Abstract

Severe periodontitis is associated with the increase of serum inflammatory markers, in a population without general diseases. The aim of the study was to determine the manner in which the response to periodontal therapy had been associated with changes in the systemic serum markers of the inflammation.

Materials and method: The study was performed on a group of 50 generally-healthy patients, yet suffering from generalized severe periodontitis, who participated to a prospective investigation developed along 12 months. The periodontal parameters and the inflammatory markers [C-reactive Protein (CRP) and Interleukin-6 (IL-6)] had been previously evaluated at 6 and 12 months, and also 6 months after the standard periodontal nonsurgical treatment. Results and discussion: 6 months after the treatment, a significant reduction of serum interleukin and C-reactive protein was observed in the subjects with a clinical response to periodontal therapy over the average, following correction of the local causal factors. Conclusions: Periodontal disease may lead to an increased systemic inflammatory load, demonstrated by an rise in the inflammatory markers in the affected subjects.

Keywords: periodontitis, inflammation, serum inflammatory markers, C-reactive protein, interleukin

INTRODUCTION

Relatively recent studies, besides those developed in the last two decades, have indicated that patients affected by severe periodontitis have high C-reactive protein and serum fibrinogen high levels, moderate leukocytosis and a higher level of serum interleukin 1 and 6, comparatively with those of the healthy persons. (1-4) More than that, in patients suffering from periodontitis, increase of the C-reactive serum protein is associated with high levels of periodontal pathogenic agents. (5)

The hypothesis according to which the inflammatory response induced by periodontitis is a significant one for subjects suffering from no other diseases is supported by at least 3 elements: periodontitis was associated with a higher risk of cardiovascular pathologies (6), with the risk of giving birth to low-weight children (7) and of having a sub-optimal control of type II diabetes (8); association between periodontitis or other chronic infections and the cardiovascular pathologies seems to have a similar magnitude (9) and experimental preclinical models indicated that chronic infection with periodontal pathogenic agents causes narrowing of the carotid’s endothelium (10) and affects foetus development (11).

Whichever the mechanisms producing it, systemic inflammation seems centred on explaining the nature between chronic infections and atherosclerosis. In this respect, the C-reactive protein represents a basic marker of the acute phase response to infection and/or inflammation. The production of C-reactive protein is usually favourized by an inflammatory stimulus, being mediated by a complex network of cytokines (mainly IL-6) (12). The C-reactive protein plays a significant part in predicting coronary events in healthy population (9).

The aim of the present interventional pilot study was of establishing whether the extent of the individual response to the periodontal treatment was associated with modifications in the serum markers of systemic inflammation, such as C-reactive protein (PCR) and interleukin 6 (IL-6), in persons suffering from no other diseases.
MATERIALS AND METHOD

The study performed was a prospective, longitudinal, simple, blind one, developed along 6 months. Participants were selected among the subjects having addressed the Department of Periodontology with various severe periodontal (periodontal pockets larger than 6 mm, resorption of the alveolar bone exceeding 30%) and generalized problems (more than 50% affected teeth). The severity levels of this pathology were established in view of an increased probability of detecting the possible systemic pathologies caused by periodontal infections. The advanced clinical conditions did not permit the formation of a reference group (ethic considerations being also had in view), so that a longitudinal cohort design was used. An initial comparative analysis of the modifications observed in the serum inflammatory markers in subjects with various levels of response to the planned periodontal treatment was performed. The exclusion criteria refer to:

- known systemic diseases,
- history and/or presence of other infections,
- systemic antibiotherapy in antecedents (3 months),
- treatment with any type of medication known as influencing the level of serum inflammatory markers
- pregnant women or women who suckle a child.

Informed consent was obtained from all patients.

Initial evaluation involved registering of the anamnesis and medical history, of the periodontal parameters and blood samples. All necessary periodontal treatments (extraction of definitely affected teeth, restorative treatments), applied prior to the periodontal treatment as such, included sanitary education, subgingival scaling and radicular levelling with a piezoelectric device equipped with subgingival insert. Further on, the patients were subjected to a non-surgical treatment of the local pathology, performed by the periodontologist.

The therapeutical phase was completed 1-3 months after the initial consultation.

The patients were re-examined 2 and 6 months after the end of the treatment.

ANALYSIS OF C-REACTIVE PROTEIN AND OF SERUM INTERLEUKIN

Serum samples were collected at various moments of time (during the initial consultation, 2 and 6 months after), then processed and stored at – 60°C, up to their standardized analysis, aimed at limiting the individual variations and the complete utilization of kits. The level of C-reactive protein was determined with an immuno-turbidimetric automated device (Cobas Integra, Roche, Mannheim, Germany; detection limit: 0.25mg/L), while interleukin 6 was measured by a high-sensitivity ELISA test (Quantikine HS, R&D system, Minneapolis, MN, SUA, mean detection limit: 0.04 ng/L)

MICROBIOLOGICAL ANALYSIS

Samples of subgingival plaque were collected during the first consultation, from 4 deep periodontal pockets, one from each quadrant. DNA was isolated by means of the DNA extraction kit (Puregene, Minneapolis, MN, USA), and with a pattern for determining the amplification of the bacterial Rrna 16S genes from 4 different periodontal pathogenic agents, on using chain multiplex-polimerases reactions. Utilization of specific primers gave 3 products with various DNA sizes for Tannerella forsythensis (Tf), Porphyromonas gingivalis (P.g) and Aggregatebacter actinomycetemcomitans (A.a). The C protein products were demonstrated on agarose gel.

The DNA genotype. To determine the genotype, DNA was extracted from the leukocytes of the subjects, from peripheral blood samples, by means of a commercial kit (Nucleon BACC2, Nucleon Bioscience, Coatbridge, UK). The oligonucleotide sequences utilized for amplifying the C-reactive protein, the size of the C-proteins had in view and the amplification program employed were described elsewhere (13).
STATISTICAL ANALYSIS

As the previous estimations of the variation in the modifications related to the associated periodontal treatment of the primary variables (C-reactive protein and interleukin 6) were available, the samples were not based on formal calculations, but on logistic and empirical considerations.

All data were loaded in a computer and analyzed with a statistical package (SAS version 8.1, Cary, NC). The modifications observed after periodontal therapy in the serum concentration of the C-reactive protein and interleukin 6 were employed as primary variables. The abnormally distributed variables were logarithmically transformed prior to their utilization in parametric analyses. The normally-distributed variables were reported as an ± average of standard deviation (SD), where the average value and the variables were used for describing the abnormally distributed data. The differences recorded – at different consults – for each inflammatory marker were recorded with a Wilcoxon test. The association between the modifications in the concentrations of C-reactive protein and interleukin 6, produced among 0 and 6 months, as well as individualization of the response to periodontal therapy were demonstrated by a generalized linear model (with SAS PROC GLM) which included the following factors: age, sex, index of bodily weight, smoking and cytokine polymorphism. Value á was established at p<0.05.

RESULTS

94 of the subjects completed the study. The subjects had an average age between of 46±9 years, 54% men and 42% were smokers, and 26% had antecedent heredocolateral cardiovascular diseases.

The average index of bodily weight was 25.3±3.7 kg/m². During a 6 month-model study, none of the patients modified the diet, medication or the smoking habits. On the average, 58±20.7% of the sites of patients’ teeth showed detectable plaque. Microbial analysis demonstrated the presence of T.f. in 76.3% of cases, P.g in 72.8% si A.a. in 43.8%, respectively. The participants showed high levels of gingival inflammation (a gingival bleeding score of 63.5±16.4%, n=94) and severe generalized periodontitis (with an average value of 77±23 periodontal lesions per subject and a clinical average of attachment loss of 4.93±1.13 mm, N=94).

Following the treatment, the significant reduction of total bleeding (45.5±16.7%, p<0.0001 t test, N=94) and of the number of periodontal pockets larger than 4 mm (57±24, P<0.0001 t test, N=94) was of 6.

The level of C-reactive protein recorded during the first consultation was of 1.9 mg/L (3.6 IQR, N=94) (fig. 1), while that of interleukin 6 was of 1.8 ng/L (1.5 IQR, N=94) (Fig. 2).
Serum improvements in interleukin 6 and in the C-reactive protein were observed, comparatively with those of the first visit and the values recorded at 6 months, in 73% and, respectively 62% of the subjects. The average change in the concentration of the C-reactive protein between the first visit and the one at 6 months was of 0.5mg/L, with a safety interval of 95% at 0.4 up to 0.7 mg/L.

The corresponding values for interleukin 6 were of 0.2 µg/L (95% CI 0.1 up to 0.4 µg/L). As the response to the periodontal treatment was not a homogenous one, the subjects were divided according to the level of their clinical response to periodontal therapy (based on the average number of residual periodontal lesions <30 and gingival bleeding <30%). Out of the subjects with a better periodontal response, 79.2% demonstrated some reduction of serum C-reactive protein.

Subsequent analysis, based on a generalized linear model with Log C-reactive protein as a dependent variable, indicated a significant association between the clinical results of the periodontal treatment and the serum level of the C-reactive protein in various moments of the treatment, after correction of other known covariates, such as age, sex and index of bodily weight.

The serum concentrations of the C-reactive protein were significantly associated with the behaviour of the functional polymorphous allele genes specific to interleukin at (-889) in the promoted regions. A similar analysis, with Log IL-6 as a dependent variable, indicates that the serum concentrations of IL6 were associated with the results of the periodontal treatment, as well as with the age of the subjects, being homozygote for allele genes 2, as due to a functional polymorphism specific to the region of genes I L-1B (-511).

DISCUSSION

In the present study, treatment of periodontitis was associated with a significant decrease of both serum CRP and IL-6 in individuals with a good general health condition, yet suffering from severe generalized periodontitis. Significant decreases of IL-6 had been already observed 2 months after the initiation of the therapy, while the C-reactive proteins decrease only after 6 months. Especially important, if considering the pilot nature of the study and the absence of a control group, was the observation that reduction of both CRP and IL-6 were significant in the subjects who respond better to the periodontal treatment. As a matter of fact, 79.2% of the subjects who respond better to periodontal therapy also show an improved level of serum CRP.

The limited data here provided apparently indicate that periodontitis contributed to the inflammatory response of the subjects, namely that the potentially maximized response to the periodontal treatment is critical in the context of the design and implementation of the study.

Improvement of CRP was observed in patients whose initial values occurred in the superior quarters of normality (an average value of 1.9 mg/L, IQR 3.6 mg/L). The analyzed data confirm some previous observations, according to which, in generally healthy patients, who suffer from chronic periodontitis, systemic inflammation moderately increases. (2) Also observed was that the levels of C-reactive protein in the normal perimeter assure a good prediction of the future coronary events in the healthy population (9, 14).

Data evidencing the role of periodontal infections are scarce. In most studies, the subjects suffering from severe generalized periodontitis were more predisposed to cardiovascular diseases. Some prospective studies associated periodontitis and teeth loss with a higher risk for carotid atherosclerosis (1 mm thickening of carotid’s endothelium) after correction of the possible factors (15, 16). In studies of cellular cultures, Porphyromonas gingivalis (Pg), one of the most important periodontal pathogenic agents, demonstrated its ability of invading the endothelial cells (17).

The periodontal pathogenic agents were identified in the aterom plaque (18). More than that, chronic inoculation with Pg in ApoE in rats increases the lipidic profiles and the formation of aterom and calcifies the atherosclerotic plaques.
Periodontitis is an infection caused by gramm-negative bacteria, present in the subgingival biofilm between the radicular surface and the junction epithelium. It is relatively not sensible to the effects of the systemic antibiotics and to the requirements of the treatment, starting with the elimination of the biofilm through mechanical means. After a successful treatment, the bacterial charge is significantly reduced, while the action of the antibodies and the affinity to specific pathogenic agents gets improved. As a result of such modifications, local inflammation considerably decreases, while the clinical parameters (pocket probing, bleeding on probing) are significantly improved.

In the present study, decrease of serum C-reactive protein was associated with half of the population which responds better to the periodontal treatment, manifested, as demonstrated by clinical parameters, in reduced infection and periodontal inflammation.

Agreeing with previous investigations, analysis of the present data shows that, apart from the significance of the results on periodontal therapy, serum C-reactive protein is associated with the various characteristics of the patient, such as smoking habits, index of bodily weight, age and presence of specific polymorphism in IL-1A and IL-6.

In terms of inflammatory cytokines, homozygote for allele genes 2, for IL-1A and IL-6 was associated with the high levels of serum concentrations.

All these observations support the hypothesis that periodontal pathology influences individual susceptibility, which amplifies the systemic inflammatory responses, such as aterosclerosis. On the other side, considering the quite reduced number of individuals involved in the study and the large number of tested covariates, such investigations are still to be extended at large scale, for producing definite results.

The conclusion to be drawn is that periodontitis seems to contribute to systemic inflammation. Mention should be also made of the potential significance of the presented proofs, of the magnitude of the observed decrease of the C-reactive protein, of the prevalence of pertodontitis in the population and of the certainty that it may be treated.

References