Microbiological survey of periodontal pockets as a diagnostic and therapeutic support, and for the prevention of periodontal disease

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ABSTRACT

Aim: *The close relationship between oral microbiota and progression of periodontal disease is established. We have the need to find a preventive/therapeutic design for the non-surgical treatment of periodontal disease and the identification of related systemic factors.*
Materials and methods: *The protocol of our study includes a periodontal microbiological examination on periodontal patients before and after SRP therapy, and a cytologic investigation using a brush on cheek mucosa. The microbiological investigation consists in the collection, by means of sterile paper cones, of sulcular fluid from the patient's periodontal pockets. We have chosen 4 sites that are more suitable for collection, the sterile paper cones have been inserted one by one in the selected pockets, and left inside for 20 seconds. An analysis was made of the bacterial DNA of the main pathogens, extracted from the paper cones. In 22 patients a cytological sampling was done using a brush, to identify the polymorphisms of the interleukin. Such polymorphisms are related to the patient's ability to interrupt the inflammatory process once the stimulus that triggered it has stopped The correlations between the presence of bacterial microorganisms and the tendency to inflammation, and the general pathologies reported in the patient's anamnesis were also analyzed.*
Results: *Twenty-eight periodontally compromised patients were examined. Of these only 20 completed the periodontal treatment plan, carrying out the microbiological analysis after SRP. All 28 patients presented with the first periodontal sampling, a high concentration of the red complex microorganisms. In 17 of 20 patients, a second periodontal microbiological analysis resulted in a lower bacterial concentration compared to the pathogenic microbial sampling.*
Conclusion: *Periodontal microbiological analysis proved useful both in periodontal diagnostic investigation and therapeutic purposes. The study highlighted some differences, compared to the data found in the literature, in the correlations between patient habits and systemic diseases and the presence of periodontal disease.*

Introduction

The modern concept of dentistry puts the dental professional in the condition to consider the oral cavity as a structure connected and related with the other systemic structures, capable of altering functionally and qualitatively health status.

The concept of biofilm intended as extended, strong/tenacious and organised polyfunctional colony of bacterial/viral matrix developed in the course of the twentieth century is accepted globally and has evolved in today's considerations of relationship that the biofilm interacts with the external environment. The new definition of microbioma takes into account the main actors of the biofilm but puts them in relation to each other, the external environment of remote structures to those of the apparatus in question and the geno-phenotypic manifestations/symptom of the host.

These considerations are indispensable in the diagnosis of the oral cavity diseases, especially on a global vision.

Periodontal disease, although differing greatly in clinical forms and course, is globally increased. The systematic reviews estimate the possibility of an incidence between 5 and 15% in the world population, not considering gender as a discerning factor.

These numbers require attention in preventive terms. The development of primary prevention, i.e. procedures to prevent the onset of the disease, is in progressive diffusion, although protocols and guidelines are very variable and often not validated by the literature.

The relationship between oral microbiota and progression of periodontal disease are absolutely undeniable although of complex definition, and are closely linked to one another by mechanical characteristics that deserve a thorough analysis.

The great use of antimicrobials and antibiotics has led to a progressive depersonalization of treatment which, on the contrary, it should be strongly patient oriented for a successful stabilization or a full cessation of the disease.

This is in the direction of progressive preservation of systemic health and increase of quality of life, as therapeutic acceptance and psychological management of the inconvenience resulting from the increase of prosthesis for loss of teeth could be entirely predictable and/or avoidable. Such consideration is true especially in the presence of co-morbidity which has demonstrated in the past a bidirectional link with periodontal disease (PD).

The interception of the patient is a crucial process for the definition of the treatment plan and establish the baseline condition of oral microbiota, major oral phlogistic markers related to integral charting evaluations, radiodigraphic assessments and mechanical sectoral instrumentation, are parameters of real quality in directing of an effective treatment plan.

In this perspective the "Prevention pack" was designed for preventive/therapeutic purposes in the non-surgical treatment of periodontal disease, including:
- recognition quality/quantity of the bacterial population of the patient;
- analysis of the main oral phlogistic markers;
- evaluation of motivation by integral charting and radiodiagnostic evaluation as support;
- mechanical removal of debris (SRP) optionally assisted.

The aim of the "Prevention pack" is to limit the initial adverse effects of periodontal disease based on quantifiable methods and qualifies as reflected in its effectiveness over time.

Materials and methods

The "Prevention pack" is a protocol that provides a periodontal microbiological examination of the patient followed by a complete periodontal study with radiographic investigations and pocket probing and four SRP cycles. The microbiological investigation consists in the collection, by means of sterile paper cones, of sulcular fluid from the patient's periodontal pockets. At first it is possible to choose the 4 sites for the cone collection. The selection criteria are related to the depth of the periodontal pocket, and to the presence of bleeding. Once the most suitable sites are chosen, the sterile paper cones are inserted one

by one in the selected pockets, and left inside for 20 seconds. After that they are extracted and placed in a test tube ready for shipment. The sampling data, i.e. depth and probing, bleeding and pus sites are noted in the request for microbiological analysis in the laboratory. The test tube with the samples is then stored in the refrigerator at + 2 ° until use. In the laboratory, an analysis is made of bacterial DNA of the main pathogens, namely Actinobacillus Actinomycetem comitans, Porphiromonas gengivalis, Bacteroides forsythus, Treponema denticola, Prevotella intermedia, Peptostreptococcus micros, Fusobacterium nucleatum, Campylobacter rectus, Eubacterium nodatum, Eikenella Corrodens, Capnocytophaga. The bacterial DNA is amplified by PCR (polymerase chain reaction) and the amplified products are analyzed by reverse dot-blot. The presence of DNA is determined by reverse hybridization (amplified gene sequences specifically hybridize to membrane-bound oligonucleotide probes). In addition, in many patients cytological sampling was done using a brush with transport medium. The brush was rubbed on the patient's dry oral cheek mucosa for 2 minutes and then reinserted into the appropriate container with the transport medium.

With this method, the laboratory analyzes the polymorphisms of the human interleukin 1 gene, precisely in position -889 of the IL-1A gene, +3953 of the IL-1B, +2018 of the human IL1 receptor antagonist gene. Here too, the regions of the genes where mutations are located are amplified by PCR and the products obtained are analyzed by reverse dot-blot. The presence or absence of the mutation is determined by reverse hybridization. Such polymorphisms are related to the patient's ability to interrupt the inflammatory process once the stimulus that triggered it has ended. The results that the laboratory sends place the patient in category A, B, C, D, depending on whether they are respectively: normal inflammatory response; slight alteration of the inflammatory response; moderate alteration; severe alteration.

Once the report has arrived, the patient is given the most appropriate antibiotic treatment based on the sensitivity of the pathogens found to specific antibiotics. Subsequently, the patient continues the therapeutic procedure of the "Prevention pack".

Furthermore, the correlations between the presence of bacterial microorganisms and the tendency to inflammation, and the systemic pathologies reported in the patient's anamnesis are analyzed.

Results

Twenty-eight periodontally compromised patients were treated using the "Prevention pack" method. Of these patients, only 20 completed the periodontal treatment with two microbiological analyses of

periodontal bacteria. It was found that 17 out of 20 patients had a bacterial concentration in the second microbiological sampling, lower than the pathogenic bacterial concentration, thus demonstrating the success of the pharmacological therapy combined with mechanical removal of calculus. All 28 patients presented at the first periodontal sampling, a high concentration of microorganisms of the red complex, in particular Porfiromonas gingivalis, Bacteroides Forsytus, Treponema Denticola, all three sensitive to metronidazole. In 13 out of 17 subjects microorganisms of the orange complex were present, in particular Prevotella Intermedia, also sensitive to metronidazole; 4 presented levels above the threshold of Actinomicetem comitans, sensitive to the combination of Metronidazole and Amoxicillin + clavulanic acid. Interestingly, 3 patients showing high levels of periodontal bacteria had the association of the two red and orange complexes. Only in one of our 28 patients treated the presence of Eikenella Corrodens was beyond the threshold parameters. Contrary to what could be thought, only 9 of these 28 patients are smokers, a factor that indicates that periodontal disease is not necessarily linked to the smoke; 18 of these 28 patients are women, since it contrasts with the prevalence rates on the population in the literature. This could also mean that there is more women's attention to periodontal health. However 7 of these women are smokers. While 2 men only among those periodontally treated were smokers, so there is a higher percentage of women smokers with periodontal problems than men, even here contrary to what one might think.

Regarding the correlation with systemic diseases we found that 5 of the total patients suffer from high blood pressure while 5 others suffer from cardio-circulatory diseases, resulting to be the most widespread pathology in this sample; 5 patients suffer from allergic problems (4 women and 1 man); 4 patients, all women suffer from G6PD deficiency. This means that in our sample 16 out of 22 patients who have been tested have an alteration of the normal inflammatory response. The data suggests that a correlation between certain types of interleukin polymorphisms and periodontal disease is possible, so it may be interesting to use the test as a screening to evaluate the patient's tendency to develop periodontal disease or not.

Discussion

Periodontal disease represents a pathology affecting the whole body in various ways. The link between alterations of connective tissue and systemic pathological conditions is widely considered in scientific literature, with many aspects to be evaluated with sufficient precision and attention to translational scientific research.

The systemic health status can affect periodontal conditions and, similarly, the chronic periodontal damage, caused by absence of correct hygiene, affects different health levels.

The idea of the "Prevention pack" is an effective and patient-oriented approach and it is targeted to deliver an efficient prevention system for patient and clinician.

Microbiological tests

Twenty patients completed the causal therapy protocol. The test was performed in first analysis for 17 patients.

There are high concentrations of microorganisms that can be considered in the red complex. In particular, Porphyromonas gingivalis, Bacteroides Forsytus, Treponema Denticola, are sensitive to metronidazole of the nitroimidazole family, particularly active towards anaerobic pathogens. In 13 patients there were microorganisms of the orange complex, in particular Prevotella Intermedia, sensitive to metronidazole; 4 patients presented high level of Aggregatibacter actinomycetemcomitans, sensitive to the Van Winkelhoff cocktail (combination of Metronidazole and Amoxicillin + clavulanic acid). Interestingly, periodontal lesions showed the association of both red and orange complexes. Only in one of the 28 patients considered in the analysys, Eikenella Corrodens was found over threshold health parameters.

Determination of bacterial colonies played a fundamental role in understanding the bacterial etiopathogenesis, classify it and orient the chemical anti-microbial adjuvant therapy.

Test for genetic polymorphisms

Periodontal brush sampling has also allowed the identification of risk in a specific category according to a risk/response model.

The polymorphisms of the human interleukin 1 gene were analyzed, in position -889 of the IL-1A

Table 1 Risk/response model

Category	A	B	C	D
Risk	Normal/adequate inflammatory response	Light alteration of inflammatory response	Moderate alteration of inflammatory response	Strong alteration of inflammatory response

gene, +3953 of the IL-1B, +2018 of the human IL1 receptor antagonist gene. Gene's regions are localized and mutations amplified by PCR. The presence of polymorphisms is connected with the ability to interrupt the inflammatory process once the stimulus that determined it has ceased.

Polymorphism analysis traces a perspective profile of the pathology responses or its stabilization with important indications on the possibility of recrudescence.

From a clinical point of view, PCR probe assays are eligible to establish a rational quantification of recall rhythms and the planning of future causal therapies. Furthermore, the tendency to remission from phlogistic alterations is also matchable for statistical purposes with the presence of pre-existent basic co-morbidity.

In the group of 22 patients analyzed, 3 types of results were found.

Six patients presented a Type A risk, with normal response to the inflammatory reaction, while 13 patients present a type B risk, with a slight alteration of the immune response. Three patients were attributed to the type of risk C, with an increased inflammatory response.

Sixteen patients in the analysis of periodontal fluid samples, presented a type D risk with significant changes in the inflammatory response determined by genetic polymorphisms. Results suggest that a correlation between specific types of interleukin polymorphisms and periodontal disease is plausible. In future it could be interesting to use the test as a screening to evaluate the tendency to develop forms of periodontal disease by refining specificity and sensitivity parameters.

Modifiable risk factors

Among the 28 patients initially subjected for analysis, presenting periodontal changes of various forms and severity, 9 were habitual users of burned tobacco; 18 patients were women. Observed data deserve future considerations and sample size extension as well as being conflicting with prevalence values reported by many authors in scientific literature.

Data found in the considered sample does not indicate an obvious role of the habitual use of tobacco smoke as a necessary condition for the development of phlogistic changes and the determinism of periodontal diseases. Forms of periodontal disease can be observed even in patients without tobacco interactions or long time dismissed smoking habits.

It is important to underline that periodontal disease presents a multifactorial etiology and the determinism of modifiable and subjective risk factors, also for a genetic/microbiological alterations, constitutes a focal objective to be achieved and consolidated.

Out of 18 female patients, only 7 are smokers. The male smoking population included 2 subjects. This is conflicting with literature findings because it suggests an increasing risk for female subjects in periodontal disease development.

Five patients out of 28 suffered from cardio-circulatory diseases, 5 presented forms of hypertension, 5 suffered from allergic problems (4 women and 1 man). In 4 female patients, the enzyme Glucose-6-phosphate dehydrogenase was found to be deficient.

Limits of this study are essentially related to the impossibility to carry out a complete antibiogram for each patient, before and after the microbiological investigation. We can not estimate data on the possible susceptibility and bacterial resistance to the antibiotics used. Based on preliminary data collected about 3 years ago, the study protocol could be re-opened by including complete antibiogram assays of the "Prevention Pack".

Conclusion

Periodontal bacterial tests and genetic tests related to interleukin polymorphisms can be considered useful as screening for the entire adult population, to evaluate the tendency of patients to develop forms of periodontal disease, in consideration of their non-invasiveness, ease of execution and low cost.

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