"OVIDIUS" UNIVERSITY FROM CONSTANȚA

MEDICINE PhD SCHOOL

PhD DOMAIN DENTAL MEDICINE

POSSIBILITIES OF USING BETA DEFENSINS IN DIAGNOSIS AND MONITORING OF PERI-IMPLANTITIS

~ ABSTRACT ~

PhD Coordinator

Prof. univ. dr. BADEA VICTORIA

PhD Student

Afef Al-Jaboobi

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Key words: beta defensins, peri-implantitis,diagnosis, monitoring

Defensins. Definition and classification.

The shortest definition of defensins shows that they are antibacterial peptides with an important role in innate immunity and which are produced by leukocytes and epithelial cells. [1, 4]

A short history of defensin discovery shows that in 1956 Skarnes and Watson identified a leukin with a bactericidal effect; concurrently Hirsch describes the presence in rabbit phagocytes of peptides with antibacterial effects. [5, 6]

Since 1980, they have been described as antimicrobial polypeptides and have been classified as components of the immune system and later called defensins. [5, 6]

Analysis of the phylogenetic evolution of the defensins showed that these peptides were perfectly preserved, being present from the eukaryotic cell, insects, plants to mammals, including the human species, [6] there being data in the literature showing that their evolution began 130 million years ago. [4]

The results of recent studies (2016) show that up to now more than 800 such peptides have been identified, being present in a very large proportion of plants and insects and only 30 of them identified in mammals. [8]

Structural features of beta defensins

Beta-defensins have a molecular weight between 4-5 kD, 38-50 aminoacids and 3 disulfide bridges, broken down as follows: cis cis1-cis5; cis2-cis4; cis3-cis6. [4, 8, 22]

The tertiary structure distinguishes four distinct types of human beta-defensins (hBD1-4). Their presence is especially associated with epithelial tissues, being present on the surface of the skin and intestinal mucosa.

In the oro-dental cavity they were identified in the gingival mucosa and on the surface of the tongue; they have also been identified in saliva in the context of various inflammatory processes of the oro-dental cavity, as well as in oral cancer. [8, 23]

Recent studies (2016) show that hBD1 and hBD-2 have been identified in various oral cancers. [8] In addition to hBD1-4 presented, two more beta-defensins, hBD5 and hBD6, whose presence was mainly expressed in epididym, were structurally delineated. [3]

Distribution of hBD-2 and hBD-3 in normal oral epithelium: basal layer for hBD-3 and superficial layers for hBD-1 and hBD-2. [38]

The definition, classification and etiology of peri-implant disease

Oral rehabilitation of the entire or partial edentulous patient using dental implants or prostheses fixed on the dental implants, completely revolutionized dental medicine and repositioned the concept of oral rehabilitation. [61]

Unfortunately, in the short or long term, complications may occur, including peri-implant disease. It is generally defined as a destructive inflammatory process of dental implant fixation and support systems. [61]

Numerous authors studied bacterial flora in the peri-implant supra and subgingival plaque in both favorable and clinically peri-implantitis patients and bacterial flora was found to be similar, with only quantitative differences in pathogenic species. [89]

Bacterial peri-implant flora in patients with favorable evolution is mainly formed from Gram positive cocci, immobile bacilli and a limited number of Gram negative anaerobic species. [90]

Mucositis is characterized by the presence of a large number of cocci, mobile bacilli and spirochetes, gingivitis-like bacterial flora. [90]

Peri-implantitis is associated with the presence of a large number of anaerobic Gram negative bacilli, similar to those identified in periodontal disease, respectively species belonging to the red complex: *Tannerella forsythia*, *Treponema denticola* and *Prophyromonas gingivalis*.

Also, the results of these studies have shown that there are no major differences in the composition of the bacterial flora identified around the natural teeth and around the implant. [89, 87, 91]

Peri-implantitis pathophysiology

With a common etiology, peri-implantitis has many common elements with periodontitis also regarding their pathogenesis. [80]

Studies performed by Berglundh T. et all. shows that the inflammatory process in mucositis is dominated by the presence of an infiltrate with B lymphocytes, T lymphocytes, PMN neutrophils, and macrophages like in gingivitis [96].

It has been found that the same cellular elements are identified in the peri-mplantitis, to which a high density of the dendritic cells is added; the cellularity described in peri-implant is similar to that identified in periodontitis, with the difference

that the density of the listed cell elements is greater in peri-implantitis than in periodontitis. [97,98]

Another important element in peri-implantant pathophysiology is related to the presence of proinflammatory cytokines IL-1β, IL-6, IL-8, TNF-α, MMP in peri-implant fluid as a response to the inflammatory process triggered by bacteria. [99, 100]

Involvement of beta defensins in peri-implantitis

Although the microbiota of the oral cavity impresses with the number of bacterial species and the density calculated in oral fluids (gingival fluid, saliva), there are levers whereby normal saprophytic and pathogenic flora are partially prevented in triggering pathological processes in this ecological niche. Thus, the quantitative balance between the bacterial species and the maintenance of the symbiosis relation with the host diminish to a certain extent the phenomenon of bacterial adhesion on the implanted biomaterial. [53, 61]

In this local defense scenario, by unspecified means, defenses also arise: alpha-defensins resulting from degranulation of PMN leukocytes attracted to the infection site and beta-defensins synthesized in the various layers of the gingival epithelium due to the presence of pathogenic bacteria.

Possible perspectives on the use of antibacterial peptides in medical practice

1. Using defensins as biomarkers

Peri-implantitis being a disease with a particular impact on dental health has been sought over time to identify and use of some markers to diagnose and monitor these conditions to limit incidence as presented in the previous chapters.

Interest in the possible use of defensins as markers of peri-implantitis diagnosis and monitoring is evidenced by the presence in the literature of the cited studies. The development of techniques as well as research equipment has allowed the deepening of genomic, proteomic and metabolomic studies, studies that have given rise to some questions about the involvement of defensins in the pathology of the oro-dental cavity. [105, 106]

Fluids from the oral cavity, respectively, saliva and gingival fluid, are important biological products in which qualitative and quantitative determination of defensins is performed along with other biomarkers used in the diagnosis of oro-dental diseases. [107]

2. Use of defensins in antibacterial treatment

Unlike classic antibiotics, antimicrobial peptides have a number of advantages that will certainly recommend them in the future as antibiotic alternatives.

Iseganan from the class of porcine cathecilins has been tested and has now reached the third phase of clinical trials for use in the treatment of ulcerative oral mucositis and it can be appreciated that the results obtained are encouraging for its use in the treatment of human oral mucositis.

There are studies showing that hBD-3 is highly active against antibiotic resistant and multidrug resistant bacterial strains, namely *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumanii*. [111]

3. Use of defensins in implantology

Future trends in genomics studies are that thru genetically engineering to be obtained effective defensin concentrations in oral cavity fluids so that the most common oro-dental or dental caries can be prevented.

It is also desirable to obtain an optimal defensin concentration so as to maintain a balance in the oral ecosystem between the saprophytic and pathogenic bacterial species in order to prevent the initiation and development of infectious phenomena including the occurrence of peri-implantitis and periodontal disease. [110, 114]

MATERIAL AND METHODS

1. STUDY GROUP

The study group was selected from a group of 290 patients present in a private dental office and at the Department of Implantology of the Faculty of Dental Medicine, Ovidius University from Constanta. A total of 1160 dental implants were inserted to them, between January 2015 and December 2016. I mention that 263 patients had a favorable evolution after dental implant insertion, namely 90,3%, and 27 had an unfavorable evolution, respectively 9.7%.

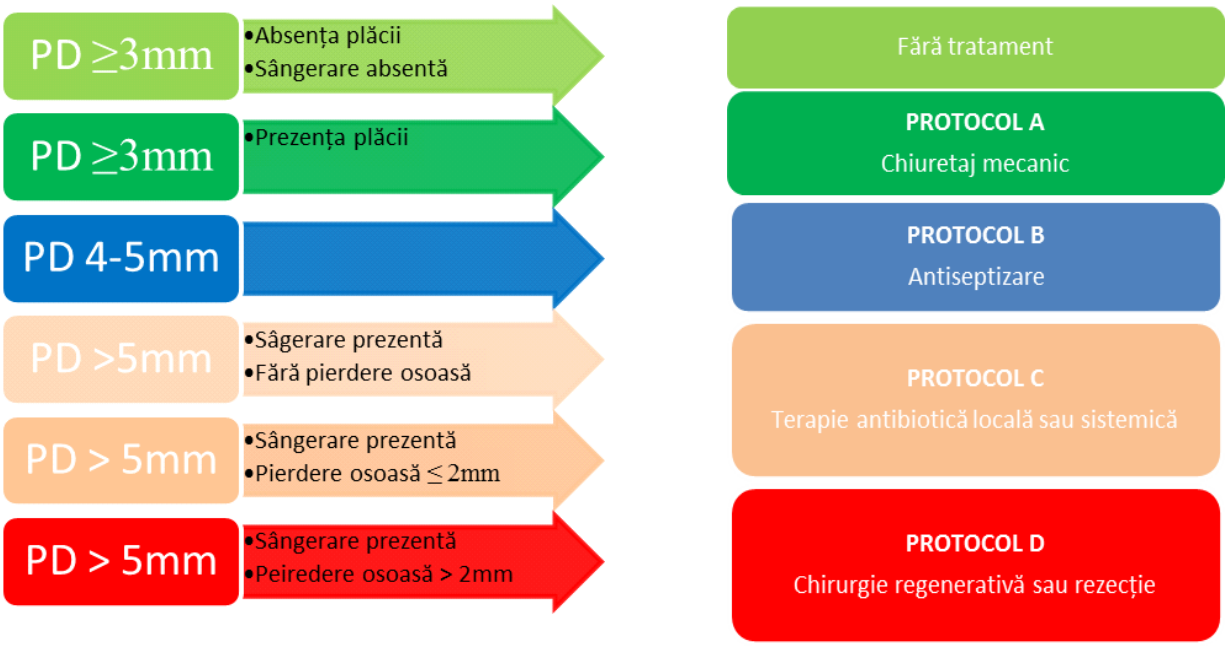
From the group of patients with favorable evolution, we randomly selected 9 patients who constituted the control group; to these we added the 27 patients with unfavorable dental implant development, thus forming the study group of 36 patients, representing 12,4% of the initial group.

The gender distribution of patients in the study group is as follows: 22 women (61,1%) and 14 men (38,9%) aged 27-70 years.

Following the orodental and radiological examination, the patients in the study group were categorized as follows: 9 patients with favorable development, 12 patients with mucositis, 15 patients with peri-implantitis.

2. EVALUATION AFTER DENTAL IMPLANT INSERTION

After the oro-dental clinical evaluation performed 7 days post-dental implant, based on the same clinical and radiological parameters, the patients were treated according to Mombelli and Lang's Cumulative Interceptive Supportive Therapy (CIST) protocols as shown in the figure below . [126, 127]



Treatment Protocols (CIST)

3. PATHOLOGICAL PRODUCTS AND HARVESTING METHODS

As shown in the literature, hBD1-3 synthesis sites are salivary duct cells, oral mucosa cells and some blood cells (PMN leukocytes). [41, 129]

There are also studies that attest to the fact that salivary mucins make it impossible to quantify hBD-2 and -3 in saliva; specifically, sialic acid residues mask hBD-2 and -3 in the proportion of 25-30% [130].

For these reasons, we decided for the quantitative evaluation of hBD-3 to use the

peri-implant fluid and saliva fluid for the quantification of IL-1 beta. Peri-implant fluid samples were harvested using sterile paper cones, which were inserted into each peri-implant ditch for 30 seconds.

4. DESCRIPTION OF THE DENTAL IMPLANT

We used NeoCMI Implant System Implant from NeoBiotech which is a screw-type implant made of pure titanium. [128] It has a denture connection system and an internal hex (IS) system. Also, the surface of the implant is of the IS-II Active type, which provides a microporous surface area to increase the area of contact with the alveolar bone. [128]

FIRST STUDY - RESEARCH ON THE POSSIBLE CORRELATION BETWEEN BETA-3 DEFENSINS AND ORODENTAL CLINICAL STATUS IN MUCOSITE AND PERI-IMPLANTITIS

Currently, dental implants are the most important method of replacing one or more missing teeth in different clinical situations.

The goal of modern dental medicine is to restore the patient's oro-dental system, enhancing the functional, comfort and aesthetic state of health, by restoring a single tooth or by replacing several teeth. [61, 80]

The development and introduction of the concept of osteointegration of endooseous implants by Dr. Branemark was an important moment in the evolution of the principles of treatment in dental medicine. Thus, the use of dental implants has increased over the past three decades, providing a solution for partial or complete edentatiosn, with many benefits for patients. [61]

At the same time, in addition to the advantages of treatment with dental implants, there is also the possibility of complications represented by mucositis and peri-implantitis. [80] Both disorders are characterized by an inflammatory reaction in the surrounding tissues of the implant. [61, 79, 80]

Mucositis has been described as a disease in which the presence of inflammation is restricted to soft tissues around a dental implant, with no signs of loss of adjacent bone tissue, with the presence of bone remodeling during healing. Peri-implantitis has been defined as an inflammatory process around an implant, which includes both soft

tissue inflammation and progressive bone loss. [61, 79, 80]

Studies performed by Mombelli et all. shows that the peri-implantitis prevalence relative to the inserted implant number is 10% and the number of dental implant patients is 20%, estimated after the patient's assessment for 5-10 years. [83]

There are different values of mucositis and peri-implantitis prevalence, between 5 and 63,4%, based on a wide variety of factors considered, such as the size of the patient batch and design of the study. [122, 123]

One of the most important factors in the etiopathogenicity of the peri-implantitis is bacterial colonization of the implant surface. Bacterial contamination of the implant generates an inflammatory response that can be evaluated from peri-implantation fluid (PICF), defined as an osmotic-mediated inflammatory exudate originating from the gingival plexus vessels. Its composition is similar to gingival fluid (GCF), containing inflammatory mediators, tissue and defensine degradation products as part of innate immunity of the host against the bacterial species present around the dental implant. [105]

WORKING HYPOTHESIS - The anti-bacterial effect of hBD-3 could influence the oro-dental clinical status of mucositis and peri-implantitis.

THE PURPOSE OF THE STUDY-was to identify the existence of a possible correlation between the quantity of hBD-3 in the peri-implant fluid and the evaluation parameters of the oro-dental clinical status in mucositis and peri-implantitis.

RESULTS

The statistical processing of the hBD-3 values obtained in the three groups shows that there are statistically significant differences between the groups of patients with mucositis and peri-implantitis, and between patients with favorable implant progression and those with peri-implantitis.

The statistical study of peri-implant denture depth (ASP) values shows that there are statistically significant differences between the three study groups ($p \leq 0{,}001$).

Statistical analysis shows that there is a high inverse correlation between ASP and hBD-3 quantified in the group of patients with favorable implant evolution. In the

batch of patients with peri-implantitis and mucositis the statistical study shows that there is a high positive correlation between the two evaluated parameters ($p \leq 0,001$).

PRELIMINARY CONCLUSIONS

1. The hBD-3 defensin quantified in peri-implant crevicular fluid can be used as a parameter for assessing the clinical status of the patient after the insertion of the dental implant.
2. Quantification of hBD-3 can be used in monitoring dental implant patients to assess the occurrence of possible complications: mucositis and peri-implantitis.
3. The evaluation of hBD-3 in the dental implant patient may be an objective argument in the correct choice of the therapeutic protocol.

SECOND STUDY - BETA-3 DEFENSINS AND IL-1 IN PERI-IMPLANTITIS

The intensity of the inflammatory process from peri-implantitis is explained by the abundance of PMN and macrophages, these cells being the main source of IL-1 synthesis [137, 138, 139]. The overwhelming role of IL-1 beta in peri-implantitis is explained by the potential of this interleukin to stimulate humoral immune response and cellular immune response as well as the ability to induce the synthesis of other interleukins, among which the most important are IL-6 and IL- 2 [100, 137, 138, 139]

WORKING HYPOTHESIS - Interleukin 1 beta is a nodal element in the peri-implantitis inflammatory process, the quantitative expression of it is related to the intensity of the inflammatory process.

THE PURPOSE OF THE STUDY - is to establish the possible correlation between quantified IL-1 beta in peri-implant crevicular fluid and clinical status in patients with favorable evolution and peri-implantitis.

OBJECTIVES OF THE STUDY

Quantitative assessment of IL-1 beta in patients with favorable evolution after dental implant insertion and peri-implantitis patients; Evaluation of the correlation between IL-1 beta and oro-dental clinical status in the two groups of patients;

Evaluation of statistical significance between IL-1 beta and beta 3 defensins quantified in peri-implant fluid in the two groups of patients in the 3 assessment moments.

RESULTS

Within this research pillar, we evaluated the two "extremes" from the clinical point of view, namely the group of patients with favorable evolution post dental implant and the peri-implantitis group, starting from the premise that between the two groups there are great differences in the intensity of the inflammatory process.

We obtained results demonstrating the existence of statistically significant differences regarding the IL1-β values between the favorable evolution and peri-implantitis patient groups in all three assessment moments ($p < 0.0001$).

The study of the possible correlation between the depth of the peri-implant pocket and IL1-β in the group of patients with favorable evolution and in the group of peri-implantitis patients after insertion of dental implants shows the existence of a high positive association between the two parameters.

PRELIMINARY CONCLUSIONS

1. Quantified IL-1β in the peri-implant fluid may be used to assess the oro-dental clinical status in the patient after the dental implant insertion.
2. IL-1β and hBD-3 can be used as biomarkers for the diagnosis and monitoring of patients after insertion of dental implants.

THIRD STUDY - BETA-3 DEFENSINS AND BACTERIAL SPECIES IN PERI-IMPLANTITIS

The role of bacteria in initiating the inflammatory process from peri-implantitis is an unquestionable reality, and many studies have been accessed in the literature, which demonstrates how bacteria initiate and maintain this condition. [61, 83] The first studies on this theme have started from the similarities between periodontitis and peri-implantitis in terms of the common variety of bacterial species generating these diseases. [142, 143]

Obviously, in time it has been demonstrated the existence of elements that make the peri-implantitis more specific from the point of view of the bacterial etiology. Thus,

the favorable post-dental implant evolution is bacteriologically characterized by the colonization of the peri-implant fluid with species from the *Streptococcus* family.

In the peri-implantitis, a colonization with saprophytic species, without pathogenicity, but later colonization occurs with gram negative bacterial species, with high pathogenic potential, among which *P. gingivalis*, *T. denticola* and *T. forsythia* are occupying a important place. [61, 80, 83]

WORKING HYPOTHESIS - Periodontal pathogenic bacteria are involved in the initiation and development of the peri-implantitis inflammatory process.

THE PURPOSE OF THE STUDY - is to identify the presence of bacterial species in the peri-implant fluid after the insertion of the dental implant in patients with favorable evolution, mucositis and peri-implantitis.

OBJECTIVES OF THE STUDY

Identification of bacterial species in peri-implant crevicular fluid in the three groups of patients; Establishing the correlation between the depth of the peri-implant sulcus and the presence of the bacterial species in the 3 groups of patients considered in the study; Establishing the correlation between the presence of bacterial species and hBD-3 in the three groups of patients considered in the study.

PROTOCOL OF MICROBIOLOGICAL ANALYSIS

Strictly anaerobic bacteria have been developed in special enclosures where anaerobiosis was performed using oxygen reducers (GEN bag anaer, BioMerioux). Identification of bacterial species was done in the API-BioMerieux system using the following kits: Rapid ID 32 A, API 20 STREP, ID 32 STAPH.

RESULTS

Taking into consideration that specialty studies show that the completion of bacterial colonization takes place two weeks after the insertion of the dental implant [52,100,138], the results of the present study show that there is an association between the depth of the peri-implant sulcus and the presence of the combination of the three

bacterial species (*Porphyromonas species*, *Fusobacterium mortiferum* and *nucleatum*, *Tannerella forsythia*) in all three groups of patients evaluated at 21 days.

The novelty of the study is related to the existence of an association with statistical significance between the depth of the peri-implant sulcus and the bacterial species considered in the study, emphasizing this association in all three groups of patients.

Also, as another novelty element of this study is the identification of threshold values for hBD-3 in the context of the presence of bacterial species considered for mucositis and peri-implantitis.

These values are "alarm signals" for the attending physician, so patients who have hBD-3 values above the estimated threshold in association with these bacterial species require monitoring and treatment according to the current therapeutic protocols. It should be noted that the association the presence of *S. aureus* signifies reserved evolving prognostic, as is mentioned in other studies. [90]

What brings novelty to the present study on peri-implantant microbiology is the identification of the correlation between Gram negative anaerobic species and the peri-implant damage level expressed by the peri-implant sulcus depth, there being statistical significance between these parameters for a critical threshold of the peri-implant sulcus of 4 mm in peri-implantitis and 3 mm in the mucositis.

PRELIMINARY CONCLUSIONS

1. The presence of pathogenic bacterial pathogens as perimplantant colonization elements defines the patient's subsequent evolution after the insertion of the dental implant.
2. The association of hBD-3 with pathogenic bacterial species requires appropriate treatment and monitoring.

FINAL CONCLUSIONS

- hBD-3 defensin quantified in peri-implant crevicular fluid can be used as a parameter for assessing the clinical status of the patient after the insertion of the dental implant.
- Quantification of hBD-3 can be used to monitor dental implant patients to assess

possible complications: mucositis and peri-implantitis.

- The assessment of hBD-3 in the dental implant patient may be an objective argument in the correct choice of the therapeutic protocol.
- IL-1β quantified in the peri-implant fluid is a biomarker that can be used to assess the oro-dental clinical status in the post dental implant patient.
- IL-1β and hBD-3 can be used as biomarkers for the diagnosis and monitoring of patients after insertion of dental implants.
- The presence of bacterial species with pathogenicity potential as peri-implant colonization elements in the peri-implant fluid are elements that define the patient's subsequent clinical development after the insertion of the dental implant.
- The association of hBD-3 with pathogenic bacterial species requires appropriate treatment and monitoring.

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