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Clinical, Biochemical and Pathophysiological Study on Inflammation in Chronic Marginal Periodontitis and Periodontal Disease

DOCTORAL THESIS SUMMARY

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INTRODUCTION

Periodontal disease, one of the oldest and most common infectious diseases, can result in the permanent destruction of dental support structures and, by evolution, can lead to tooth loss. Chronic marginal periodontitis is an inflammatory condition of the tooth-supporting apparatus, the so-called marginal periodontium, which also affects the gingiva and the deep structures of the periodontium, namely the periodontal ligaments and the alveolar bone.

The aetiology of periodontal disease can be attributed to an uncontrolled process of inflammation, with microbial factors inducing a series of host defence responses. Along with the mechanisms associated with cholesterol, the crucial role of inflammation among the mechanisms of systemic heart disease in all stages of atherosclerotic lesion development is scientifically accepted (1). Under this “inflammatory hypothesis”, periodontal disease could contribute to systemic inflammation and therefore to atherogenesis.

Motivation for choosing the research topic

I chose this topical issue due to the fact that the pathophysiological connection between chronic marginal periodontitis and ischemic heart disease is still a medical field under continuous research. The oral cavity is a point of connection between the external environment and the internal part of the human body. Basically, the oral cavity is a passageway for multiple types of microorganisms as well as the interface between the two environments.

The general part comprises three chapters and represents a review of the current general knowledge on the subject under study.

Chapter 1. Chronic marginal periodontitis – nosological framework

As a common form of periodontal disease, chronic marginal periodontitis has different stages of severity, ranging from reversible to irreversible. It is accepted that the microorganisms in the bacterial plaque that exist in the form of biofilms are the primary etiological agents of periodontal disease. Both local and general, systemic, factors are involved in the aetiology of periodontal disease.

Bacterial pathogens associated with periodontal disease have been identified in the mentioned subgingival biofilms. The microbiota associated with periodontitis consists of a complex mature ecosystem, different from those found in healthy periodontium or in gingivitis. Most of the bacteria

involved are gram-negative anaerobic bacilli. They include *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum*, *Eikenella corrodens, Campylobacter rectus*, *Parvimonas micra* and *Streptococcus intermedius*. Three species - *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, designated as part of the “red complex”, are involved in the progression of chronic periodontitis (1).

An important factor in bacterial invasion is the release by periodontopathogenic bacteria of formations from the outer membrane of the gram-negative wall, the so-called “vesicles”. Due to their size, they can cross the epithelial barrier to the connective tissue, thus being an attack factor by releasing enzymes and inducing the factors responsible for the production of inflammatory processes by endotoxin. Such vesicles are produced by bacteria of the species *Porphyromonas gingivalis, Aggregatibacter actynomicetecomitans* and *Treponema denticola* (1).

The human microbiota contributes directly and indirectly to the normal development of the body's nutritional and defense functions. It exists in harmony with the host, a situation in which both parties benefit from this association (symbiosis). Disturbance of balance in this relationship (dysbiosis) leads to the colonization of external microorganisms, often pathogenic

There are two assumptions that lie at the basis of periodontal disease as an etiological factor in general health. The first one is related to the bacteria released from biofilms located in periodontal pockets that can pass into the bloodstream through the ulcerations of the epithelium of the periodontal pocket, colonising other areas of the body (2). The second one is related to the periodontal pathogens that cause inflammatory reactions in the affected tissues, stimulating the release of inflammatory cytokines or acute phase proteins, contributing to systemic inflammation, possible atherogenesis and other pathologies. The oedematous growth and the increased tendency of the gingival and periodontal tissues to bleed facilitate the transmission of these compounds into the blood, and it is possible to measure high concentrations of mediators in the peripheral blood (3).

In order to establish a correct diagnosis in chronic marginal periodontitis, an objective clinical examination as well as complementary examinations are performed, and periodontal disease indices are calculated. Thus, we have the possibility to identify the aetiology of the disease.

Chapter 2. Ischemic heart disease - etiopathogenesis, production mechanisms and systemic biomarkers

Etiopathogenesis of ischemic heart disease

Ischemic heart disease is caused by an imbalance between the state of myocardial energy and the coronary blood flow. The causes of ischemic heart disease can be: coronary atherosclerosis, coronary vasculitis, congenital abnormalities of the coronary circulation. Atherosclerosis is the thickening of the tunica intima, the inner layer of the blood vessels, and the tunica media, the underlying layer, which contains smooth muscle and elastic tissue.

Pathophysiological mechanisms in ischemic heart disease

The onset of ischemic heart disease is based on an initial pathophysiological mechanism that consists in the interruption of an atherosclerotic plaque (rupture) with the subsequent aggregation of platelets and the formation of an intracoronary thrombus. The mentioned events occur through the processes of haemostasis in order to repair the lesion.

Endothelial dysfunction, inflammation and coronary atherosclerosis

Endothelial dysfunction is induced by decreased epithelial cell function. It leads to the decrease in the action of vasodilators and the increase in that of vasoconstrictors at the level of vascular endothelium. Under physiological conditions, endothelial cells prevent thrombosis through various anticoagulant and antiplatelet mechanisms.

The chronic vascular inflammatory process influences the ability of the endothelium to produce proinflammatory cytokines, adhesion factors and molecules. Certain cytokines and factors, such as Interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), are released by the endothelium, stimulating adhesion molecules and increasing vascular risk. Increased serum levels of IL-6 and C-reactive protein may reduce NO production, facilitate thrombus formation and, consequently, increase the risk of cardiovascular events (4,5).

Oxidative stress

In the vascular wall there are a variety of reactive oxygen species, such as NADPH (nicotinamide adenine dinucleotide phosphate), oxidase, xanthine oxidase, mitochondrial respiratory chain enzymes (6). The alteration of the balance between increased production of

reactive oxygen species in cells and tissues, which exceeds the ability of the antioxidant system to neutralise them, leads to the installation of oxidative stress (7).

Chapter 3. Relationship between chronic marginal periodontitis and ischemic heart disease

Hypotheses of the relationship between periodontal disease and cardiovascular disease

Among a number of complementary hypotheses, there are two more important ones: the first predicts that bacteria and their toxins have a direct impact on the vessel wall during bacteraemia; the second assumes that the cytokines and inflammatory mediators released during chronic periodontal inflammation can potentially affect the vessel wall (8, 9).

Bacteraemia and pathogenic effects on the myocardium in ischemic heart disease

In the periodontal hotbed, a series of substances with hormonal effects (endocrine-like substances) are eliminated and immune complexes are formed that amplify the inflammation both in the dental-periodontal region and in the heart tissues (10). The affected marginal periodontium is a constantly renewed reservoir with the permanent elimination of toxic substances in the bloodstream, inducing and perpetuating systemic pathological effects and disorders of internal organs, including the heart (11).

High-level bacteraemia can initiate a host response that alters coagulation, endothelial integrity of the vascular wall, and platelet function causing possible thromboembolic events.

Bacterial DNA of oral origin has been frequently detected in atherosclerotic lesions, which suggests that they may influence atherogenesis and other systemic pathological conditions. The most common and highest levels of periodontopathogens identified in atheromatous plaques have been *Porphyromonas gingivalis* and *Treponema denticola* (12).

Systemic inflammation-associated biomarkers and periodontal disease

Biomarkers have been defined as “cellular, biochemical, or genetic alterations” molecules by which a simple normal or abnormal biological process can be recognised or monitored (13). Bacteria associated with periodontal diseases can colonise atheromatous plaques and injure them by inducing local inflammation, which results in the spread of inflammatory events leading to the

formation, development and possible rupture of the atherosclerotic plaque. At the same time, an increased systemic inflammatory level could result from bacteraemia or as a consequence of proinflammatory cytokines generated at the site of periodontal injury, with direct access to blood flow.

II. Personal contribution

Chapter 4. Research hypothesis and scientific research objectives

The *research hypothesis* started from the premise of the connection between cardiovascular disease and chronic marginal periodontitis. The oral cavity, being a favourable environment for the development of different types of bacterial strains, favours the installation of periodontal disease. Consequently, these bacteria can enter the bloodstream and cause an inflammatory immune response.

The *goal* of the present study was to investigate a connection, in terms of inflammation, between periodontal disease and ischemic heart disease through complex clinical investigations. In this regard, I performed anamnesis, diagnosis, laboratory tests with blood collection, gingival microbial samples collection, and I applied a prophylaxis questionnaire to patients with ischemic heart disease.

The *general scientific objective* was to identify, based on the epidemiological, diagnostic, clinical, paraclinical and prophylactic characteristics of periodontal disease and ischemic heart disease, a connection between the two diseases in terms of inflammation.

The *specific scientific objectives* were related to highlighting the degree of awareness of dental prophylaxis in patients diagnosed with ischemic heart disease, the frequency of detection and the quantity of marker bacteria associated with periodontitis in patients with ischemic heart disease, and to assessing the differences, in terms of clinical and paraclinical markers, between clinically healthy patients with chronic marginal periodontitis, patients with ischemic heart disease and normal periodontal status and patients with both pathologies.

Chapter 5. General research methodology

My personal research presented in the doctoral thesis includes several clinical studies conducted in order to raise awareness of the role of dental prophylaxis in patients with cardiovascular disease, and to investigate a possible inflammation-based correlation between heart disease and periodontal disease, from the identification of the types and prevalence of gram-negative germs in bacterial plaque to the identification of possible changes in the general medical tests in patients with one or both pathologies.

Study 1- Systematic synthesis on the degree of correlation between periodontal disease and ischemic heart disease

In this chapter a meta-analysis on the degree of correlation between the two pathologies was performed.

Study 2 - Statistical study on the changes in clinical and laboratory parameters in patients with chronic marginal periodontitis, ischemic heart disease and both pathologies

I evaluated a number of 468 patients (208 were diagnosed at the “Sfântul Ioan” Emergency Clinical Hospital in Bucharest and 260 were diagnosed at private dental offices). Out of the total number of patients evaluated, 118 met the selection criteria. They were those included in our study. Subsequently, I formed three groups of patients: the first group consisted of 31 patients with ischemic heart disease, the second group consisted of 40 patients with ischemic heart disease and chronic marginal periodontitis, and the third group consisted of 47 patients with chronic marginal periodontitis. The first group included patients from the “Sfântul Ioan” Emergency Clinical Hospital in Bucharest (31 patients), and groups 2 and 3 included patients from private dental offices (87 patients).

Study 3 – Study on the degree of awareness of the role of dental prophylaxis in patients with ischemic heart disease

I applied a questionnaire related to oral-dental prophylaxis to a number of 57 patients with ischemic heart disease, who presented for specialised treatments in different dental offices and who met the selection criteria.

Statistical analysis

The statistics of the obtained data was made using the R project, a language and environment for statistical computing and graphics, version 4.0.2, a software that can run on UNIX, Windows

or MacOS platforms (10). Descriptive statistical analyses were performed for the analysed characteristics, the results obtained being expressed as maximum, minimum, average and standard deviations for numerical variables and as frequency for categorical variables, for each of the three groups of patients studied. The following tests and methods of statistical analysis were applied: testing for normality of the distribution - Kolmogorov-Smirnov test; testing for homogeneity or multivariate dispersion - Levene test; testing for the differences between the three groups of patients at the level of each systemic variable - ANOVA test (analysis of three Bonferroni variants), to analyse the differences between the group averages; testing the difference between groups two by two - T test in pairs. The correlations between the continuous variables were determined with a linear regression model, the Pearson Chi correlation analysis, in which $p < 0.1$ was considered statistically significant. The correlation was analysed using three methods: Pearson, Kendall-τ (tau) and Spearman-ρ (rho), with a significance level of 5% ($p < 0.05$).

For the statistical analysis of the bacterial load, I performed a dichotomised non-parametric evaluation of the results transmitted by the laboratory, in which I gave: score 0 for undetectable bacterial species, score 1 for low levels of bacterial load and score 2 for high levels of bacterial load.

Chapter 6. Systematic synthesis on the degree of correlation between periodontal disease and ischemic heart disease

Introduction

The goal of the present study was to identify the degree of correlation between chronic marginal periodontitis and ischemic heart disease by researching various studies addressing the two inflammations as well as the biochemical, pathophysiological and clinical associations between the two pathologies.

Material and methods

I researched in the literature from the PubMed, Science Direct and Scopus databases relevant studies published during the past 5 years, to answer the following question: "What are the interrelationships and correlations between periodontal inflammation and ischemic heart disease?"

Working hypotheses

In an attempt to examine the relationship between periodontal disease, ischemic heart disease, and biochemical and pathophysiological data, the studies were selected in order to investigate the correlations between periodontal disease and vascular atherosclerosis, endothelial dysfunction, chronic heart failure, stroke and myocardial infarction.

The factors considered in order to find correlations between periodontal disease and ischemic heart disease are summarised in Figure 6.1.

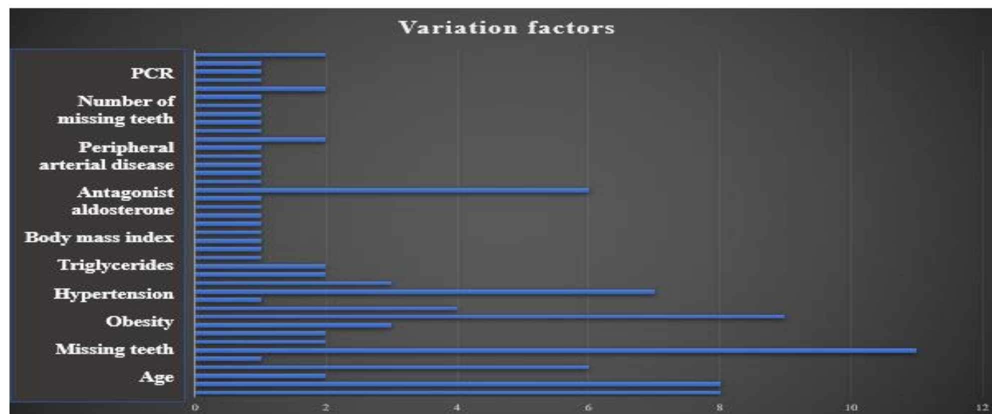

Figure 6.1. Variation factors tested in the studies

Results of analysed studies

In the studies included in our meta-analysis, statistically, severe periodontitis was significantly associated with: subclinical atherosclerosis in women over 65, alcohol users, non-smokers; vascular dilation at 60 s measured in the brachial artery or absence of dilation, regardless of differences in sex, age and metabolic risk; increase in the thickness of the atheromatous plaque and its calcification; diagnosis of ischemic heart disease.

The number of teeth and the number of deep periodontal pockets were significantly related to future cardiovascular disease and heart failure.

Discussions and conclusions

The prevalence and severity of periodontal disease is higher in patients with chronic heart disease as opposed to the general population in the case of ischemic heart disease. Periodontal

disease could be interpreted as an early clinical predictor of acute coronary heart disease complications in patients with diabetes who are smokers (14).

Simple, non-invasive periodontal therapy can improve the health of the patients with diabetes, vascular disease and other comorbidities. Oral health, mainly expressed in the number of teeth, was connected to episodes of myocardial infarction and heart failure (15).

I am of the opinion that future research should consider the following directions:

- the choice of study groups large enough to compensate for the covariates;
- the preparation of appropriate treatment regimens to test the impact of periodontal infection on the measured outcome (e.g., systemic antibiotic adjuvant versus mechanical therapy);
- the longitudinal monitoring to examine the duration of the therapeutic effect on the systemic biomarkers;
- the selection of subjects with concomitant moderate to severe periodontal disease and high systemic levels (> 3.0 mg / l) of C-reactive protein, which may be necessary to clarify whether periodontal therapy has a real impact on cardiovascular risk;
- the uniform classification by correlating the severity of chronic marginal periodontitis with cardiovascular disease;
- the specification of the type of population on which the analysis was performed.

Chapter 7. Statistical study on the modifications of the clinical and laboratory parameters in patients with chronic marginal periodontitis, ischemic heart disease or both pathologies

Introduction

Evidence has recently been provided that patients with periodontal disease are at increased risk for cardiovascular disease. This risk is independent of other known behavioural and medical risk factors. However, it is proportional to the severity of periodontal disease.

Results

The chapter was structured according to four types of analysis: analysis of epidemiological characteristics; statistical analysis on the predominance of gram-negative germs in periodontal

pockets; statistical analysis of the modifications in blood / serum parameters; and analysis of the correlation between selected clinical (periodontal) and blood / serum parameters.

1. **Analysis of epidemiological characteristics**

The variables “age”, “sex”, “active smoker”, “history smoker”, “non-smoker”, “BMI” were analyzed (Figures 7.1, 7.2, 7.3, 7.4, 7.5, 7.6)

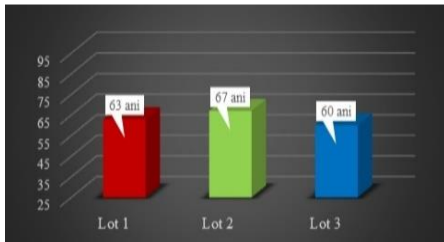

Figure 7.1. “Age” variable for the 3 groups

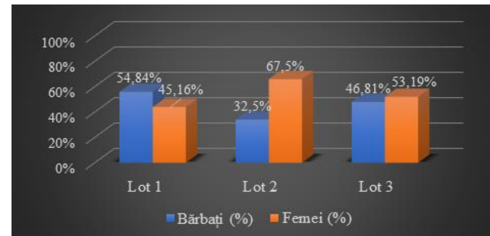

Figure 7.2. “Gender” variable for the 3 groups

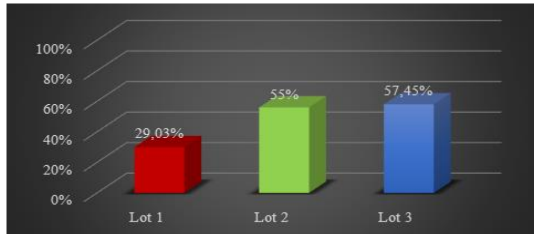

Figure 7.3. “Active smoker” variable for the 3 groups

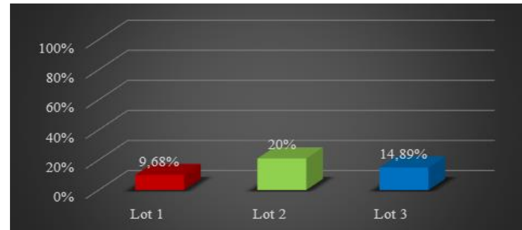

Figure 7.4. “Former smoker” variable for the 3 groups

Figure 7.5. “Non-smoker” variable for the 3 groups

Figure 7.6. “BMI” variable for the 3 groups

In group 2 there are patients with the highest average age (67 [± 0.021]), and in group 3 there are patients with the lowest average age (60 [± 0.087]). Statistically, there is a significant difference between the three groups. The statistical analysis of the three groups resulted in an increased percentage of female patients with ischemic heart disease.

The highest percentage (57.45%) of active smokers was found in the group with chronic marginal periodontitis, and the lowest percentage (29.03%) was noted in the group with ischemic heart disease. The "former smoker" variable had the highest percentage (20%) in the group of patients with chronic marginal periodontitis and ischemic heart disease (group 2), and the lowest percentage (9.68%) in the group with ischemic heart disease.

The analysis of "non-smoker" variable highlighted the fact that the highest percentage (61.29%) of non-smokers were in group 1 (patients with ischemic heart disease), and the lowest (25%) percentage in group 2 (patients with both pathologies).

The highest value of the average body mass index (BMI) was in the patients in group 1 - patients with ischemic heart disease (29.5 kg/m^2) and the lowest in group 3 - patients with chronic marginal periodontitis (26.35 kg/m^2). Statistically, there was a significant difference between the mentioned study groups 1-3 ($p < 0.001$).

2. Statistical analysis of the predominance of gram-negative germs in periodontal pockets

This subchapter included two assessments: periodontal assessment and microbiological assessment.

2.1. Periodontal evaluation

I performed a complete assessment of the dental-periodontal status considering the following: number of teeth; presence or absence of dental plaque; bleeding on probing (BOP). Therefore, I determined the Community Periodontal Index of Treatment Needs (CPITN) for each patient, taking into account 6 variables for each tooth.

In the case of group 2, I noticed that the average depth of the periodontal pockets was higher than in groups 1 and 3. Statistically, there were significant differences between groups 1-2 ($p < 0.001$) and 1-3 ($p < 0.001$).

Regarding group 2, I noticed that the clinical attachment loss was higher than in groups 1 and 3. Statistically, there were significant differences between groups 1-2 ($p < 0.001$) and 1-3 ($p < 0.001$).

The highest mean value of the "number of teeth" parameter was found in patients in group 1 - patients with ischemic heart disease 22 (± 0.2) and the lowest in group 3 - patients with chronic marginal periodontitis 11 (± 0.6). Statistically, there were significant differences between all three groups ($p < 0.001$).

The highest mean value of the "BOP" parameter was in group 3 - patients with chronic marginal periodontitis (38%) and the lowest in group 1 - patients with ischemic heart disease (30%).

The highest mean value of the "CPITN" parameter was in group 2 - patients with ischemic heart disease and chronic marginal periodontitis (4.2 [± 0.2]) and the lowest in group 1 - patients with ischemic heart disease (2.4 [± 0.6]). Statistically, there were significant differences ($p < 0.001$) between the three groups.

With regard to severe periodontitis, the highest mean value was in group 2 - patients with ischemic heart disease and chronic marginal periodontitis (72.5%). As for moderate periodontitis, the highest mean value was in group 3 - patients with chronic marginal periodontitis (55.32%).

2.2. Microbiological evaluation

I identified pathogens that exceeded the significance threshold, I analysed the distribution of bacterial species by test groups, I evaluated the composition of the bacterial flora in the studied groups and I tested the possibility of a correlation in terms of the bacterial flora composition between the three groups. The periodontal agents collected were: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Eikenella corrodens*, *Campylobacter rectus*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Prevotella nigrescens*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Capnocytophaga gingivalis*

When investigating the composition of the flora in the gingival sulcus in the three studied groups, the following observations resulted:

- *Aggregatibacter actinomycetemcomitans* was present in high concentration in the bacterial plaque of the group with cardiac pathology and reduced in the other two test groups;

• *Porphyromonas gingivalis* showed the highest levels in the group with marginal periodontitis, being followed, with small differences, by the group with both pathologies and the one with cardiac pathology;

• *Treponema denticola* was high in group 3 and low in group 1;

• *Tannerella forsythia* was present in high but equivalent concentrations in groups 2 and 3 and very low in group 1;

• *Eikenella corrodens* had the highest levels in the group with both pathologies;

• *Campylobacter rectus* had a very high level in the group with periodontal pathology and a very low one in the group with cardiac pathology;

• *Prevotella intermedia* had increasing concentrations from group 1 to group 3;

• *Fusobacterium nucleatum* had equivalent concentration levels in all three studied groups;

• *Prevotella nigrescens* was present in high concentration in the group with chronic marginal periodontitis (group 3) and in low concentration in the group with cardiac pathology (group 1);

• *Capnocytophaga ochracea* showed equally high levels in the group with both pathologies (group 2) and in the one with periodontopathic symptoms (group 3);

• *Capnocytophaga sputigena* was present in equally large amounts in all groups;

• *Capnocytophaga gingivalis* was present in equally large amounts in all groups.

Following the comparative analysis of the distribution of bacterial species between groups, the following results were obtained:

- Significant differences were present between groups 1 and 2 for *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, *Tannerella forsythia*, *Capnocytophaga ochracea*.
- Between groups 2 and 3 there were statistically significant differences for *Aggregatibacter actinomycetemcomitans, Treponema denticola, Tannerella forsythia, Campylobacter rectus, Prevotella intermedia, Prevotella nigrescens, Capnocytophaga ochracea*.
- Between groups 1 and 3, there were statistically significant differences for *Campylobacter rectus, Prevotella nigrescens, Capnocytophaga sputigena*.
- In terms of correlation, considering the group with both pathologies and the one with chronic marginal periodontitis, the Pearson index $r = 0.60774$ showed a higher degree of correlation for 36,9% of cases $y = 0.5498x + 0.4615$ $R^2 = 0.3693$ (Figure 7.7).

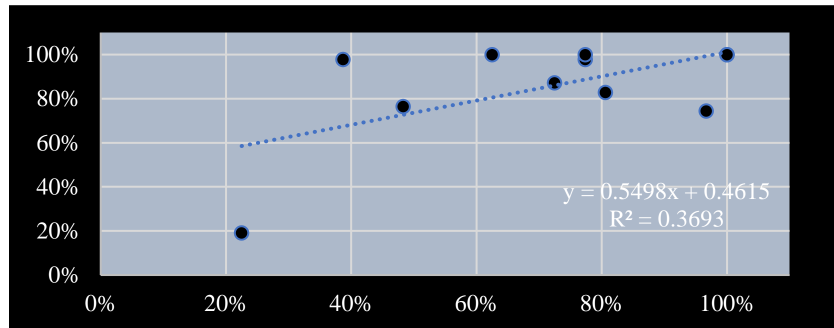

Figure 7.7. Correlation between group 2 and group 3

3. Comparative analysis of changes in blood / serum parameters

The data obtained from the records of the values of the blood / serum investigations were centralised and statistically processed for the three studied groups.

Following the analysis of the mean values of the complete blood count, the values that exceeded the normal value limits were considered as follows:

- Group 1 - International Normalized Ratio (1.23[±0.37]), serum urea (48.46[±0.65]), CRP (0.99[±2.07]), hsCRP (0.87[±0.43]), serum creatinine (1.34[±2.26]) and fibrinogen (465.54[±98.94]);
- Group 2 - average erythrocyte haemoglobin concentration (32.50[±0.57]), average red blood cell volume (97.78[±1.63]) and eosinophil count (1.09[±0.27]), serum urea 43.85(±1.93), VSH 31.58(±0.83), CRP 1.34(±1.86), hsCRP 2.77(±0.36), serum creatinine 1.21(±1.296) and fibrinogen 426.70(±102.95);
- Group 3 - mean corpuscular haemoglobin (32.35[±0.78]), average red blood cell volume (96[±5.52]), eosinophil count (0.95[±0.13]) and basophils percentage (1.29[±0.84]), serum urea (40.10[±0.79]), CRP (1.18[±0.63]) and hsCRP (2.56[±0.43]).

4. Analysis of the correlation between selected clinical (periodontal) and blood / serum parameters

After testing the correlation between periodontal indices and selected serum parameters, for group 1 there were statistically significant correlations between the number of teeth and hsCRP,

between BOP and the number of lymphocytes and the number of platelets, as well as between CPITN and the number of platelets (Table VII .1).

Table VII.1. Periodontal and blood / serum indices with significant p - group 1

Periodontal indices	Serum blood / serum	Pearson	Kendall	Spearman
Number of teeth	hsCRP	0.569 p<4.137E-04	0.299 p<0.012	0.381 p<0.017
BOP	Lymphocytes	-0.317 p<0.041	-0.231 p<0.037	-0.316 p<0.042
	PLT	-0.52 p<0.001	-0.35 p<0.003	-0.506 p<0.002
CPITN	PLT	-	-0.364 p<0.004	-0.487 p<0.003

For group 2, there were statistically significant correlations between the depth of the periodontal pocket and CRP, CK-MB respectively, between CAL and the number of neutrophils, between the number of teeth and the number of lymphocytes (Table VII.2).

Table VII.2. Periodontal and blood / serum indices with significant p - group 2

Periodontal indices	Serum blood / serum	Pearson	Kendall	Spearman
APP	CRP	-	-0.226 p<0.039	-0.28 p<0.040
	CK-MB	-	0.237 p<0.032	0.288* p<0.036

CAL	Neutrophiles	-0.29 p<0.035	-0.289 p<0.008	-0.368 p<0.010
Number of teeth	Lymphocytes	-	-0.198 p<0.040	-0.29 p<0.035

As for group 3, there were statistically significant correlations between the depth of the periodontal pocket and CK-MB, between CAL and CK-MB, between the number of teeth and fibrinogen, between BOP and the number of lymphocytes, neutrophils respectively, and between CPITN and WBC (Table VII.3).

Table VII.3. Periodontal and blood / serum indices with significant p - group 3

Periodontal indices	**Serum blood / serum**	**Pearson**	**Kendall**	**Spearman**
APP	Neutrophiles	-0.277* p<0.030	-	-
	CK-MB	0.256 p<0.041	0.253 p<0.019	0.303 p<0.019
CAL	CK-MB	0.264 p<0.036	0.211 p<0.039	p<0.267 p<0.035
Number of teeth	Fibrinogen	-	0.182 p<0.045	-
BOP	Lymphocytes	-	0.174 p<0.050	0.253 p<0.043
	Neutrophiles	-	0.181	0.253

			p<0.043	p<0.043
CPITN	WBC	0.267 p<0.035	0.224 p<0.034	0.27 p<0.033

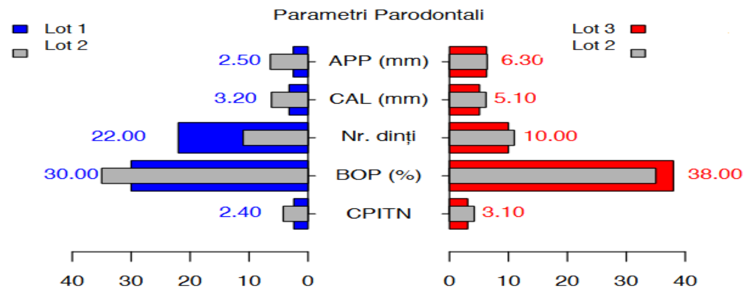

Figure 7.8. Pyramid graph for periodontal parameters

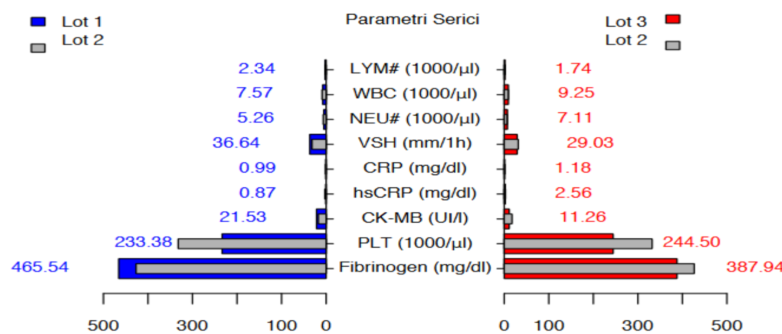

Figure 7.9. Pyramid graph for blood / serum parameters

Differences can be observed for the parameters "number of teeth" and "BOP" in groups 1 and 2, and in terms of blood / serum parameters, it is worth noting the parameters PLT and fibrinogen for both groups 1-2 (Figure 7.8) and groups 2-3 (Figure 7.9).

Discussions

In group 1 (patients with ischemic heart disease), as the values were lower than in the other two groups, we can assume the lower presence of bacterial flora in this group. Thus, the heart disease, not being associated with chronic marginal periodontitis, did not potentiate the destruction of the

bone support. Although the clinical attachment loss was higher in groups 2 and 3, no statistically significant differences were found in the comparison between those groups.

In our study, the number of missing teeth on the arches was different between the three groups (1-2, 1-3 and 2-3) with statistical significance, the influence of each pathology, or their association, on dental-periodontal health causing different degrees of edentulousness - a result that showed the different influence of the two pathologies and their association on the marginal periodontium.

The value of the bleeding on probe index was not statistically significantly different between the studied groups, but the highest value was found in group 3, where we only had the damage of the marginal periodontium, in the area where the local circulation was affected. Although close in terms of percentage, the value of the bleeding index in the group with ischemic heart disease was lower; the mentioned result showed a lower influence of inflammatory factors in the case of periodontal circulation than in the case of impaired coronary circulation (group 1 - patients with ischemic heart disease). Thus, the BOP index could not be considered as representative in the correlation between the two pathologies.

The increased presence of bacteria in periodontal tissue causes the release of exotoxin, endotoxins, proteolytic and hydrolytic enzymes as well as toxic metabolic products, not only through direct mechanisms but also through indirect mechanisms such as antigen-antibody reactions and complement activation (16).

The significant percentage of severe periodontitis in group 2 compared to those without associated heart disease indicates the possibility that deep periodontal pockets and advanced bone resorption may be a risk factor for heart disease, a conclusion that is consistent with Latronico's 2007 study (17).

The correlation between the group of patients with ischemic heart disease and the one with both pathologies was very weak, while in 36.9% of cases a correlation could be established between the flora of the associated pathology and that found in the group of patients with chronic marginal periodontitis. It can certify the possibility of the etiological connection between the two pathological entities.

The patients with aggressive periodontitis had statistically significant increases in serum CRP levels compared to subjects with healthy periodontitis. Increased CRP in these subjects may show the periodontal infection contribution to the systemic inflammation. In patients with aggressive

periodontitis, high CRP levels may be an indicator of both periodontal tissue condition and the risk of systemic diseases such as ischemic heart disease.

Statistically, CK-MB concentrations were significantly correlated with the depth of the periodontal pocket in groups 2 and 3, as well as with CAL parameter in group 3, providing some evidence with regard to the influence of oral health on the evolution of ischemic heart disease.

The high values of APP parameters and the number of platelets are specific to patients with both pathologies highlighting the severity of the two inflammatory diseases, by affecting the periodontal status and increasing the risk of developing blood thrombi. Platelet growth in chronic marginal periodontitis may partially explain the association of the two inflammatory diseases and may indicate a pathophysiological connection between chronic marginal periodontitis and an increased risk of ischemic heart disease.

Conclusions

1. The predominance of severe chronic marginal periodontitis was noticed in group 2, which can be considered a risk factor for the cardiovascular system meeting the degree of severity criteria to favour systemic complications. At the same time, we can state that the presence of severe CMP in a patient with ischemic heart disease aggravates the condition of the periodontium.
2. A higher mean CPITN score in groups 2 and 3 placed these patients in class III periodontal therapy, requiring more complex periodontal treatment, compared to a lower mean CPITN score in group 1, which included the patients in class II periodically necessary therapy, requiring a programme of oral-dental prophylaxis.
3. As for *Aggregatibacter actinomycetemcomitans*, there was a statistically significant difference between groups 1-2 and 1-3, which shows the specificity of this bacterium for periodontal disease.
4. The group with the most changes in the values of the complete blood count parameters (which exceeded the significance threshold) was group 2, with the two pathologies, which suggests the influence of ischemic heart disease and chronic marginal periodontitis on these parameters by their association.

5. Comparing the complete blood count parameters between groups, statistically significant differences were found in all three groups in terms of haematocrit, platelet count, eosinophil count and lymphocyte and neutrophil percentage.

6. Statistically significant differences were found in all combinations of groups for the following variables: serum sodium, TGP, serum triglycerides, serum urea, ESR, hsCRP, serum uric acid, blood glucose and LDH.

7. Increased values of APP parameters and platelet count were specific to patients with both pathologies highlighting the severity of the two inflammatory diseases, by affecting (degrading) periodontal status and increasing the risk of developing blood thrombi.

Chapter 8. Study on the degree of awareness of the role of dental prophylaxis in patients with ischemic heart disease

Introduction

The goal of the present study was to identify the level of awareness of the role of dental prophylaxis in patients with cardiovascular diseases and the level of acceptance of the manoeuvres required by it.

Material and method

In order to meet the intended goal, I developed a questionnaire consisting of 8 questions (items), applied to a number of 57 patients known to be suffering from ischemic heart disease, who presented for specialised treatments in dental offices.

Of the 57 subjects participating in the study, 37 (representing 67.91%) were males, while the remaining 20 (representing 35.08%) subjects were females.

Results

Following the evaluation of the answers to the 7 questions (7 existing items in the questionnaire), the following aspects resulted:

- To the first question in the questionnaire, 38 (representing 66.66%) of the patients involved in the study answered affirmatively to point a. of the item (correct answer), while 19 (representing 33.33%) patients answered affirmatively to point c. of the question.

- For the item no. 2 of the questionnaire, all the subjects involved in the study provided the correct answers (they answered affirmatively to points a., b. and e. of the question).
- To the question no. 3 of the questionnaire, 50 (representing 87.71%) of the patients involved in the study answered affirmatively to points a., b., d. of the question (correct answers), while 7 (representing 12.28%) subjects answered affirmatively to points c. and d. of the item (figure 6.3.)
- After studying the item no. 4 of the questionnaire, the distribution of the patients involved in the study, depending on the types of their characterisations presented in the item, was as follows: 12 (representing 21.05%) subjects fell into the category described in point a. of the item, 20 (representing 35.08%) subjects fell into the category described in point b., 10 (representing 17.54%) subjects fell into the category described in point c., while 15 (representing 26.31%) subjects fell into the category described in point d. of the item.
- To the question no. 5 of the questionnaire, all the subjects involved in the study provided the correct answers (they answered affirmatively to points a. and d. of the question).
- To the item no. 6 of the questionnaire, 48 (representing 84.21%) of the patients involved in the study answered affirmatively to the variants a. and c. of the question (correct answers), while 9 (representing 15.78%) patients answered affirmatively to all points of the item: a., b., c.
- To the last item of the questionnaire (question no. 7), 26 (representing 45.61%) of the patients involved in the study answered affirmatively to a. and b. (correct answers), while 31 (representing 54.38%) of the subjects answered affirmatively to all points of the question: a., b., c., d.

Discussions

The patients with heart disease are more susceptible to periodontal disease because of their health condition. More than half of the patients included in the study understood the connection between certain microorganisms that can be found in the aetiology of not only gingival disease but also atheromatous plaques and ischemic heart disease. All patients understood that, as a prophylactic measure, it is important they should properly remove microbial dental plaque, dental tartar as well as food debris. As for dental prophylaxis, most patients knew that this operation can be performed both at home and at the dental office, by a team consisting of a dentist and a nurse

specialised in oral and dental prophylaxis. Therefore, all patients participating in the study were aware that, in order to maintain good oral hygiene at home, classic or electric toothbrushes should be used, as well as adjuvants such as dental floss, interdental brushes, mouth irrigators.

Patients are more effectively enrolled into active therapy if they understand the etiology of periodontal diseases, treatment options, consequences of nontreatment, the direct benefits of therapy and also the value of treating periodontal infection in relation to their overall health (18).

Conclusions

1. The majority of patients involved in the study (66% representing 2/3) agreed on the microbial component in the development of ischemic heart disease, understanding the importance of oral-dental prophylaxis in this case, while the remaining 33% agreed on the implementation of oral-dental prophylactic manoeuvres.
2. Over 85% of the patients involved in the study received all the pieces of information on oral and dental prophylaxis.
3. 35.08% fell into the category of patients who removed their dental microbial plaque relatively well and conscientiously, followed by those who fell into the category of patients who were not interested in oral hygiene, who did not have a regular toothbrushing, or who did not follow the correct tooth brushing techniques (26.31%), then by those included in the category of patients who performed the correct tooth brushing and who thoroughly removed the dental bacterial plaque, without damaging their soft tissues (21.05%), and by those patients classified in category of very dynamic, aggressive patients, who removed dental plaque effectively, but with repeated and sometimes extremely accentuated soft tissue damage, which favoured bleeding (17.54%).

Chapter 9. Conclusions and personal contribution

9.1. Final conclusions

1. The conclusions of the systematic synthesis made in the documentation of the present doctoral thesis, regarding the correlation between the two pathological entities, show that periodontal disease is highly prevalent in chronic heart disease regardless of its cause.

2. Chronic marginal periodontitis can be evaluated as a risk factor for the cardiovascular system, but periodontal injury must be within the confirmed severity levels and the literature to have multiple systemic and cardiovascular implications in particular.
3. Severe forms of chronic periodontitis marg represent a certain statistical factor revealed by the cardiovascular system, and especially the pathophysiological phenomena and the pathogenesis of ischemic heart disease enhance the destruction of bone support, ligament, soft structures of the periodontium.
4. Chronic marginal periodontitis, being an infectious disease with inflammatory effects, can lead to the development and instability of atheromatous plaques in the arteries. Thus, because of the systemic inflammation, endothelial dysfunction may represent the link between the two pathological entities.
5. Ischemic heart disease, associated with chronic marginal periodontitis, potentiates the destruction of bone support.
6. Prevotella nigrescens may be a marker of cardiac tissue damage, with a statistically significant difference between groups 1-3 and 2-3.
7. The factors that differentiate the group of patients with chronic marginal periodontitis from those with associated cardiovascular disease are: age, smoking history, average number of teeth present, periodontal damage, concentration of *Campylobacter rectus, Prevotella nigrescens, Capnocitofaga sputigena, Tannerella forsythia, Capnocytophaga ochracea*.
8. The factors that differentiate the group of patients with ischemic heart disease from those with associated pathology are: smoking history, periodontal pocket depth, average bone level, average number of remaining teeth, CPITN score, concentration of Actynomicete comitans, *Treponema denticola*.
9. In 36,9% of the cases, a correlation can be established between the flora in the group with associated pathology and that found in the group with chronic marginal periodontitis, which can certify the possibility of the aetiological connection between the two pathological entities.

9.2. Personal contribution

The innovative contribution of the present thesis is supported by the studies performed on the three groups of various patients (1 group with chronic marginal periodontitis, 1 group with ischemic heart disease and 1 with the two diseases). The mentioned studies were complemented

by a study on the oral-dental prophylaxis applied to a group of patients with ischemic heart disease, which was intended to highlight the inflammatory connection between the two diseases. The originality of the present research is provided by the results obtained from the studies performed on patient groups, where we have blood / serum markers that could signal the association of periodontitis with cardiovascular disease and, moreover, could establish a correlation between the flora in the group with associated pathology and that found in the group with chronic marginal periodontitis.

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Scientific papers developed during the doctoral study programme

I. Articol ISI

1. **Voinescu I**, Ferechide D, Cristache CM, Burlibasa L, Burlibasa M. Ethical and legal aspects in periodontal disease diagnosis and therapy. Rom J Leg Med 27(1):57-64, 2019. doi: 10.4323/rjlm.2019.57, p 0,488.

II. Articole BDI

1. **Voinescu I**, Ferechide D. Statistical Study Regarding the Presence of Gram Bacteria in Patients with Cardiovascular Disease and Periodontal Disease. Modern Medicine. 2019, 28(4):407-411, 2021. https://doi.org/10.31689/rmm.2021.28.4.413
2. **Voinescu I**, Ferechide D, Perieanu V, Costache MG, Zara DM, Perieanu MV, Burcea CC, Malița M, Beuran IA, Burlibasa L, Marcov N, Iorgulescu G, Dina MN, Burlibașa M, Oancea L, Ionescu I, Marcov EC. The therapeutic behavior of the dentist in patients with atherosclerosis – theoretical and practical aspects. Romanian Medical Journal, 67(2):172-178, 2020. doi: 10.37897/RMJ.2020.2.12.
3. **Voinescu I**, Dumitru Ferechide D, Beuran IA, Burlibașa M, Dumitru SG, Costea R, Babiuc I, Eftene O, Perieanu VS, Donciu I, Perieanu MV, Andrei OC, Stănescu R, Marcov N, Marcov EC, Popoviciu O. The need for oral and dental hygiene in patients with ischemic cardiomyopathy-preliminary study. Revista Medicală Română, 66(4):376-383, 2019. doi: 10.37897/RMJ.2019.4.15.
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