endoscopic implants.

Procedure for use:

In periodontal disease

A volume of 0.2 ml per 2-3 mm is introduced into the gingival sulcus.
A volume of 0.3-0.5 ml per 1-2 teeth is introduced into the mobile mucosa
Period: 4 sessions with a break between 7-8 days in the same place (gingival groove, mobile mucosa, interpapillary, in the periodontal pocket, after performing all necessary dental procedures: hygiene, occlusal rebalancing, restoration of overflow seals, closed), field closure/open, guided tissue regeneration techniques.

Autologous plasma injections are the final regenerative stage of periodontal treatment.

In alveolitis

Alveolar curettage followed by antiseptic washes and plasma injection into the groove and margins.
In peri-implantitis the injections are made in the marginal area of the gum within 2 teeth.
When implants are inserted, they are injected the same day or the next day 0.1-0.2.

Method effectiveness

- Stop inflammatory processes
- Reduce pain syndrome
- Speed up implant integration process
- Stop bleeding gums
- Accelerate tissue healing in patients with diabetes

Conclusions

Tissue regeneration using growth factors is a revolutionary method in all fields of medicine but it is still new, there is much more to discover in this method. The exact mechanism of action of the different factors is not yet fully understood.

7.2. Antioxidant and anti-inflammatory properties of melatonin and micronutrients in patients with periodontal disease

(Acatrinei et al., 2021)

Periodontal disease is a chronic disease with a complex etiology that includes bacterial colonization, excessive inflammation and oxidative stress. The hormone melatonin has antioxidant properties and may help alleviate chronic conditions by reducing stress. The aim of this study was to investigate and analyse the effect of exogenous melatonin on periodontitis patients. More detailed studies are needed to extend the therapeutic possibilities of melatonin as a premedication in dentistry.

Purpose

The aim of this study was to investigate the anti-inflammatory and antioxidant properties of melatonin supplements by measuring the clinical status of selected periodontitis patients. Improvement of inflammatory and antioxidant parameters.

Method

The study was conducted on 9 patients (4 men and 5 women) with moderate gingivitis and periodontitis resistant to treatment. The ages were between 30 and 70 years old. They were asked to rinse with a solution containing melatonin and 2 tablets of Melatonin 3 mg (Melatonin 5 mg., bulking agents (Dicalcium phosphate, Microcrystalline cellulose), stabilizer (Hypromellose), anti-caking agent (Vegetable Magnesium Stearate) to put under tongue one hour before bedtime for 8 weeks followed by a reassessment of the periodontal status. Patients were reminded every evening by a written message to take the pills.

2.Materials and methods

2.1 Materials

Consultation records showing gum disease, inflammation and periodontal pocket status.

Micronutrient deficiency analysis sheets (nutrigenetic test).

Consultation kits

Treatment prescriptions

2.1. Subjects

To find out the micronutrient DNA deficiencies, nutrigenetic tests were performed on 9 patients (4 men and 5 women) with gingivitis and moderate periodontitis aged 36-71 years old.

All subjects were systemically healthy, without symptoms of infection and had not taken antibiotics for at least 3 months prior to the experiment.

Vitamin therapy has been started — almost all vitamins are used: vitamin A, vitamin C, vitamin E, in the form of polyvitamins and Tarosin, which contains vitamins C + P. Vitamins activate capillary circulation, optimize vascular permeability, metabolic changes and oxidation-reduction reactions, cell regeneration.

It started administration of melatonin 3mg, 2 sublingual pills before one hour before bedtime, melatonin acts on antioxidant levels and inflammation at the periodontal level.

All subjects were informed about the purpose of the study and voluntarily enrolled.

2.2. Procedure

2.2.1. Dentist oral examination with the preparation of the periodontal chart in which the gingival status and periodontal pockets before treatment were noted (example Fig:1, 2, 3)

Fig.1 P.F. Patient no. 1 P.M. (66 years old, sex M). Oral hygiene indices assessment

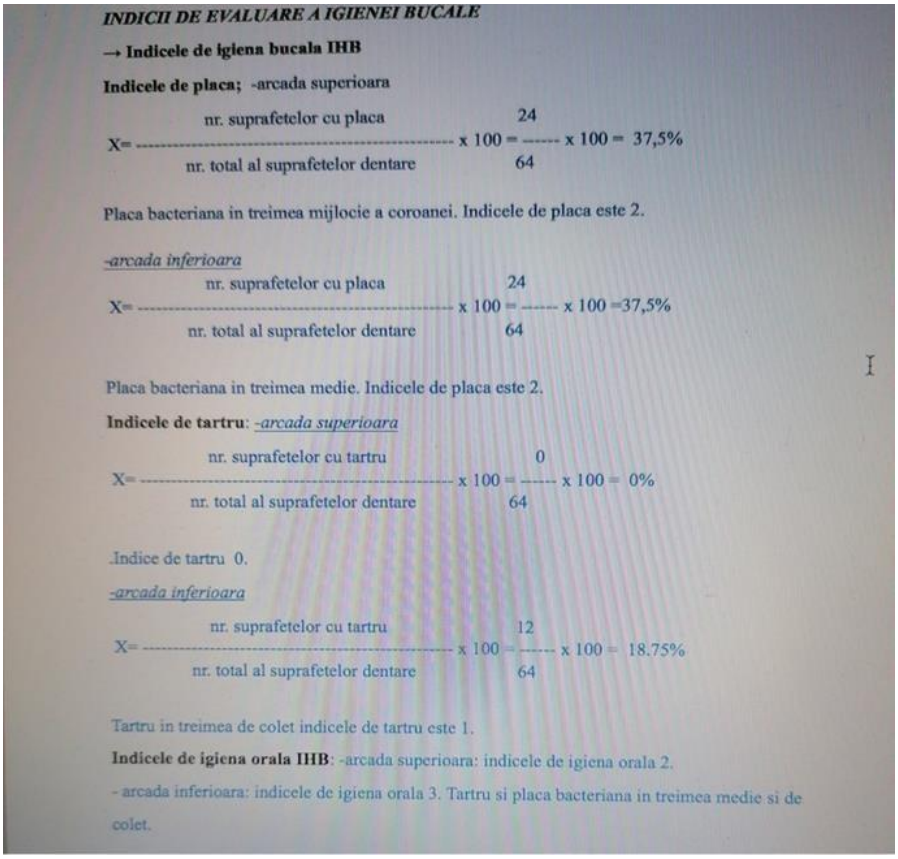
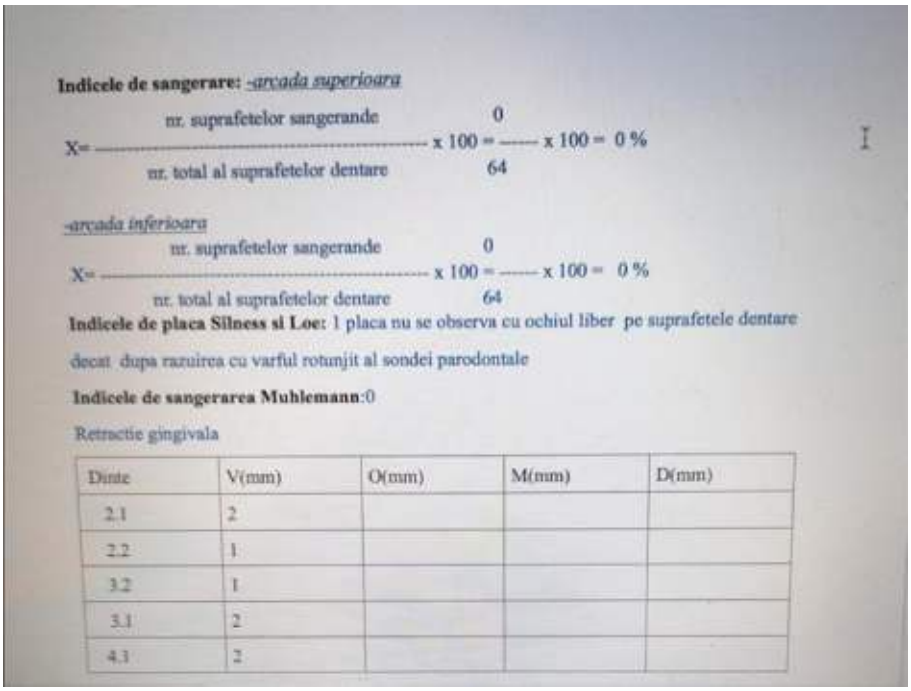


Fig. 2 P.F. Patient no. 1: P. M. (66 years old, sex M). Oral hygiene indices assessment continued fig.1 and bleeding index



Indicele de sangerare: *-arcada superioara*

nr. suprafetelor sangerande 0
X= ---------- x 100 = ----- x 100 = 0 %
nr. total al suprafetelor dentare 64

-arcada inferioara

nr. suprafetelor sangerande 0
X= ---------- x 100 = ----- x 100 = 0 %
nr. total al suprafetelor dentare 64

Indicele de placa Silness si Loe: 1 placa nu se observa cu ochiul liber pe suprafetele dentare decat dupa razuirea cu varful rotunjit al sondei parodontale

Indicele de sangerarea Muhlemann:0

Retractie gingivala

Dinte	V(mm)	O(mm)	M(mm)	D(mm)
2.1	2			
2.2	1			
3.2	1			
3.1	2			
4.1	2			

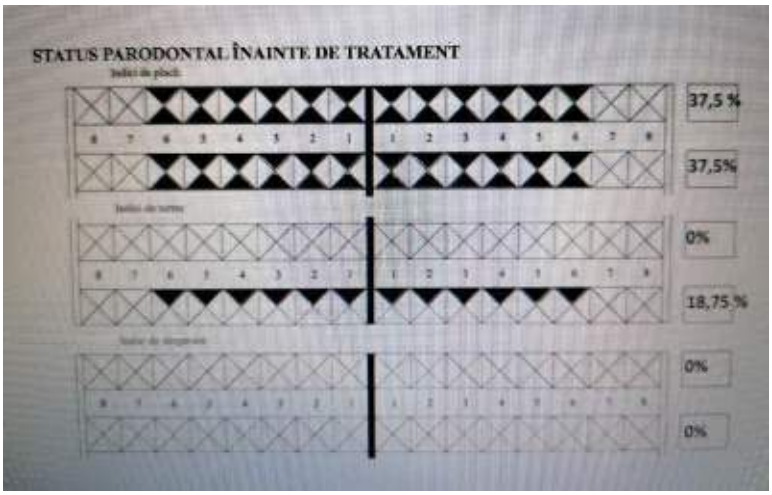
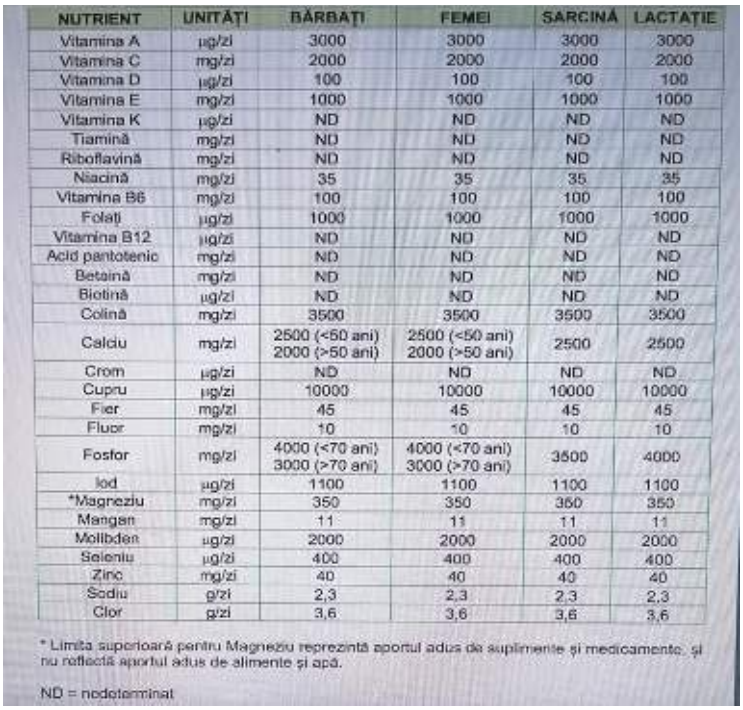


Fig.3 P.F. Patient no. 1: P.M. (66 years old, sex M). Periodontal status assessment before treatment

Carrying out nutrigenetic test. A simple saliva sample is taken, after which a report is generated by the testing laboratory that includes information about the patient's deficiencies.

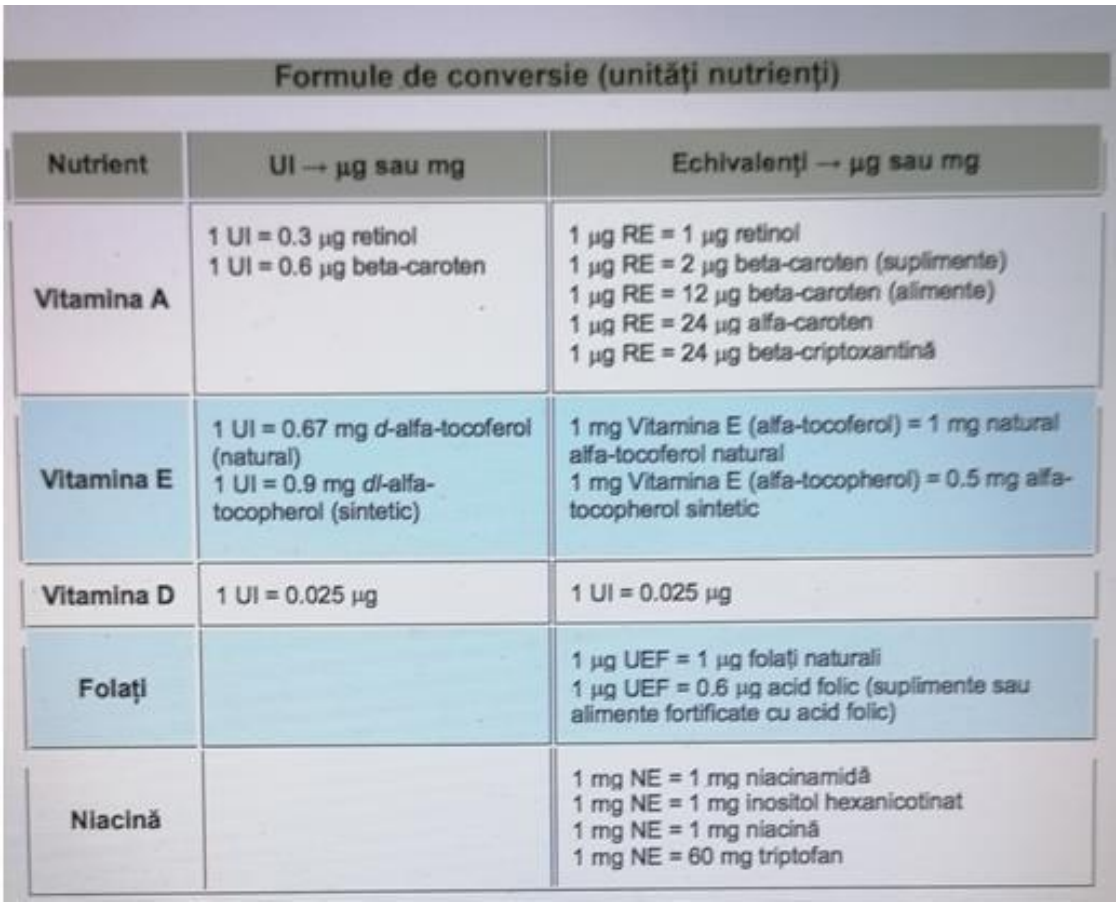


NUTRIENT	UNITĂȚI	BĂRBAȚI	FEMEI	SARCINĂ	LACTAȚIE
Vitamina A	µg/zi	3000	3000	3000	3000
Vitamina C	mg/zi	2000	2000	2000	2000
Vitamina D	µg/zi	100	100	100	100
Vitamina E	mg/zi	1000	1000	1000	1000
Vitamina K	µg/zi	ND	ND	ND	ND
Tiamină	mg/zi	ND	ND	ND	ND
Riboflavină	mg/zi	ND	ND	ND	ND
Niacină	mg/zi	35	35	35	35
Vitamina B6	mg/zi	100	100	100	100
Folați	µg/zi	1000	1000	1000	1000
Vitamina B12	µg/zi	ND	ND	ND	ND
Acid pantotenic	mg/zi	ND	ND	ND	ND
Betaină	mg/zi	ND	ND	ND	ND
Biotină	µg/zi	ND	ND	ND	ND
Colină	mg/zi	3500	3500	3500	3500
Calciu	mg/zi	2500 (<50 ani) 2000 (>50 ani)	2500 (<50 ani) 2000 (>50 ani)	2500	2500
Crom	µg/zi	ND	ND	ND	ND
Cupru	µg/zi	10000	10000	10000	10000
Fier	mg/zi	45	45	45	45
Fluor	mg/zi	10	10	10	10
Fosfor	mg/zi	4000 (<70 ani) 3000 (>70 ani)	4000 (<70 ani) 3000 (>70 ani)	3500	4000
Iod	µg/zi	1100	1100	1100	1100
*Magneziu	mg/zi	350	350	350	350
Mangan	mg/zi	11	11	11	11
Molibden	µg/zi	2000	2000	2000	2000
Seleniu	µg/zi	400	400	400	400
Zinc	mg/zi	40	40	40	40
Sodiu	g/zi	2,3	2,3	2,3	2,3
Clor	g/zi	3,6	3,6	3,6	3,6

* Limita superioară pentru Magneziu reprezintă aportul adus de suplimente și medicamente, și nu reflectă aportul adus de alimente și apă.

ND = nedeterminat

Fig.4 Tolerable Upper Intake Level. The table below shows the daily nutrient upper intake levels for adults according to age, sex and physiological status. Unless advised by a doctor, these levels should not be exceeded.



Formule de conversie (unități nutrienți)

Nutrient	UI → µg sau mg	Echivalenți → µg sau mg
Vitamina A	1 UI = 0.3 µg retinol 1 UI = 0.6 µg beta-caroten	1 µg RE = 1 µg retinol 1 µg RE = 2 µg beta-caroten (suplimente) 1 µg RE = 12 µg beta-caroten (alimente) 1 µg RE = 24 µg alfa-caroten 1 µg RE = 24 µg beta-criptoxantină
Vitamina E	1 UI = 0.67 mg *d*-alfa-tocoferol (natural) 1 UI = 0.9 mg *dl*-alfa-tocopherol (sintetic)	1 mg Vitamina E (alfa-tocoferol) = 1 mg natural alfa-tocoferol natural 1 mg Vitamina E (alfa-tocopherol) = 0.5 mg alfa-tocopherol sintetic
Vitamina D	1 UI = 0.025 µg	1 UI = 0.025 µg
Folați		1 µg UEF = 1 µg folați naturali 1 µg UEF = 0.6 µg acid folic (suplimente sau alimente fortificate cu acid folic)
Niacină		1 mg NE = 1 mg niacinamidă 1 mg NE = 1 mg inositol hexanicotinat 1 mg NE = 1 mg niacină 1 mg NE = 60 mg triptofan

Fig.5 Conversion formulae (nutrient units)

The tables show the deficiencies in the 9 tests of the patients before the drug treatments. The patients were tested only once in order to highlight the deficiencies and to draw up treatment plans with supplements, and a new set of tests was carried out after the end of each treatment.

From the nutrigenetic tests we were able to create some statistics on: vitamin and melatonin deficiency according to age, sex, and age which showed that both sexes are deficient in vitamin D but the higher percentage is in the male sex (100% male/60% female). Also from this, it appears that both sexes are deficient in vitamin C (50% male/40% female). In females compared to males, a deficiency of vitamin A and E was found. Also from these blood tests, melatonin deficiency was found in both sexes with a higher percentage in females (100% F and 80% M).

4. Discussions

The symptoms and treatment of oral inflammatory diseases are mainly influenced by micronutrient intake or supplements. Micronutrients are necessary to maintain the health of the oral mucosa and structures, as they possess anti-oxidant and anti-inflammatory properties. Deficiency of certain micronutrients may modulate the risk of oral inflammatory diseases. Melatonin supplements along with micronutrients may be effective in improving oxidative and inflammatory status. Therefore a melatonin and micronutrient therapy may be recommended as part of the therapeutic approach in the control of periodontal disease. However, more studies are needed with micronutrient and bioactive compound administration in halting the progression of periodontal disease.

7.3. The action of micronutrient and bioactive compound treatments in halting the progression of periodontal disease

(Acatrinei D. et al., 2020)

Purpose

The aim of this study was to address the controversy surrounding the relationship between periodontal disease and changes in periodontal tissues and to assess the type of changes in periodontal disease with nutrients by measuring the clinical status of selected periodontitis patients.

Method

The patients selected for the study were among those who sought periodontal treatment in private practice between August 2019 and July 2020, with symptoms specific to periodontal disease: changes in shape, color or texture of gum tissue, edema, mild or spontaneous bleeding, gingival pain and tenderness, gingival itching, tooth mobility, pockets of varying depths, hyper-growths of varying degrees, recessions, halitosis.

A total of 9 patients (4 men and 5 women) with moderate/severe chronic periodontitis aged 36-71 years old.

Vitamin therapy — almost all vitamins are used: vitamin A, vitamin B complex, vitamin C, vitamin D, vitamin E, and vitamin PP, in the form of polyvitamins and Tarosin, which contains vitamins C + P. Vitamins activate capillary circulation, optimise vascular permeability, metabolic exchanges and oxidation-reduction reactions and cell regeneration.

Data was collected by clinical appearance of patients before and after nutrient therapy. Periodontal control with examination of gums and periodontal pockets was performed.The study is carried out until the end of each patient's nutrient therapy.

1.Introduction

Oral diseases are worldwide common public health problems [Acatrinei et al., 2020]. Oral diseases of inflammatory origin include periodontal diseases: gingivitis and periodontitis, inflammation of the oral mucosa, pulpal and periapical lesions [Acatrinei et al., 2019]. Periodontal disease is one of the most common chronic inflammatory disorders [Civitelii et al., 2002]. Untreated periodontitis and dental cavities leading to pulp and periapical lesions are the most common cause of teeth loss in adult life [Vical et Brussee, Kribbs, 1992]. Oral inflammatory pathologies negatively influence quality of life, leading to impairment of daily activities [Miley et al., 2009].

2.Materials and methods

2.1 Materials

Consultation records showing gum disease and periodontal pocket status.

Micronutrient deficiency analysis sheets (nutrigenetic test). Consultation kits

Treatment prescriptions

Subjects

To find out the micronutrient DNA deficiencies, nutrigenetic tests were performed on 9 patients (4 men and 5 women) with gingivitis and moderate periodontitis, aged 36-71 years old.

All subjects were systemically healthy, without symptoms of infection and had not taken antibiotics for at least 3 months prior to the experiment.

Vitamin therapy was started - almost all vitamins are used: vitamin A, B complex, vitamin C, vitamin D, vitamin E, and vitamin PP, in the form of polyvitamins and Tarosin, which contains vitamins C + P. Vitamins activate capillary circulation, optimize vascular permeability, metabolic changes and oxidation-reduction reactions as well as cell regeneration.

All subjects were informed about the objective of the study and voluntarily enrolled.

Procedure

Dentist oral examination with the preparation of the periodontal chart in which the gingival status and periodontal pockets before treatment were noted (example Fig:1, 2, 3).

ORAL HYGIENE INDICES ASSESSMENT

Oral Hygiene Index (OHI index)

Dental plaque index: *superior arch*

$$X = \frac{\text{plaque areas}}{\text{total dental areas}} \times 100 = \frac{24}{64} \times 100 = 37.5\%$$

Bacterial plaque in the middle third of the arch. Plaque index is 2.

inferior arch

$$X = \frac{\text{plaque areas}}{\text{total dental areas}} \times 100 = \frac{24}{64} \times 100 = 37.5\%$$

Bacterial plaque in the middle third. Plaque index is 2.

Tartar index: *superior arch*

$$X = \frac{\text{tartar areas}}{\text{total dental areas}} \times 100 = \frac{0}{64} \times 100 = 0\%$$

Tartar index is 0.

inferior arch

$$X = \frac{\text{tartar areas}}{\text{total dental areas}} \times 100 = \frac{12}{64} \times 100 = 18.75\%$$

Tartar index is 1.

OHI index (Oral Hygiene Index):

superior arch: oral hygiene index: 2

inferior arch: oral hygiene index: 3

Tartar and plaque in the middle third and pack.

Bleeding index: *superior arch*

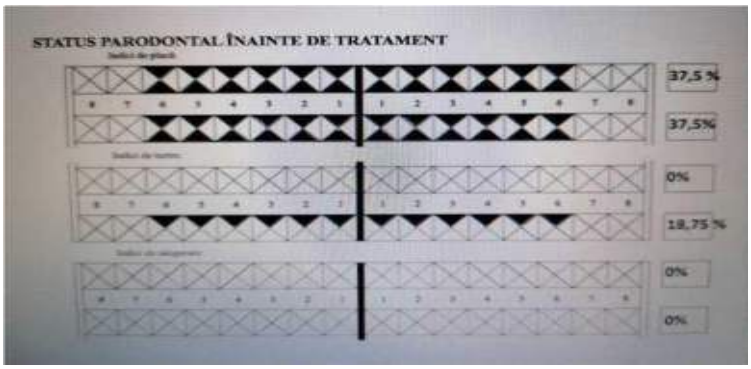
$$\text{bleeding areas} \quad 0$$

$$X = \frac{\text{dental areas}}{} \times 100 = \frac{}{64} \times 100 = 0\%$$

inferior arch

$$X = \frac{\text{bleeding areas}}{\text{dental areas}} \times 100 = \frac{0}{64} \times 100 = 0\%$$

Silness and Loe plaque index: 1 plaque is not visible to the naked eye on dental surfaces until after scratching it with the periodontal probe rounded tip.



Muhlmenann bleeding index: 0
* The upper intake limit for magnesium resulted from supplements and medication that does not reflect food and water intake. ND = not determined

Deficiencies of the 9 patient tests before drug therapies were highlighted in the tables. They were tested once to highlight deficiencies and to draw up therapy plans with supplements, leaving a new set of analyses to be carried out after completion of each therapy.

From the nutrigenetic tests we were able to create some statistics on: vitamin deficiency according to age, sex, and age which showed that both sexes are deficient in vitamin D but the higher percentage is in the male sex (100% male/ 60% female).Also from this it appears that both sexes are deficient in vitamin C (50% male/40% female). Vitamin B deficiency is higher in females than in males (60% female/50% male). In females, compared to males, a deficiency of vitamin A, vitamin E and Cu was found, whereas in males a deficiency of Zn was found, but not found in females.

We highlighted the deficiency by age groups in 4 tables and we found out that in the 30-40 and 40-50 years old ranges, we have a 100% deficiency in vitamin D and also a 100% deficiency, but in the 50-60 and 60-70 years old ranges, deficiencies shown in vitamin B2, vitamin B3, vitamin B6, vitamin B12.

3. Discussions

The symptoms and treatment of oral inflammatory diseases are mainly influenced by micronutrient intakes or supplements. Micronutrients are necessary to maintain healthy mucous membranes and oral structures, as they possess antioxidant and anti-inflammatory properties. Deficiency of certain micronutrients may modulate the risk of oral inflammatory diseases. Also, deficiency of some B-complex vitamins helps in the early detection of certain anaemias. Research has also confirmed that deficiency of certain trace minerals also worsens certain oral diseases in the presence of chronic systemic pathologies. Periodontitis is an inflammatory and progressive disease affecting the connective tissue around the teeth whose role, among others, is to support the teeth.

7.4 *Porphyromonas gingivalis* bacteria interaction with other periodontal disease bacteria and valid therapies

(Acatrinei D. et al., 2020)

Purpose

- This paper highlights *Porphyromonas gingivalis* interaction with other bacteria in determining periodontal disease and valid therapies (PRP, systemic and local antibiotics, antiseptic solutions and antimicrobial plant derivatives) (Al-

Haroni et al. 2006, Bidault et al. 2007, Charton et Mouton 2003Paster et al, 2001, Rams et al. 2011, etc). In our research we proposed biochemical and histological study on the use of new therapeutic methods in some oral and facial pathologies. Thus, in our previous work we aimed to demonstrate that tissue regeneration using growth factors is a revolutionary method in all medical fields and skin attachments: juvenile/vulnerable acne, alopecia, androgenic alopecia, dentistry, traumatology, rehabilitation medicine; gynecology). At the basis of autologous platelets use is the discovery that platelets contain protein factors (PRP FACTORS) that initiate cell regeneration processes.

Method

- A total of 6 patients (2 males and 4 females) with moderate/severe chronic periodontitis, aged 27-51 years old.
- Subgingival plaque was collected from patients with moderate and severe chronic periodontitis in a specific sterile kit to identify *Porphyromonas givalis* and other periodontogenic bacteria.

Material

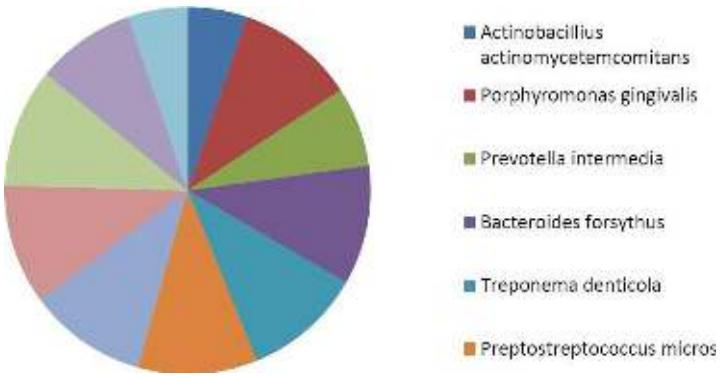
- Medical consultation kits

- Prophylaxis kits (ultrasound handles, loops, brushes) - Special collection kits

- Laboratory where the submitted samples are analysed - Special collection kits and blood centrifuge
- Antibiotics

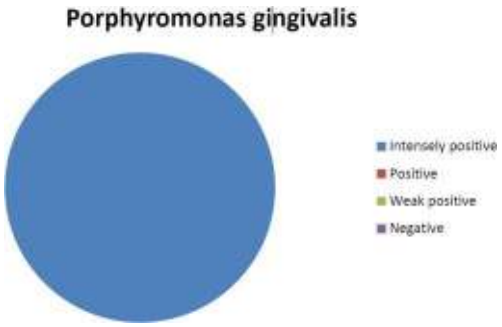
Procedure

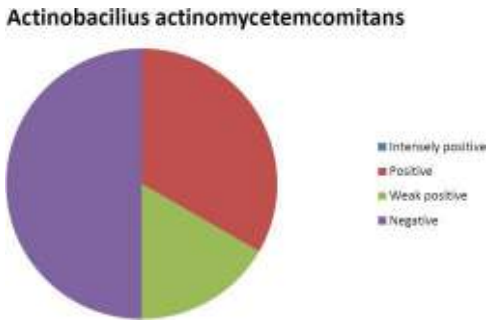
- The sampling was done by the dentist with the help of the paper swabs contained in the collection kit, necessary before mechanical or antibiotic therapy. Using sterile tweezers, a stick was inserted into the periodontal pocket until it reached the base (the depth of the periodontal pocket should be at least 4 mm); this procedure was repeated in all 4 patients. The stick remained in place for 10 seconds, after which it was withdrawn and inserted into a transfer tube. Up to 4 samples were harvested from different periodontal bags. All sticks from the same patient were inserted into a single transfer tube. The tube was placed in the blue collection kit together with a file containing the patient personal details and transported to the laboratory where microbiological analyses were performed.



Germs associated with periodontitis

Of the 6 patients tested, all 6 were positive for *porphyromonas gingivalis*.



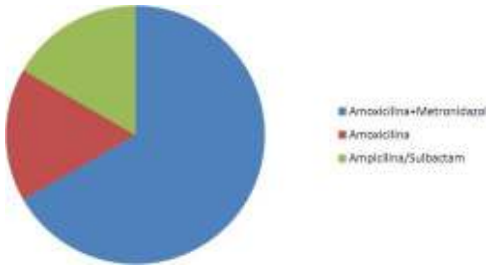


Preparation

The freshly obtained plasma was injected into each patient's periodontal pockets and mobile mucosa, with each procedure repeated 4 times for each at an interval of 7-12 days (the period during which platelets are viable).
This therapy was carried out after debridement, washing with antiseptic solutions and under antibiotic control. In our study, PRP showed antimicrobial properties against fresh isolates of Porphyromonas gingivalis by minimal inhibitory concentrations and adherence resistance tests.

Antibiotic sensitivity tests

This graph highlights that more than half react to Amoxicillin + Metronidazole therapy if they are not allergic to one of the substances.



Natural sources

In this study, plants proved to be a good source for finding new bacterial agents. Patients were advised to consume proanthocyanidins-rich blueberry and cranberry juice, as these two have been shown to have activity against P. gingivalis biofilm. It was found that after some time the inflammatory activity decreased.

Findings

The clinical study included 6 cases diagnosed with generalized periodontitis. Out of the total number of 6 patients, the

gender distribution of the patients in the group included 2 males and 4 females Table 1).

Gender distribution of patients in the group

Cases diagnosed with generalized periodontitis had a wide age range. In terms of age group distribution, case 1 was a patient aged 20-29 years old, 2 cases were patients aged 30-39 years old, 2 cases were patients aged 40-49 years old and 1 case was a patient aged 50-59 years old, thus finding an identical incidence in 30-39 and 40-49 years old ranges. (Table 2)
The youngest patient diagnosed with generalized periodontitis was 27 years old and the oldest, 51 years old.

Age distribution of patients in the group

In the age ranges 20-29 and 40-49 years old, the *Actinobacillus actinomycetemcomitans* germ is completely absent. Other germs that are 50% absent in the age interval 30-39 years old are *Prevotella intermedia* and *Capnocytophaga spp*, and in all the same age group 40-49 years old, the germ *Eubacterium nodatum* is absent.

Percentage of presence or absence of germs according to age

	Age 20-29	Age 30-39	Age 40-49	Age 50-59
Porphyromonas gingivalis	1	2	2	1
Prevotella intermedia	1	1	2	1
Peptostreptococcus micros	1	2	2	1
Fusobacterium nucleantum	1	2	2	1

In the following table we see that the male segment has a much higher presence of all germs than women. The only germ in males that is 50% absent is *Prevotella intermedia*. In females, the fewest germs present are 25% of *Actinobacillus actinomycemcomitans* and *Capnocytophaga spp.*

Percentage presence of periodontitis-associated germs by sex

Actinobacillus	25% present	100%present
Porphyromonas gingivalis	100%present	100%present
Prevotella intermedia	100%present	50% present
Bacteroides forsythus	100%present	100%present
Treponema denticola	100%present	100%present
Peptostreptococcus micros	100%present	100%present
Fusobacterium nucleantum	100%present	100%present
Eikenella corrodens	100%present	100%present
Eubacterium nodatum	75% present	100%present
Capnocytophaga spp	25% present	100%present

Discussions

It was found that the germ present in 100% proportion, regardless of sex or age, was *Porphynomonas gingivalis*. In addition, 100% present, regardless of sex and age are *Bacteroides forsythus, Treponema denticola, Peptostreptococcus micros, Fusobacterium nucleantum, Eikenella corrodens* and *Capylobacterium nodatum*, in which the percentage of intensity differs, ranging from intensely positive to weakly positive. Antibiogram results showed low sensitivity of pathogenic microorganisms in generalized periodontitis to amoxicillin in combination with metronidazole, i.e. 66.66%. 4 out of 6 patients responded positively to amoxicillin in combination with metronidazole therapy, which seems to have the ability to reduce inflammation and inhibit MMP-2 activity.

- There are other adjacent therapies that give results such as PRP injected into the gum pockets and mobile mucosa. In our study, PRP showed antimicrobial properties against Porphyromonas gingivalis germs by minimal inhibitory concentrations and adherence resistance tests.
- Plants have proven to be a good source for finding new antibacterial agents. Proanthocyanidins rich blueberry and cranberry juice showed activity against Porphyromonas gingivalis biofilm and decreased inflammation.
- Further studies are needed to decipher the relationships between antibiotics and the patient's local response to individual variations in the synthesis and release of inflammatory or matrix-modulating effectors.

7.5 Comparative study on the non-surgical therapy of periodontal disease: SRP, laser therapy *vs.* plasma

(Acatrinei et.al., 2023)

Introduction

Periodontal disease is a chronic, progressive and complex condition that affects the teeth's supporting structures: gums, ligaments and bone. The complexity of this condition is determined by a number of characteristics both in terms of etiology and the diversity of clinical forms or evolutionary and therapeutic features. Thus, periodontal disease is a pathological manifestation characterised by (Charton et Mouton, 2003, Carranza et al. 2006):

- Multifactorial etiology, being caused by both determinants and promoters
- Clinical forms of periodontal disease are numerous and polymorphic: acute or chronic, reversible or irreversible
- Evolution is often chronic, with periods of remission and exacerbation

The etiological factors involved in periodontal disease are:

- Local factors (determinants or predisposing factors)
- General (systemic) factors

Nowaday, the microbial factor is considered the main reason for the development of periodontal disease. In addition to this, there are other local irritant or functional factors, such as:

- Predominantly harsh diet putting pressure on periodontal ligaments
- Bacterial plaque retentive areas (fillings and dental neck cavities)
- -Type of diet, oral hygiene quality and regularity
- Unprotected dentures
- Occlusal trauma, malocclusion, occlusal dysfunction
- Bruxism or other parafunctional habits

The general conditions most commonly associated with periodontal disease are as follows:

- Endocrinological disorders
- -Hematological disorders
- Malnutrition (hypovitaminosis, nutritional deficiencies)
- Metabolic diseases (diabetes, obesity, metabolic syndrome)
- Connective tissue diseases

In the etiology of periodontal disease, the triggering factor is microbial, represented by bacterial plaque. Associated favouring factors influence the evolution and prognosis. The clinical manifestation of periodontal disease is the result of the cumulative interaction of the following factors:

- Species of micro-organism involved and its aggressiveness
- Each patient background and constitutional predisposition
- Presence of local enabling factors
- Patient's pathological history
- Patient’s immunological status (Mysak et al. 2014)

The most important microorganisms in the periodontal field are the following (Acatrinei et al. 2019):

1. *Porphyromonas gingivalis* is a Gram-negative bacterium involved in the etiology of periodontal disease and one of over 500 bacterial species that colonise the oral cavity. It usually colonises the oral cavity, but has the potential

to become virulent when the situation allows (immune system decline). Together with *Treponema denticola* and *Tannerella forsythia* it makes up the red complex of periodontal bacteria. (Acatrinei et al. 2019, Adrian et al. 2007, Bodet et al. 2007)

2. *Aggregatibacter actinomycetemcomitans* is a Gram-negative bacterium usually found in the microbial flora of periodontitis patients, both in those with localized forms and chronic periodontitis. Less commonly, it has also been intercepted in patients with endocarditis. *A. actinomycetemcomitans* was first discovered by Danish periodontist Jørgen Slots, professor and microbiologist at the University of South Carolina School of Dentistry. (Nesbit, 2007)

In recent times, various treatment methods have been tried to halt the progression of periodontitis, including:

1. Scaling Root Planning — is the central element when it comes to treatment methods for marginal periodontitis and it is based on the mechanical removal of bacterial plaque and hard deposits present on the root surface and the disruption of the local niche they create. It also removes toxins secreted by pathogenic bacteria and helps activate the immune system. The aims of SRP are as follows:

— plaque and tartar removal - both of them are primary goals of a successful therapy

— healing influence and reduction of periodontal pockets by non-surgical therapy

— inflammation reduction prior to surgical periodontal therapy, if required (Albandar and Rams, 2002)

2. Another adjuvant method in periodontal disease is laser therapy. The use of this technology allows us to:
— safely remove damaged tissues from periodontal pockets without damaging healthy ones
— clean away soft or calcified bacterial plaque debris that contributes to periodontal inflammation, thereby achieving local decontamination
— stimulate fibrinogenesis and tissue coagulation, producing a decrease in bleeding and promoting epithelial reattachment to tooth root.
— seals the periodontal pocket and reduces the risk of reinfection after treatment (Vipin et Mahindra, 2011)

3. There are various forms of platelet-enriched concentrates such as PRP-platelet-rich plasma and PRF-platelet-rich fibrin. These have been used for tissue regeneration in various in vivo and in vitro studies. The benefits of PRF have been considered superior to PRP because it involves easy handling, low cost price, low anticoagulant concentration which reduces biochemical alteration and risks associated with bovine thrombin. During the last three decades, PRF has been successfully used in the field of dentistry with regenerative effects, but it can also be used in other fields, such as oromaxillofacial and orthopedic surgery (Armitage, 2003).

Purpose

The purpose of this study was to demonstrate the regenerative capacity of the body under the influence of tissue growth factors found in autologous plasma. Although the results are also encouraging in patients undergoing only SRP and laser therapy, on the long term, it was observed that those who also received injectable autologous plasma sessions had more stable results over time and a lower risk of relapse.

Tools and method

The study involved 35 patients, 19 men and 16 women. They are patients of "Super Dent 92" Clinic of Aesthetics and Implantology in Constanta and the results were recorded and interpreted in the same clinic. These patients ages range between 25 and 50 years old and were divided into two therapy groups. The first group of patients underwent SRP and diode laser therapy and the second group underwent SRP, diode laser therapy and autologous plasma injection therapy. The study was conducted over a 3-year period, and patients were monitored weekly for the first 3 months, then monthly, and every 3 months in the last year. The following parameters were followed: gingival bleeding index, bacterial plaque index, tartar index, CPITN index and periodontal inflammation stage.

The working method for the group of patients who received SRP therapy with laser therapy was as follows:

1. Initial clinical and paraclinical consultation in which the targeted indices are measured, the depth of the periodontal pockets is measured, panoramic X-ray or CT scan is taken, as appropriate, and the therapy plan is

established. The patients had their initial periodontal analyses taken with the help of the kit for testing the microbial species present in the periodontal bacterial flora.

2. Teeth hygiene is performed during session number two:

   - Bacterial plaque is revealed using pellets with bacterial plaque developer. With the EMS Perioflow Guided Therapy Biofilm machine using 400 micrometre powder for supragingival areas and 14 micrometre powder for areas where periodontal pockets have been detected.

   - Supragingival scaling is performed with a P1 scaling loop attached to the ultrasonic unit and subgingival scaling with a PERIOFLOW Nozzle.

3. SRP therapy is performed on all teeth present on the arch. Gracey 1/2, 3/4, 5/6, 7/8, 9/10, 11/12, 13/14 and 15/16 curettes were used. For irrigation 3% hydrogen peroxide and 0.12% chlorhexidine gluconate were used.

4. For very deep periodontal pockets, the use of a periodontal diamond loop with a long active side is required to finish the root surfaces.

5. Apply Hyben-X local decontaminant.

6. Irrigate the affected area with 3% hydrogen peroxide an aqueous solution, then introduce the diode laser fibre at the level of the periodontal pockets, the proposed effect being to decontaminate the bacterial load.

7. Apply Metronidazole antibiotic and Coe-Pak periodontal cement.

8. Patients are advised to keep the cement in the field for 24 hours, eat semi-homeless food and remove it the next day, when the first periodontal check-up is performed.

For the group of patients who also received autologous plasma injections, the steps followed are the same, except that after completion of the closed-field curettage (SRP) protocol using Gracey curettes and diode laser decontamination, venous blood is collected according to the well-known protocol.

- Fit the tourniquet to the hand of interest, disinfect the elbow crease
- Fit the needle to the vacutainer and insert it into the vein
- Fit the vial and collect the desired amount of blood
- Spin at the following parameters: 1200 revolutions 14 minutes

  The resulting plasma is injected into the gingiva at the level of the mobile mucosa and into the periodontal pockets.

Patients are monitored at the established intervals and a new analysis of the bacterial species present in the periodontal microflora are sampled in the third month post Scalling Root Planning therapy. The aim of this analysis is to detect quantitative changes in the germs involved in periodontal disease, such as *Porphyromonas gingivalis*, *Actinomycetens comitans*, etc.

The purpose is also to prevent bleeding, reduce the depth of periodontal pockets, reduce inflammation and mobility. Tools used according to the previously described work method are the following:

1. Periodontal Bacteria Test Kit

Using this test allows microbiological analysis of bacteria present in the patient's oral cavity. To collect it, a paper cone from the packet is inserted in each representative area or in areas with deep periodontal pockets.

The micro-Dent A test makes it possible to determine the five important periodontitis markers *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedio, Bocteroides forsythus* and *Treponema denticola*. In some cases of periodontitis, the diagnostic spectrum can be extended by determining 6 other germs, such as: *Peptostreptococcus micros, Fusobacterium nucleatum/periodonticum, Eikenella corrodens, Campylobacter rectus, Eubacterium nodatum* and *Capnocytophago spp* (micro-IDent B test).

As a conclusion, micro-IDent tests are useful for:

— identify risk areas and early recognition of relapses;

— choose the appropriate antibiotic and documenting therapeutic success,

— assess the risk of implant failure before therapy.

Moreover, knowledge of the microbiological situation allows early therapy to prevent disease progression.

Sampling is done by the dentist using the paper cones contained in the collection kit, necessarily before antibiotic therapy. A sterile forceps is used to grasp a sterile cone in the periodontal pouch up to its base (the depth of the periodontal pouch must be at least 4 mm). The cone remains in place for 10 seconds, after which it is removed and inserted into a transfer tube. A maximum of 4 samples can be sampled from different periodontal bags. All cones from the same patient are placed in one transfer tube. The tube is placed in the blue collection kit together with the patient personal details file.

The method of obtaining the results is a multiplex polymerase chain reaction (PCR) with colorimetric detection.

2. Perioflow teeth hygiene device

This device is very powerful and extremely useful in the prophylactic and curative treatment of gum disease because it allows clinical detection of bacterial plaque deposits using a plaque indicator. This indicator can be in various forms: pellets, chewable tablets, liquid, etc. The one used in the study is marketed by EMS and is distributed in the form of blue pellets. On contact with plaque, it is coloured pink (recent plaque) or blue (old plaque).

3. Dental Scaler

For scaling, the EMS No Pain system loops are used, which attach to the ultrasonic part of the Perioflow unit.

The set consists of 10 thin and flexible tips able to adapt to the topography of periodontal or peri-implant pockets.

The PS Piezon No Pain tool is thin and smooth like a probe. It is also minimally invasive, maximally preventative and gives the doctor an excellent tactile sensation when working subgingivally. The biggest advantage of using it is the fact that it preserves the epithelium due to its absolute linear movements and painless as long as the recommended protocol is followed.

4. Drugs with specific action on bacteria involved in periodontal disease.

The following drugs were used on each of the two groups of patients:

— Hyben-X-substance with decontaminating role on oral tissues. It is applied to the lesions for 20 seconds, then washed with plenty of water.

— Metronidazole 10mg/0.5mg/g, gingival gel with the active substance metronidazole as metronidazole benzoate 10 mg and chlorhexidine digluconate solution 20%, 0.5 mg/1g gel

— Parodium gel, gum gel with rhubarb extract with anti-inflammatory action

5. DoctorSmile laser

The DoctorSmile laser operates at the following parameters: wavelength 810-980 nanometres and a power of 5 Watts, and is classified as medical class IIB. For the decontamination function, the fibre tip is inserted into the periodontal pocket in contact with the mucosal wall and 20 vertical movements are performed so as to minimise the amount of necrotic tissue. Avoid contact with the root surface of the teeth to avoid thermal necrosis of the pulp tissue. For a superior antimicrobial effect, it is recommended to irrigate the working surfaces with a 3% hydrogen peroxide aqueous solution.

6. Radiology equipment

All necessary radiological investigations were carried out in the Clinic. It allows paraclinical investigations such as panoramic X-rays and computed tomography. With the help of these additional tests, there is a possibility of confirming the clinical diagnosis of chronic marginal periodontitis. X-ray scans show bone defects, widening of the lamina dura, the presence of infectious intraosseous granuloma/cyst formations or lateral periodontal abscesses.

The following formulae are used to measure the indices that we follow in each of the two groups of patients:

1. CPITN index (community periodontal index of treatment needs)

CPITN is a community indicator of periodontal disease of treatment needs. It establishes periodontal treatment needs (TN) according to clinical status (CPI).

Determination method:

— dental arches are divided into sextants: 18-14, 13-23, 24-28, 38-34, 33-43, 44-48. A sextant is examined only if there are at least 2 teeth that do not show an indication for extraction. Otherwise, the sextant is excluded from the study and the remaining tooth is included in the next sextant.

— periodontal probe with a blunt tip is used by inserting it with medium pressure into the gingival sulcus in 6 points, until slight ischaemia of the mucosa

— benchmark teeth are analysed in epidemiological studies: 17, 16, 11, 26, 27, 37, 36, 31, 46, 47

— it is determined:

- presence/absence of bleeding gums
- presence/absence of suprasubgingival tartar
- presence/absence of periodontal pockets

CPITN score

0 = healthy periodontium

1 = bleeding on probing

2 = supra/subgingival tartar

3 = periodontal pockets up to 5 mm deep

4 = periodontal pockets over 6 mm deep

Treatment needs

0 = no treatment needed

1 = health education + professional brushing

2 = health education + scaling + professional brushing

3 = health education + scaling + professional brushing + periodontal curettage

4 = complex periodontal surgical treatment

2. Gingival index (GI)

The evaluation is from 0 to 3.

0 = normal gums;

1 = mild inflammation with colour change, mild oedema, no bleeding;

2 = moderate inflammation, red gums, oedema, bleeding on palpation with probe;

3 = severe inflammation, congestion, marked oedema, ulceration, spontaneous bleeding;

GI refers to pathological changes in the gums that are reversible (in the case of gingivitis).

3. The Periodontal Index (PI) — introduced in 1956 — assesses both gum and supporting periodontium condition, using a scale from 0 to 8:

0 = absence of inflammation, retraction and bone lesions;

1 = mild gingivitis with partial, localized, loose gum inflammation;

2 = moderate gingivitis, with circumscribed, total inflammation, false pockets - epithelial insertion has not migrated, the bottom of the pocket is at the level of the anatomical core;

4 = gingivitis and periodontitis with true pocket formation; teeth are well implanted and without pathological mobility; bone atrophy is up to 25% of alveolar bone height;

6 = gingivitis and periodontitis with deep pockets, bone atrophy between 25 - 50%.

8 = gingivitis and periodontitis with deep pockets, bone destruction more than 50% mobile, migrated teeth with reduced functional capacity

The PI index is higher than the GI gingival index.

4. PDI - Periodontal Disease Index (introduced in 1959) — periodontal disease index that rates the severity of periodontal disease based on inflammation and pocket depth on a scale of 0 to 6:

0 = normal appearance;

1 = mild gingivitis that does not circumscribe the tooth;

3 = severe gingivitis with ulceration and bleeding;

4 = gingivitis plus periodontitis with pockets up to 5 mm below the gum;

6 = severe gingivitis with periodontitis with pockets over 6 mm.

As the assessment of the PDI index over the whole arch is difficult, it is applied to a small number of 6 teeth: 16; 21; 24; 36; 41; 44.

Since the most severe periodontal lesions are found only on the mesial surfaces, only lesions on the mesial surfaces of the 6 teeth mentioned are considered.

5. Tooth Mobility Index (TMI) (1950) — assesses tooth mobility on a scale from 0 to 3.

0 = no mobility;

1 = pathological mobility in vestibulo-oral direction;

2 = pathological mobility in vestibulo-oral and mesio-distal direction;

3 = pathological mobility in vestibulo-oral, mesio-distal and vertical direction.

6. Oral hygiene indices: plaque index and tartar index.

Bacterial Plaque Index (PI). Since the existence of bacterial plaque in periodontal disease and the correspondence between disease severity and plaque abundance has been demonstrated, the assessment of periodontal status based on the evaluation of the amount of plaque accumulated was proposed.

The IP assessment ranges on a scale from 0 to 3:

0 = absence of plaque;

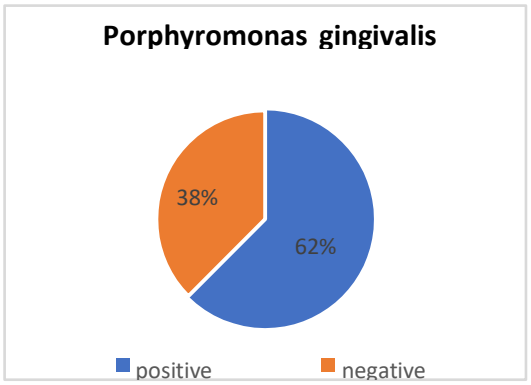
1 = dental plaque in the cervical 1/3 of the buccal or oral surface of the tooth;

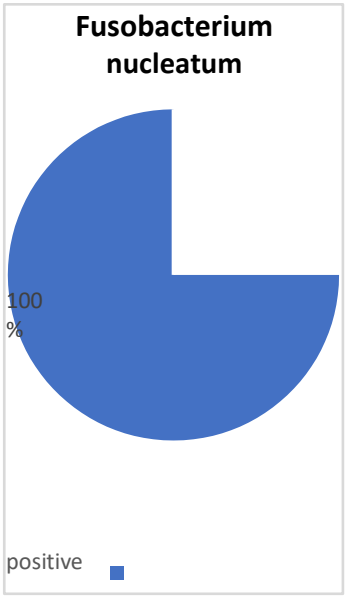
2 = dental plaque up to ½ of the area;

3 = dental plaque greater than ½ of the area.

Simplified PI is only performed on 6 teeth and can be assessed using organic stains or simple inspection.

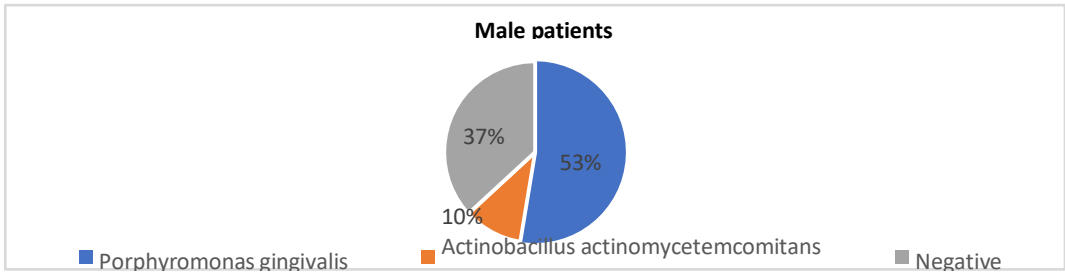
The Tartar Index uses the same rating scale.





Results and discussions

Regarding the results of the qualitative and quantitative analysis of the bacteria present in the bacterial plaque collected using the micro-Dent A test, in the group of female patients the presence of *Porphyromonas gingivalis* was found in 10 out of 16 patients, i.e. 62.5%. Of these, all were positive for *Fusobacterium nuacleatum*.

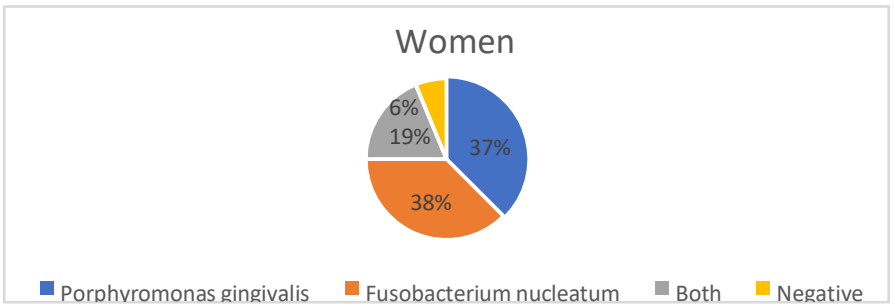


Concerning the group of male patients, among the 19 patients, 10 patients showed intensely positive values for *Porphyromonas gingivalis* bacteria, i.e. 52.63%, 2 patients showed elevated values for *Actinobacillus actinomycetemcomitans* serotype B, i.e. 10.52% and 7 patients were considered negative for the presence of periodontal bacteria, i.e. 36.84% of patients.

It can be noticed that both groups of patients had an increased susceptibility especially to the presence of *Porphyromonas gingivalis*, 62.5% of females and 52.63% of males, and a lower percentage to *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*. This result reinforces the theory that periodontal disease is mostly caused by well-known periodontal bacteria.

Following the treatments mentioned above, namely Scaling Root Planning therapy, diode laser therapy and autologous plasma injections, the analysis was repeated using the micro-Dent A test and the results showed a trend of decreasing bacterial load both for *Porphyromonas gingivalis and* other species.

In the female patients group the final results showed 6 patients positive to *Porphyromonas gingivalis*, 6 to *Fusobacterium nucleatum*, 3 to both species and one negative and in the male patients group 7 were positive to *Porphyromonas gingivalis*, 1 to *Actinobacillus actinomycetemcomitans*, 2 positive to both species and 9 were negative.



In terms of the indices analysed, both groups showed a decrease in the gingival and periodontal mobility indices. However, a higher percentage of patients who received plasma (6 out of 16, i.e. 37.5%) had GI values of 0 compared to those who did not (5 out of 19, i.e. 26.31%). These results reinforce the theory that plasma has a beneficial complementary role to the classical SRP and laser therapy.

In the first group of patients, the CPITN index decreased to 2 or 1, and in the second to 0 or 1.

The mobility index was reduced to values of 1 or 0 in both batches, and the plaque and tartar indices to values of 0 in 90% of cases.

Overall, the values showed an improvement in periodontal health in both groups of patients.

Conclusions:

1. Both treatment options can be considered beneficial in reducing the symptoms of periodontal disease.
2. For patients who also opt for autologous plasma treatment, periodontal pockets more than 5 mm deep were sometimes even completely reduced compared to those who did not (reduction of a few mm, but persistence of pockets).
3. Connective periodontal regeneration is induced and mobility disappears due to these new connections between tooth and bone.
4. The quality of the gums improves in terms of tone and colour.
5. No bleeding is obtained on probing at regular check-ups, which indicates that periodontal disease has stopped progressing.
6. The values of the bleeding, periodontal, CPITN and gingival indices decreased following the therapies.
7. The previously amputated interdental papillae regenerate and close the interdental spaces.
8. In the long term there is a low risk of relapse.
9. Depending on the main and additional hygiene measures the patient practice at home, the results obtained may be negatively influenced.
10. For monitoring and maintaining the results, regular check-ups every three months and deep cleansing with the EMS Perioflow GUIDED THERAPY BIOFILM are recommended.

GENERAL CONCLUSIONS

From the analysis of collected data during the experiments carried out at the SuperDent 92 Clinic, on

BIOCHEMICAL AND HISTOLOGICAL RESEARCH — ORAL REHABILITATION METHODS AND TECHNIQUES, the following assessments, comments and conclusions can be formulated:

Tissue regeneration using growth factors is a revolutionary method in all fields of medicine but it is still new, there is much more to discover on this method. The exact mechanism of action of the different factors is not yet fully understood.

The symptoms and treatment of oral inflammatory diseases are mainly influenced by micronutrient intake or supplemens. Micronutrients are necessary to maintain the health of the oral mucosa and structures, as they possess anti-oxidant and anti-inflammatory properties. Deficiency of certain micronutrients may modulate the risk of oral inflammatory diseases. Melatonin supplements along with micronutrients may be effective in improving oxidative and inflammatory status. Therefore a melatonin and micronutrient therapy may be recommended as part of the therapeutic approach in the control of periodontal disease. However, more studies with micronutrients and bioactive compounds administration are needed to halt the progression of periodontal disease.

Recent periodontitis studies have reached the following conclusions:

- oxidative stress leads to destruction of the periodontium(destruction of bone and adjacent connective tissue);
- there is an association between periodontitis, cardiovascular disease and diabetic metabolic syndrome;
- there is a reduction in the body's ability to heal, caused by a significant decrease in the amount of antioxidants in the gum tissue; therefore, antioxidant therapy should be considered;
- diet rich in antioxidants is a key factor.

In periodontitis, antioxidant therapy can neutralize reactive oxygen species (toxins) and thus can be used to treat this chronic inflammatory disease involving increased oxidative stress.

Vitamin supplements, antioxidants, minerals, omega 3 and phytonutrients can reduce periodontal inflammation. In addition, bioflavonoids have antimicrobial and anti-inflammatory activities. In addition, nutritional therapy should be considered as a new adjuvant treatment for both regeneration of damaged periodontal tissue and reduction of gingival inflammation.

It was found that the germ present in 100% proportion, regardless of sex or age, was *Porphynomonas gingivalis. In addition, regardless of sex and age are 100% present Bacteroides forsythus, Treponema denticola, Peptostreptococcus micros, Fusobacterium nucleantum, Eikenella corrodens* and *Capylobacterium nodatum*, in which the percentage of intensity differs, ranging from intensely positive to weakly positive. Antibiogram results showed low sensitivity of pathogenic microorganisms in generalized periodontitis to amoxicillin in combination with metronidazole, i.e. 66.66%. 4 out of 6 patients responded positively to amoxicillin combined with metronidazole therapy, which seems to have the ability to reduce inflammation and inhibit MMP-2 activity.

There are other adjacent therapies that give results such as PRP injected into the gum pockets and mobile mucosa. In our study, PRP showed antimicrobial properties against *Porphyromonas gingivalis* germs by minimal inhibitory concentrations and adherence resistance tests.

Plants have proven to be a good source for finding new antibacterial agents. Proanthocyanidins-rich blueberry and cranberry juice showed activity against *Porphyromonas gingivalis* biofilm and decreased inflammation.

Further studies are needed to decipher the relationships between antibiotics and the patient's local response to individual variations in the synthesis and release of inflammatory or matrix-modulating effectors.

Both treatment options, laser PRS and laser PRS with PRP therapy can be considered beneficial in reducing periodontal disease symptoms.

For patients who also opt for autologous plasma treatment, periodontal pockets more than 5 mm deep were sometimes even completely reduced compared to those who did not (reduction of a few mm but persistence of pockets). Periodontal connective regeneration is induced and mobility disappears due to these new tooth-bone bonds.

The quality of the gums improves in terms of tone and colour.

No bleeding is obtained on probing at regular check-ups, which indicates that periodontal disease has stopped progressing.

The values of the bleeding, periodontal, CPITN and gingival indices decreased following the treatments.

The previously amputated interdental papillae regenerate and close the interdental spaces.

On the long term there is a low risk of relapse.

Depending on the main and additional hygiene measures the patient practice at home, the results obtained may be negatively influenced.

For monitoring and maintaining the results, regular check-ups every three months and deep cleansing with the EMS Perioflow GUIDED THERAPY BIOFILM are recommended.