

RESEARCHES REGARDING THE MONITORING COMPLEMENTARY TREATMENT IN PERIODONTITIS BY USING SALIVARY 8-OHdG BIOMARKER

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Abstract

The aim of this study was to evaluate the efficiency of complementary methods – apitherapy, in patients with chronic periodontitis, by using salivary levels of 8-OHdG biomarker.

The study group was formed of 32 patients with chronic periodontitis (superficial and deep), that were treated 6 weeks by using: conventional methods (16 patients) and by conventional methods associated with complementary methods using an ethanolic mixture of propolis and royal jelly (16 patients). The salivary level of oxidative stress produced by bacteria species involving chronic periodontitis was determined by assessing the salivary 8-hidroxy-2-deoxy guanosine (8-OHdG) before and after the treatment using an ELISA method. Patients with chronic superficial periodontitis were cured clinically, in parallel with the decrease of the salivary level of 8-OHdG for the both types of treatment studied. At the patients with chronic deep periodontitis treated by conventional methods associated with complementary methods was observed a higher regression of periodontitis, correlated with lower levels of salivary 8-OHdG, compared with the patients treated only by the conventional method.

The results suggest that apitherapy can be successfully used as a complementary method in the treatment of periodontitis. The study demonstrates the existence of a high correlation between the values of salivary 8-OHdG biomarker and the status of periodontal tissue after different ways of treatment.

Rezumat

Scopul acestui studiu a fost evaluarea eficienței metodelor complementare de tratament – apiterapia, aplicată la pacienții cu parodontită cronică, utilizând nivelurile salivare ale biomarkerului 8-hidroxi-2-deoxi guanozina salivară (8-OhdG).

Grupul de studiu a fost format din 32 pacienți cu parodontită cronică (superficială și profundă), care au fost tratați 6 săptămâni prin metode convenționale (16 pacienți) și metode convenționale asociate cu metode complementare, folosind un amestec de propolis și lăptișor de matcă într-o soluție alcoolică (16 pacienți).

Nivelul stresului oxidativ salivar produs de speciile bacteriene implicate în producerea parodontitei cronice s-a evaluat prin determinarea biomarkerului salivar 8-OHdG înainte și după tratament, utilizând metoda ELISA.

Pacienții cu parodontită cronică superficială s-au vindecat clinic, în paralel cu normalizarea nivelului salivar al biomarkerului 8-OHdG pentru ambele modalități de tratament studiate. La pacienții cu parodontită cronică profundă tratați prin metode convenționale asociate cu metode complementare s-a observat o ameliorare clinică evidentă a bolii parodontale, concomitent cu niveluri salivare mai mici ale biomarkerului 8-OHdG, comparativ cu pacienții care au fost tratați numai prin metode convenționale. Rezultatele sugerează că apiterapia poate fi folosită cu succes ca metodă complementară în tratamentul parodontitei. Studiul demonstrează existența unei corelații între valorile biomarkerului salivar 8-OHdG și starea țesutului parodontal după diferite modalități de tratament.

Keywords: apitherapy, 8-OHdG, periodontitis.

Introduction

Periodontal disease can be defined as an inflammatory disease that affects the supporting structures of teeth [7]; in the present, many researchers appreciated that periodontal disease is one of the major public health problem in the world and is the most common cause of tooth loose in adults [7, 15].

Periodontal disease is associated with anaerobic and microaerophilic bacteria that produce enzymes and toxins that can cause directly tissue damage to periodontal tissue [8, 14, 15, 21].

Therefore, in the periodontal tissue the presence of parodontopathogenic bacteria will activate phagocytes that release reactive oxygen species; their presence will generate destructive processes in the periodontal tissue and cause DNA and protein damages, lipid peroxidation [12, 17]; an important marker for the DNA damage present in saliva is 8-hidroxy-2-deoxy guanosine (8-OHdG), that is the result of a guanosine oxidation, process induced by reactive oxygen species [18, 19, 22, 23].

Conventional therapy in periodontitis consists of mechanical therapy, surgical treatment, and antibiotics - indicated to prevent locally or systemic complications [6].

On the other side, antibiotic therapy has some disadvantages such as: toxicity problems, possibility to develop allergies, resistance of anaerobic bacteria species that became in the present a serious problem worldwide and sometimes a high cost [6, 7].

Therefore, new strategies for treatment of periodontal disease must be developed and especially the discovery of an alternative treatment that will partially or totally exclude the use of antibiotics [2, 4, 9].

In this context, special attention has been accorded to natural medication, among which apitherapy (therapy can include the use of honey, propolis, pollen, royal jelly and bee venom), that could be a good alternative [9, 11, 16].

Having a very good anti-inflammatory effect, honey can be used in periodontitis treatment; antioxidant effect of honey was demonstrated by their protective activity to the periodontal tissue against damage of free radicals produced following inflammatory response [2].

Royal jelly contains 17 amino acids, vitamins and trace elements like zinc, copper and manganese; these elements are endogenous components of the detoxifying enzymes and furthermore, they contain a substance with antibiotic effect named royalisin [2, 11].

Modern research has shown that propolis is composed of more than 150 individual substances (flavonoids, phenolic compounds, aromatic acids) that have many biological effects such as: anti-inflammatory, antimicrobial, antioxidant, immunomodulatory and analgesic effects [1, 2, 3].

Among these effects, antimicrobial activity is focused against many pathogenic microorganisms such as bacteria, viruses and fungi; also, was reported that propolis has significant antimicrobial activities in saliva samples from periodontitis patients, suggesting that propolis can be used to inhibit oral microbial species [5, 10].

Because periodontitis is an infectious disease in which the bacterial oxidative stress is a very important process, our study aimed to compare results in periodontitis treatment by conventional methods with the mixed treatment (conventional and complementary methods - apitherapy), using the 8-OHdG salivary biomarker.

Materials and Methods

Study group

The study group consisted of 32 patients (18 males; 14 females), aged 35-44 years, living in Constanta, Romania, who had varying degrees of periodontitis. Ethical permission to conduct this study was given by the Professional Ethical Committee of Ovidius University, Constanta, in order to respect the ethical principles for medical research involving human subject, given by the World Medical Association Declaration of Helsinki. After periodontal examination including: BOP (bleeding on probing), API

(approximal plaque index), PPD (probing pocket depth), the patients were separated in 2 groups:

- chronic deep periodontitis-16 patients (8 males; 8 females)
- chronic superficial periodontitis-16 patients (10 males; 6 females).

In the end of clinical evaluation, each group was divided in 2 subgroups likewise:

-chronic depth periodontitis - first group (4 male; 4 female), that were treated by conventional therapy (mechanical scaling, root planning, surgical treatment) and second group (4 male; 4 female) were mixed treated, in combination - conventional therapy and apitherapy.

- chronic superficial periodontitis - first group (5 male; 3 female), that were treated by conventional therapy (mechanical scaling, root planning, surgical treatment) and second group (5 male; 3 female) were mixed treated, in combination-conventional therapy and apitherapy.

Clinical orodental status was assessed before treatment and was repeated 3 weeks and 6 weeks after treatment by two examiners in the Department of Preventive Dentistry Ovidius University Constanta, Romania, Faculty of Dental Medicine.

Apitherapy

A mixture of 100 mg propolis, 150 mg royal jelly, in 7.5 mL 12% ethanol was used as complementary treatment; the treatment consisted of mixture application in certain periodontal pockets once a week for 6 weeks, using a syringe.

Saliva sampling

Saliva samples were collected using a standard method, compatible with analysis of biomarkers, namely passive collection in sterile containers.

The samples were obtained before the treatment, in the same appointment with the clinical exam, but right before the oral examination (to avoid blood contamination of the saliva samples). The saliva collection was repeated in the same conditions 6 weeks after the treatment.

Saliva samples were used for measuring the level of oxidative stress by assessing the salivary level of 8-OHdG biomarker.

8-OHdG analyses

The salivary 8-OHdG determination was performed using an ELISA kit (Cayman Chemical, USA). This assay is based on the competition between sample 8-OHdG and 8-OH-dG-acetylcholinesterase conjugate for a limited amount of 8-OHdG monoclonal antibody.

Statistical analyses

Statistical analysis was performed using SPSS 14.0 for Windows and MedCalc 11.3.0. Differences between the levels of 8-OHdG before and after

treatment were analyzed by using Student's t-test, $p < 0.05$ (two tail). Analysis of the correlation between variables was made by measuring Pearson coefficient. All statistical analyses were performed using statistics software; the statistical significance of the results was defined: $p < 0.05$.

Results and Discussion

The patients with superficial chronic periodontitis, in the both studied groups were clinically cured and in parallel the salivary level of 8-OHdG decreased:

-in the conventional treatment, the decrease of 8-OHdG was from 2.48 ± 0.26 ng/mL to 1.75 ± 0.13 ng/mL;

-in the mixed treatment, conventional treatment associated with apitherapy, the decrease of 8-OHdG was observed from 2.48 ± 0.26 ng/mL to 1.58 ± 0.08 ng/mL.

Clinical healing was made quickly after the mixed treatment (3weeks) and after 6 weeks using only the conventional treatment.

Statistical processing of the data showed a significant difference in the mean value of salivary levels of 8-OHdG before and after the treatment ($p < 0.001$) in the both types of treatment; similarly, there is a significant difference in the mean value of salivary levels of 8-OHdG in periodontal patients mixed treated compared with conventional treated patients ($p = 0.011$) (Table I).

Table I

The 8-OHdG level according to the type of treatment in chronic superficial periodontitis

8-OHdG (ng /mL)	n	Range	Mean \pm SD	SE mean	p
Conventional treatment	8	0,35	1.75 ± 0.13	0.04	0.011 (*)
Conventional treatment & Apitherapy	8	0,22	1.58 ± 0.08	0.02	
* $p < 0.05$					

For the groups of patients with deep chronic periodontitis, a clinical improvement was observed in parallel with the decrease in salivary levels of 8-OHdG.

-in conventional treatment, the decrease of 8-OHdG was from 3.87 ± 0.12 ng/mL to 3.09 ± 0.51 ng/mL;

-in mixed treatment, conventional treatment associated with apitherapy, the decrease of 8-OHdG was made from 3.87 ± 0.12 ng/mL to 2.54 ± 0.47 ng/mL.

Statistical processing of the data showed a significant difference in the mean value of salivary levels of 8-OHdG before and after the treatment ($p < 0.001$) for the both methods of treatment; also, a statistical difference was observed in the mean value of salivary levels of 8-OHdG in periodontal patients mixed treated compared with conventional treated patients ($p = 0.021$) (Table II).

Table II

The 8-OHdG level according to the type of treatment in chronic deep periodontitis

8-OHdG (ng /mL)	n	Range	Mean \pm SD	SE mean	p
Conventional treatment	8	1.43	3.09 \pm 0.51	0.18	0.021 (*)
Conventional treatment & Apitherapy	8	1.39	2.54 \pm 0.47	0.16	
* $p < 0,05$					

Very important for the clinical evaluation was the periodontal pockets depth, that shows a significant decrease in chronic periodontal deep from 5.44 \pm 0.87 mm to 4.61 \pm 0.05 mm after the conventional treatment and to 4.27 \pm 0.08 mm after the mixed treatment.

The analysis of the correlation between the individuals salivary levels of 8-OHdG and the pocket depth (Pearson coefficient) shows that there is a high significant relationship ($r = 0.990$) between this two variables in mixed treatment (Table III).

Table III

The correlation of the 8-OHdG level and periodontal pockets in deep chronic periodontitis

8 OHdG (ng/mL)	Probing depth (mm)	
Conventional treatment	Sig.(2-tailed) ¹	Pearson Correlation / Sig.(2-tailed)
Conventional treatment & Apitherapy	0.000 (*)	0.990 (**) / 0.000 (*)
* $p < 0,001$, ¹ Paired Samples T test , ** Correlation is significant at the 0.01 level (2-tailed)		

In the same time, there were not significant differences between the gender and the salivary levels of 8-OHdG no matter the method of treatment used in all groups studied ($p > 0.05$).

Periodontitis is recognized as a major public health problem in the world and is the most common cause of tooth loss in adults according to the World Health Organization [20].

In this context, many researches have reported the benefits of apitherapy in periodontal disease and suggested that, in the future, apitherapy should play a key role in periodontitis treatment [1, 2, 9].

On the other side, a direct proportional relationship between salivary level of 8-OHdG biomarker and the severity of periodontal disease was demonstrated in many studies [19, 22, 23].

According to our results, the association between propolis and royal jelly in alcoholic solution with conventional treatment of periodontal disease was demonstrated to have benefic effects in the evolution of periodontal disease.

So, in the both groups of chronic superficial periodontitis, the salivary level of 8-OHdG decreased and became normal in the same time with clinical healing, but clinical healing was achieved quickly after mixed treatment (3 weeks) compared with conventional treatment (6 weeks).

At patients with deep chronic periodontitis mixed treated, salivary levels of 8-OHdG decreased rapidly and it was associated with a greater clinical improvement in comparison with the patients treated only by conventional method.

It is very difficult to compare the results of different researchers, because there are differences in the composition of propolis and in the modalities of preparing the ethanol extracts from propolis.

So, Renata Cairo *et al.* [13] showed a regression of 95% gingivitis and suppuration in all the teeth irrigated with 10% Brazilian green propolis gel after 5 weeks of treatment. Their results suggest that propolis gel could be used as an adjuvant therapeutic method assigned for the treatment of chronic periodontitis.

Feres M. *et al.* [3] reported that propolis showed significant antimicrobial proprieties in saliva samples from periodontally healthy and diseased subjects, suggesting that this substance may be used therapeutically in the future.

Although results obtained in our study are similar, a significant correlation was found between salivary levels of 8-OHdG and clinical status in all forms of periodontitis, therefore recommended propolis and royal jelly can be used in the treatment of chronic periodontal disease. In this regard, the results of our study suggest that 8-OHdG may provide useful information regarding periodontal status before and after treatment.

Finally, the most important results of this study regard the patients treated with these natural products mentined above. They cured quickly the superficial chronic periodontitis and improved the deep chronic periodontitis.

Because periodontitis is, according World Health Organization one of the most important problems of the oral health in the world, it is necessary to discover a new strategy for a more effective treatment based on natural products.

Our study focused only on chronic periodontitis, but further studies using this mixture of natural products are needed for a large number of subjects in all age groups and with other form of periodontitis for established the benefits of this natural therapy.

Conclusions

Ethanollic mixture of propolis and royal jelly used in periodontitis treatment showed a significant effect in the remission of this disease, suggesting that this complex may be used as an adjuvant to periodontal treatment - results after mixed treatment are more effective than conventional treatment, results evaluated by clinical and biochemical parameters.

The study demonstrates the existence of a correlation between the values of salivary 8-OHdG biomarker and the status of periodontal tissue after different ways of treatment. Future research with a greater number of subjects is needed to establish the efficiency as an alternative method for treatment of periodontitis.

The natural remedies, including apitherapy, are expected to be widely used in the future. That is the reason why a research strategy for new pharmaceutical results based on natural products is necessary.

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