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FACULTY OF DENTAL MEDICINE

COMPLEX STUDIES ON THE
ASSESSMENT OF RISK MARKERS IN
PERIODONTAL PATHOLOGY

PhD THESIS ABSTRACT

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INTRODUCTION
ARGUMENT OF THE RESEARCH TOPIC

The Periodontal disease can have different forms, with a vast area of manifestation, from affecting the gingival tissues until the destruction of the periodontium, which can even lead to the teeth loss in certain cases. The manifestations and evolution of the periodontal diseases vary for each form in part and consequently, the most thorough understanding of the aetiology and of the existent risk factors in order to establish an accurate algorithm of diagnosis and a therapeutic plan is required as a necessity.

In the last few years extraordinary progress was achieved in understanding the causative factors associated with the appearance of the periodontal diseases. It was clearly established that these diseases are by their condition contagious and they appear in the presence of the deposits of supra and subgingival bacterial plaque.

The studies regarding the risk factors are extremely complex being necessary to take into consideration multiple factors that can significantly affect the resistance of the host, the association of the factors possibly varying in different periods of an individual’s life.

The risk factors are considered as modifiers of the disease activity. In association with the sensitivity of the host and of the variety of the local and systemic conditions, they influence the inception and the progression of periodontitis, aspects that are reflected in the modifications of some biomarkers. The identification of these biomarkers is a challenge not only for the clinician but also for the specialty researchers, being of a real use in the early tracking of the disease evolution and also the setting up of some compelling measures having as a goal the efficiency of the therapy. Regarding the choice of these biomarkers, it is essential that these could fulfil their rightful role in routine practice, so that their connection with the disease worsening and severity mechanism and also the therapeutic attitude are fully understood.

A biological marker, respectively the biomarker, in accordance with the most updated definition, is a substance (parameter) which is objectively measured and assessed as an indicator of the normal biological processes, or of the pharmacological responses to a therapeutic intervention. Taking into account the fact that saliva and GCF (gingival fluid) are fluids easily to collect and that they contain local markers and systemic derivatives of the periodontal disease, in the current study, the gingival fluid was chosen, as an interface in assessing the level of specific biomarkers for the condition of periodontal alteration, of different degrees of severity.

CHAPTER I ELEMENTS OF ETIOPATHOGENESIS IN THE PERIODONTAL DISEASE

The periodontal disease is a multifactor disease characterized by chronic inflammatory processes initiated by the presence of the anaerobic micro-organisms that are Gram-negative alongside which we can associate pathological conditions as for example dental cavities, tophus, vicious habits, dento-maxillary anomalies, lack of teeth, traumatic occlusion.

The power of the microbial attack depends on the micro-organisms virulence, the quantity and composition of the plague, the ability to invade tissues and on their metabolic products. The host’s capacity to respond to the bacterial challenge will determine the severity of the gingivitis, the initiation of the periodontitis and the fastness with which the periodontal tissues will be destroyed. [35]
The particularly important role of the micro-organisms from the biofilm was emphasized through the research carried out by Waerhaug, Glickman, Slots, Socranski, and Carranza. However, the attitude of some well-known vernacular researchers as Victor Babeş who tried to explain a possible involvement of the bacteria in “gingival-periodontal “osteo-periostitis “[236] is also meritorious. These opinions appeared in the first discourse of microbiology from the world “Les bacteries “appeared in Paris in 1866, being written together with Cornil. In 1894 the article “About a bacillus that induced gingivitis and bleeding in scurvy” appeared from the “Magazine of Romanian Medicine”. [48,129]. Even more, in the last 20-25 years, the researchers and clinicians offered different aspects of the periodontal disease not only concerning the disease’s condition, but also the sensitivity, aetiology and pathogenesis. It is known that CMP (chronic marginal periodontitis) is a multifactor disease with a wide range of local modifications, and also generalized ones, the risk factors modifying the host’s answer to the main etiological incriminated factor – dental plaque. [146,307,352]

Anyway, although the existence of micro-organisms is vital, the host factor is extremely important. The more the aetiology has evolved, the newer forms of periodontal diseases were met. [26]

The specialty studies noticed that the systemic factors cannot provoke by themselves an inflammatory response of the gum. They have the role to decrease the resistance of the periodontal tissues or to provoke certain tissue phenomena, by making them more vulnerable in front of the local factors. Among these, we cite the nutritional, neuropsychological deficiencies, cardiovascular diseases, diabetes, small weight upon preterm birth for children and the diseases of the respiratory system. [14, 118, 121]

Four main pathogenic links belonging to the host contribute to the self- conveyance of the local inflammatory response from within the periodontal disease, reflected subsequently upon systemic level. These four elements being in permanent interrelation represent: epithelial answer, inflammatory cytokines, leukocytes and monocytes and each of them can be bacteriologically conditioned.

All specialty studies came to the conclusion that periodontitis is an infectious chronic disease that affects gingival tissues, the periodontal ligament and the alveolar bone, possibly being a source that can generate systemic inflammation, the association between the presence and the severity of the disease influencing the diffusion and extension of inflammatory biomarkers.

**CHAP.II DEFINITION OF THE CONCEPT OF RISK FACTOR IN PERIODONTAL DISEASE**

The periodontal disease designates the integrity of the pathological manifestations that refer to the cover periodontium, these presenting a plurifactorial aetiology (determinant and favourable factors), knowing a chronic evolution (with periods of activity and inactivity), and requiring a complex and long term treatment. The term of periodontal disease, in its strictest meaning, refers to gingivitis and periodontitis. The latter are constituted in destructive forms of the periodontal disease, as a consequence of interactions among the microbial factors (determinant) and those of the host but also possible influences of the environment (favourable factors). [4]

Assessing the periodontal risk has as objectives their identification in order to avoid, reduce or control them.
The periodontal risk can be identified in terms of risk factors, risk indicators and risk predictors that can be associated to some external factors (tobacco, social-economic status, therapeutic compliance), internal factors (age, bacterial agents or their derivatives, genetic factors, systemic troubles, hormonal and medicinal products influences, stress) to which we can correlate morphofunctional and retention factors.

The risk factor is considered to be determinant for the appearance of the disease because it fulfils two criteria: it presents biological plausibility as a causative agent and the prospective clinical studies support its association with the periodontal disease, respectively they demonstrated that it precedes the appearance of the disease (smoking, for ex.). [11]

Multiple specialty studies were carried out up to now on the risk factors and the possibilities of tracking down the patients with a high risk.

Recent epidemiological studies have provided important information that led to a better understanding of the periodontal disease pathogenesis. We mustn’t forget the existence of some risk factors (FR) and their involvement as an integrating part of the causative chain of the periodontal disease.

The presence of risk factors implies the growth of the probability as the periodontal disease could intervene, as there is the inverted possibility as well through which the periodontal disease represents an aggravating factor of some systemic diseases in certain clinical-pathological conditions. The recognition and understanding of the risk factors is of great importance in the management of the periodontal disease. [45, 46.]

CHAP. III THE UTILITY OF THE BIOLOGICAL INFLAMMATORY MARKERS IN THE DIAGNOSIS & PROGNOSIS OF THE PERIODONTAL DISEASES

In the last years, an increased number of epidemiological studies indicated that the patients with periodontitis can have an increased risk of cardiovascular diseases. The association between periodontitis and cardiovascular diseases actually remains an issue for debate.

Critics underlined the fact that both periodontitis and atherosclerosis collaborate from the point of view of risk factors, but this association, even if it is established, could be fake.

In the case that the association was well established, the critical problem regarding its condition in possible terms of causability will remain central, in order to understand the medical significance of the periodontal infections.

The individuals affected by this disease can present common polymorphisms of the specific genes considered important in the settlement of the proteins responsible with the installation of the inflammatory response.

Even more, in patients with severe periodontitis, high levels of reactive C protein can be registered, Fibrinogen, moderate leucocytosis, and also high serum levels of IL-1 & IL-6 (in comparison with non-affected populations). [16]

Among the associated risk factors, there is also smoking, systemic diseases such as hyperglycaemia that also occur in the area of etiological agents for cardiovascular diseases.

*It is thus evident that severe periodontitis in healthy individuals is associated with an inflammatory systemic response.*

Regarding the association of periodontitis, the systemic inflammation and cardiovascular diseases, there were suggested two hypotheses that can explain the etiological ways of association and the mechanisms of signalling:
SUMMARY

COMPLEX STUDIES ON THE ASSESSMENT OF RISK MARKERS IN PERIODONTAL PATHOLOGY

- One is based on the chronic infectious load which the periodontitis can present in the body through the access of the microorganisms or of the endotoxins;
- Another theory considers that the sick periodontal is like a source of systemic inflammatory mediators. No matter the basic mechanism/s, the systemic inflammation might be central in order to explain the condition of the bond between the chronic infections and atherosclerosis. [27, 42]

CHAP.IV RESEARCHES CONCERNING THE BIDIRECTIONAL RELATION BETWEEN THE PERIODONTAL DISEASE AND HYPERLIPIDAEMIA

Despite the multiple analyses and the effort made for a long period of time, the cardiovascular disease remains one of the main causes of morbidity and mortality in the world. The atherosclerotic process, a biological response that is related with the inflammation [55], is found many times at the base of the cardiovascular diseases.

Although the fact that dyslipidaemia is an important determinant of the atherosclerosis has been recognized for a long time, recent researches suggest that the chronic inflammation has an important significance. The systemic markers of inflammation, such as IL-6 and C-reactive protein (CRP) register a high level in the patients’ plasma with atherosclerosis [60]. The inflammation is an important part of the immune congenital response against the pathogen agents.

These correlations are at the base of the concept through which the chronic infection, respectively the chronic inflammation, advances and accentuates atherosclerosis. Indeed, a variety of infectious agents, especially Chlamydia pneumoniae, Helicobacter pylori, Trypanosoma cruzi and the virus Herpes simplex, were connected in the last year to atherosclerosis. [15]. These studies determined a re-examination of the risk factors list for the cardiovascular diseases, initially elaborated by Framingham in the 1960s [12].

Although the resemblances tend to be modest, periodontitis was correlated with a variety of systemic diseases, including the metabolic syndrome, the resistance to insulin and the preterm birth / small weight at birth. During the last decade, a considerable number of researches regarding the connection between the periodontal disease with atherosclerosis and the vascular disease [30] have been carried out. Such studies present reliable insertions in specialized literature that proves the liaisons between infection, chronic inflammation and atherosclerosis.

A great number of studies from the specialty literature investigate the effects of cardiovascular treatment on the incidence and severity of the periodontal disease. Although some researchers suggest the contrary, the majority of studies indicate the fact that the treatment not only reduces clinical indications of the periodontal disease but, it also ameliorates the modifications of lipid metabolism and consequently atherosclerosis.
After establishing the presence of periodontal alterations, the doctor must determine which clinical diagnosis best describes the impairment type at each patient in part: chronic, aggressive, mortifying periodontitis etc. Because the present classification is based on a combination between the clinical status, the disease progression rate and the model of domestic aggregation of the cases in the absence of a systemic cause for the clinical observations, it is demanded to take into consideration the following aspects:

The range of the periodontal diseases extends from the periodontal benign disease types known under the name of gingivitis, to the chronic and aggressive forms of the periodontal disease, that not only threaten the dentition, but can be, also, a threat to the general state of health. All the types of periodontal inflammatory disease are associated with the chronic inflammation, resulting at the same time the periodontal ligament’s destruction as well as the bone.

If it is left untreated, the disorder can lead to the significant tissue destruction; the affected teeth can become mobile and can be lost if the disease continues to be active. The periodontal disease prevalence in the world is very high, the reports indicating degrees of approximately 90 % of the adult population with minor changes of gingivitis type, 60% with signs of chronic periodontitis and variable rates between 5-15% with aggressive periodontitis. [12, 13]

V.2. THE AIM OF THE STUDY

For the purpose of assessing the involvement of risk markers in the pathogenesis of the periodontal disease, we approached in a multidimensional way the human sample group enclosed in the research through clinical, paraclinical, statistic and biochemical investigations.

Our results can constitute the starting base in the reasoning and subsequently the development of a relationship between the presence of an infection at the level of oral cavity and the disruption of the homeostatic mechanisms that influence the level of inflammatory level. The study is also supported by previous data from the specialty literature regarding the connection between the periodontal disease and the high levels of the C reactive protein, Fibrinogen and the number of white cells.

V.3. RESOURCE AND METHOD

The conducted study targeted the following analysis directions:

a. The assessment of the periodontal status in the patients taken in the study
b. The assessment of the clinical indicators of the periodontal disease (respectively the bacterial plaque index, parameters that attest the degree of gingival inflammation-bleeding indices, the loss of the attachment, alveolar bone lysis) in patients with severe systemic diseases and their comparison with those from the control group.
c. The comparative assessment of the periodontal index values at the teeth level - Ramfjord (respectively 16, 21, 24, 36, 41, and 44) at patients from the study groups, with those from the control group.
d. The comparative assessment of the inflammatory markers values from the peripheral blood (C reactive protein, Fibrinogen, ESR, leukocyte count) at patients taken into the study.
e. The correlation of the clinical indicators’ level as current diagnosis elements in the periodontal disease with the systemic markers values.

V.4. RESULTS

V.4.1 The results of the clinical periodontal assessment

The results were obtained by carrying out some comparative clinical-statistical studies on a personal database regarding the health state and impairment state of the marginal and deep periodontium, in people susceptible or affected by the periodontal chronic disease and with aggressive character.

The medical charts were filled in for all the patients, including: personal data, medical and dental status history.

We tried to assess possible correlations between the local impairment degree of the patient and the type of periodontal disease.

The diagnosis of the aggressive generalized periodontitis (GAP=AgP) takes into account the fact that this type of disease usually affects people under 30 years old (but they can be older) in comparison with CP which affects prevailingly the age groups over 30 years old.

Group A/B/C

Men / Women

Absent Periodontal pockets/ Periodontal pockets 4-6mm

Diagram V. 1. Assessing the presence and depth of the periodontal pockets according to groups

Tabel V.1. The Prevalence of the periodontal indices of the studied groups
### V.4.2 The Results of assessing the inflammatory markers

#### V.4.2.1 PCR, ESR, assessment Fibrinogen

The medium level of CRP was of 1.9 mg/l with an IQ range of 3.6 mg/l, in time. A significant difference in the serum level of IL-1 and IL-6 was registered at the study groups, the differences being noticeable according to the type and severity of the periodontal alterations.

**Table V.2.** The Distribution of the inflammatory markers value, on groups of study

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C reactive protein</td>
<td>1.56±2.1</td>
<td>5.02±6.0</td>
<td>1.46±2.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(mg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSH (mm/h)</td>
<td>6.3±5.7</td>
<td>7.4±10.6</td>
<td>4.3±5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>264.0±73.7</td>
<td>305.7±54.1</td>
<td>218.0±62.7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Diagram V.2.** Values of the inflammatory indices on groups of study
Patients with periodontitis have higher levels of CRP & IL-6 in comparison with periodontal healthy populations [94]. This disturbance of the physiological homeostasis is also accompanied by a smaller number of erythrocytes and increased concentrations of the haemoglobin, higher values of the haptoglobin, moderate leucocytosis and increased levels of cholesterol, LDL and glucose [221]. Nevertheless, it became clear that some additional factors (for example: smoking) can lay at the base of these associations.

The results of the present study established the presence of a causability connection between the periodontitis and the inflammatory systemic response. Even more, there is a correlation between the severity of the periodontitis and the serum concentrations of CRP, probably the type and grade of severity of the periodontitis reflecting at the level of the analysed systemic markers.

**V.4.2.2 The results of assessing proinflammatory cytokines IL-1, IL-6**

Periodontal pockets abundantly contain gram-negative bacteria that come into contact with the conjunctive tissue and blood vessels from the periodontium. This infection in its turn leads to chronic subclinical bacteremia as a result of the periodic release of cytokines in circulation, among these IL-1, IL-6 being frequently investigated and assessed.

Cytokines play an important role in the pathology associated with chronic inflammatory diseases. Due to pro-inflammatory and chemotactic (on neutrophils) characteristics, some of the cytokines, such as interleukins of type IL-1, IL-6, can play an important role in the pathogenesis of the periodontitis. The biological effects of the two mentioned interleukins are relevant in this respect.

In our study, the values of the interleukin 1 are situated at much higher levels in the case of young individuals with severe periodontal injuries, the average level in this case being almost double compared to the one registered in individuals with chronic impairment of the periodontium. Although limited due to the relatively small sample of work (the number of patients with aggressive periodontitis who addressed to the dental clinic being lower, compared to those with chronic periodontitis injuries, localized or generalized), the present study supports the importance of markers of IL-1 type in certifying the degree of severity of periodontal territory alterations, and therefore, pointed hereinafter the assessment of the possibilities to use such parameters in the consideration of the feasibility for the treatment algorithm in patients with various distortions of the Periodontal area.

**Diagram V.3.** The average of the interleukin recordings 1 on subgroups
The same measurements and recordings were also made for IL-6, known as being often secreted along with other pro-inflammatory cytokines during acute phase reactions. First reports in this regard have shown that IL-6 levels from the gingival crevicular fluid were elevated during the chronic progressive periodontal disease [124] as well as in refractory periodontitis.

Both inflammatory markers had a positively asymmetric distribution curve. An average of the values that was significantly reduced of IL-6 in serum was found in patients without periodontal disease (test of Wilcoxon, p=0.021, 0.006 respectively).

The difference in the average concentration of IL-6 was of 0.18 ng/l (0.02-0.44, 95% CI) between the group with chronic periodontitis and that with aggressive periodontitis 0.22 ng/l (0.06-0.44, 95% CI).

**Diagram V.4** Average of the interleukin recordings 6 on the analysed subgroups, according to the periodontal diagnosis

Patients with severe periodontitis have elevated levels of C-reactive protein, hyperfibrinogenemia, moderate Leucocytosis and also elevated serum levels of IL-1 and IL-6 as compared to control unaffected populations [94].

**V.5. DISCUSSIONS**

Recent epidemiological studies have shown that periodontal infection is a risk factor for a number of diseases and systemic conditions. In addition to the risk factors, chronic infections and the subsequent generating of a generalized inflammatory response can be conventionally associated with this increased risk.

Innate defence systems include epithelial, conjunctive tissue elements, and soluble products which are antimicrobial, in health status being able to protect themselves against infectious diseases that include periodontal alterations as well. There were reported imperfections of the compounds in epithelial tissues, connectives (fibroblasts, cement), from the tissue fluids (saliva) or of the enzymatic type (e.g. alkaline phosphatase), which may be relevant for early-onset periodontitis.
As a first approach, at this stage, our study aimed at determining the level of certain inflammatory markers (of proteins or cytokines type) in various forms of periodontal alteration, so that later on, we could evaluate whether the surgical periodontal treatment could affect the serum levels of the interleukins IL-1, IL-6 and of C-reactive protein (CRP), in patients with chronic periodontitis and also in the case of those with aggressive periodontitis.

It should be mentioned that, among the patients who came for dental check-up, we also included in the study groups (A) the individuals with generalized severe chronic periodontitis (the Criteria adopted at the Workshop of international classification for periodontal diseases and conditions, 1999), with the loss of clinical attachment larger than 5 mm and with more than 50% of the situs involved. These levels of disease severity were chosen in order to increase the probability of detecting the systemic load, probably induced through a local periodontal infection.

Thus, in this study, we evaluated the degree of "leakage" for proinflammatory cytokines, such as interleukins (IL-1, IL-6) in the systemic circulation, the systemic inflammatory response generated by the severity of periodontal disturbances produced in the periodontal territory (of different levels in localized or generalized chronic periodontitis, or aggressive), finally aiming at the analysis of the changes brought by periodontal surgical therapy in the systemic concentration of cytokines and other acute phase reacting substances (CRP).

The number of white blood cells in generalized aggressive periodontitis is bigger than in the localized one and then in the control group. The correlation between CRP and periodontal health state is evident in the widespread aggressive periodontitis, being a correlation between the forms of the disease and the number of leukocytes.

CRP, IL-6 and the neutrophils can contribute in part to the chronic infections association mechanisms with cardiovascular diseases. CRP can activate the complement in the destroyed walls of blood vessels, IL-6, having proinflammatory and procoagulant properties which may contribute to the pathogenesis of aggressive syndromes.

The high levels of the reactive C protein in the blood, of leukocytes, Fibrinogen and other markers of inflammation, which were often associated with a consecutive risk for the occurrence of cardio-vascular imbalances, demonstrate that the high levels of inflammation markers may contribute to the formation of the atheroma plaque. C-reactive protein (CRP) is thus an ideal marker of inflammation, not being produced or consumed in inflammatory processes. It is synthesized by hepatocytes in response to stimulation through cytokines released by macrophages, endothelial cells (the capacity of aortic and coronary endothelial cells is proved to synthesize CRP, the process being stimulated by the action of cytokines secreted by macrophages, T lymphocytes and NK).

Periodontal pockets contain gram-negative bacteria in abundance, and come into contact with the connective tissue and the blood vessels from periodontium, these infections leading, in their turn, to chronic subclinical bacteraemia, as a result of the periodic release of cytokines in circulation.

This study conducted to assess the systemic inflammatory load determined by chronic or aggressive periodontitis in individuals otherwise "healthy" and also to assess whether periodontal therapy could bring a change in serum markers of systemic inflammation, equally indicates that both
generalized severe periodontitis and aggressive periodontitis seem to contribute to systemic inflammation.

A limitation of this study represents the relatively insufficient sample size of patients, especially with regard to the identification of those with aggressive periodontitis. Furthermore, although we have excluded from the study the smokers, the obese individuals, and, to the possible extent, the individuals with any other infections, we didn’t evaluate the effects of some possible changes in cholesterol, blood pressure, educational status, and it is not excluded that these factors could have influenced our data. The results of this phase of the study reveal the importance of the identification and early diagnosing of cases with severe periodontal destructions, and the need of oral health education.

V.6. CONCLUSIONS

1. Our results support the idea that the CP and AgP are accompanied by a systemic imbalance, reflected in the acute phase of the reactants level, of pro-inflammatory cytokines (IL-1, IL-6), very likely, the pathogenesis of periodontal alterations being mediated at the level of these mediators.
2. The prevalence (%) of markers – periodontal health indicators, in patients with periodontal aggressive disease compared to the control group shows a higher value at the study group unlike the control one.
3. Inflammatory markers like C reactive protein, VSH, Fibrinogen, have high values at the groups of study in comparison with the control group (PCR 5.02±6.0 vs 1.56±2.1, (p<0.01) (VSH 7.4±10.6 vs 6.3±5.7, p <0.001), (Fibrinogen 305.7±54.1 vs 264.0±73.7; p<0,05)
4. The leukocytes number is higher in the group with generalized aggressive periodontitis, followed by the localized aggressive periodontitis and the control.
5. Our expectations converge towards the idea that periodontal therapy may lead to reductions in serum levels of proinflammatory cytokines, the acute phase reactants level, and, although none of the available protocols for periodontal treatment is specifically designed to improve the systemic status, the therapeutic algorithm being used at present, can be upgraded to handle simultaneously the periodontal and systemic sequelae distortions.
6. Although the results of this phase of the study reveal the importance of early identification and diagnosis of cases with severe periodontal destructions, and the need of oral health education, the use of groups of larger sizes and additional studies in order to significantly contribute to the identification of the most optimal therapeutic measures and to avoid systemic complications (among which the cardiovascular ones are not to be neglected at all), shall be imposed as a necessity.
VI.1. INTRODUCTION - PLASMATIC LIPID LEVELS AT PATIENTS WITH PERIODONTAL DISEASE

Most specialty studies support the concept of an association between periodontal disease and changes of lipid metabolism in various diseases, such as atherosclerosis or coronary artery diseases.

Although chronic inflammation plays a clear role in the pathogenesis of studied diseases, changes in lipoprotein metabolism and levels seem to also have an impact in the modulation of the oral alterations in periodontal territory.

The evidence so far suggests that periodontal disease may induce changes in the metabolism of lipoproteins with low-density, proatherosclerotic, so that, very likely, patients with periodontitis present an increased risk of developing cardiovascular diseases.

VI.2. THE AIM OF THE STUDY

This study was conducted on a sample of periodontal patients compared to healthy ones at the oral level, in order to comparatively assess the level of serum lipids (surrogate biomarkers for periodontal and coronary disease) correlated with various forms of periodontal disease.

The research goal was focused on the record level of total cholesterol, low density lipoproteins (LDL), high-density lipoproteins (HDL) and triglycerides in the Group of subjects with periodontal disease and in healthy individuals without periodontal alteration, as well as the degree of association between an altered lipid profile and the type of periodontal diseases.

VI.3. MATERIAL AND METHODS

The groups of patients enrolled in this study, assessed in the required assistance in the Department of Periodontology, the Faculty of Dental Medicine of the UMF Iaşi and Dentesse clinic-private practice offices of Iaşi, consisted of:

- **Study group**
  - 52 patients with periodontal disease (20 women and 32 men, with the age between 20-65 years old, average of 41.4±3.1)
  - Patients were divided in 2 subgroups:
    - **Subgroup 1** consisting of **29 patients**, aged between 20-65 years, at which the periodontal diagnosis included marginal chronic periodontitis of different degrees
    - **Subgroup 2** consisting of patients with aggressive periodontitis - **23 patients**

- **Control group**
  - 20 patients in the control group – without periodontal alteration (8 women and 12 men, aged between 30-60 years old, average 54.4±2.8).

The database has also included a questionnaire concerning general medical and social status, which all the subjects have completed with or without periodontitis, enrolled in the study.
No individual has taken any hypercholesterolemicant medications and patients with more than 3 Periodontal pockets, with a depth of probing >4 mm have been included in the Group of subjects with periodontal disease (study group).

The balance of the oral status of patients was performed with the help of the following indices:

- CPITN Index
- Gingival inflammation index - Løe and Silness
- Plaque index

**Measurements of blood glucose and lipid profile**

After being enrolled in the study group with periodontitis, both these patients and the subjects in the control group were collected fasting blood samples for plasma lipids recording of the concentrations of glucose in the blood.

**VI.4. RESULTS**

**VI.4.1 The general characteristics of the study groups**

The medical charts were filled in for all the patients including: personal data, medical and oral history.

52 patients with periodontal disease (23 women and 29 men, aged between 20-65 years, the average of 41.4±3.1) and 20 patients in the control group (8 women and 12 men, aged between 22-60 years, the average 44.4±2.8) participated in this study.

**VI.4.2 The periodontal characteristics of the study groups**

The analysis of clinical indices was followed by the assessment of some possible correlations between the extent of local damage of the patient and the type and severity of periodontal deteriorations.

The results of our study show a direct relationship between the degree of plaque formation and the age and level of oral hygiene of the studied groups, but with significant variations within the groups with CP and AgP.

An increase in the prevalence of total and subgingival scale deposit along with the patients’ age was noticed.

In some subjects in the control group, Periodontal pockets of moderate depth (8.7 ± 8.5% of sites) were present, but deep Periodontal pockets were not present.
Diagram VI. 1 The analysis of the size of the periodontal pockets and their frequency in the total study group

Bleeding in probing was more frequent in patients with periodontal disease (25.1 ± 6.3%) compared to the control group patients (5.0 ± 6.9%, p = 0.0001). The latter, had significantly fewer absent teeth than patients with periodontitis (7.9 ± 5.7 compared to 11.2 ± 7.4, p = 0.03).

A qualitative estimation of microbial plaque accumulation didn’t reveal a significant difference between patients with periodontitis, and the patients in the control group (50 ± 19% unlike 48 ± 23%).

Diagram VI. 2 The assessment of the oral hygiene degree, comparatively in the study group with the control group

The assessment of the degree of periodontal alteration according to the depth of the periodontal pockets highlighted for most of the patients a moderate level of impairment: 47.2 ± 24.6% of sites had a depth of probing of 4-5 mm and 8.6 ± 16.3% of sites have had a depth of probing of more than 5 mm.

Diagram VI. 3 The degree of attachment loss, in regard to age
We mention that in most cases of AgP, the level of periodontal destruction seems to be higher than estimated, very likely because of the cumulative action of local factors, the observation not being valid for all the subjects.

Diagram VI.4 The prevalence of teeth with Gingival recession

In general a discrepancy between local factors and the level of tissue degradation indicates either the presence of an infection with etiologic agent characterized by a particular microbial virulence or the existence of a high susceptibility at the host level. Moreover, the percentage of subgingival scale areas with at least 1 mm thickness is greater in the group with chronic periodontitis patients, patients with rapidly progressive periodontitis presenting more surfaces with subgingival scale in relation to subjects with chronic periodontitis.

VI.4.3 The Characteristics of lipid profiles at the study groups

Serum lipid parameters including ★total cholesterol, ★LDL cholesterol ★HDL, ★triglycerides, represent as many factors, which may vary depending on various systemic conditions. Our study targeted on registering the values of these parameters in patients with diverse periodontal alteration, highlight changes depending on the presence of periodontal affected territories.

Within multiple researches on the multivariate regressive models in which the evaluation takes into account associated factors or risk factors such as age, smoking, alcohol consumption, certain mental states or chronic consumption of certain pharmacological agents (aspirin for ex.), the reports related to the periodontal disease were associated with significantly elevated levels of triglycerides (30% higher among cases with periodontal disease compared to subjects without periodontal alteration) cholesterol (11%), LDL cholesterol (11% higher). [72,111]
SUMMARY

Diagram VI. 5 The level of lipids and blood lipoprotein fractions in groups of parodontopathy

The analysis of the frequency of pathological values of the lipid profile between the two large groups of study, with and without periodontal alteration, showed significant differences, the frequency of hypercholesterolemia in periodontal patients being approximately two times higher than that registered at the control group.

This was both valid for total cholesterol and also "bad" cholesterol-LDL. Pathological levels of triglycerides were about 12% more frequent in the Group of patients with periodontitis compared with subjects in the control group, while the levels for HDL cholesterol fraction were comparable in the two research groups.

Therefore, the results of the actual parameters of records of the lipid profile and of the variation in frequency of occurrence of abnormal (pathological) levels in the blood of the subjects in the two groups, in which the major distinction represents the altering of the periodontal territory, indicate: higher average plasma levels of lipid parameters: cholesterol, triglycerides and LDL-C to subjects with periodontitis when compared with individuals in the control group, the level of HDL-C registering close values, without significant statistical differences.

Moreover, significant changes between the subjects with periodontitis and those without injuries of the periodontal territory were also observed when the frequency of plasmatic pathological lipid concentrations, hypercholesterolemia being twice more common in subjects presenting various forms of oral tissue of periodontal destruction, was estimated.

Many studies in the recent years regarding the evolution of periodontal disease according to various conditions have focused on the investigation of pro-and anti-inflammatory cytokines. However, the relatively new work also suggest that periodontal therapy improves Dyslipidemia, one of the recent reports from the literature indicates that intensive periodontal therapy reduces total cholesterol and LDL-C, but has no effect on HDL-C and TG when tested at 6 months after the completion of therapy [176]. But there are enough studies that argue for favorable changes both in LDL-C and in HDL-C after periodontal therapy [154,189,190].
A great number of studies in recent years regarding the evolution of periodontal disease according to various conditions have focused on the investigation of pro-and anti-inflammatory cytokines. However, the relatively new studies also suggest that periodontal therapy improves Dyslipidemia; one of the recent reports from the literature indicates that intensive periodontal therapy reduces total cholesterol and LDL-C, but has no effect on HDL-C and TG when tested at 6 months after the completion of therapy [176]. But there are enough studies that argue for favorable changes both in LDL-C and in HDL-C after periodontal therapy [154,189,190].

These data mark the relationship between periodontitis and the changes in lipid profile, a positive correlation being observed between the severity of periodontitis and the total concentration of cholesterol, triglycerides and LDL cholesterol. In contrast, the levels of protective lipoproteins against atherosclerosis do not correlate with the periodontal status, very likely, chronic Hyperlipidemia being able to modulate the body's resistance to infection by bacterial of periodontopathogenic type.

VI.5. DISCUSSIONS

The specialty literature contains contradictory results regarding the relationship between serum lipid levels and the periodontal status.

Our study, which aimed, on one hand, at recording the level of total cholesterol, low density lipoproteins (LDL), high-density lipoproteins (HDL) and triglycerides in the group of subjects with periodontal disease and in healthy subjects without periodontal alteration, as well as the degree of association between an altered lipid profile and periodontal diseases on the other hand, indicates the presence of an altered lipid profile in subjects with oral periodontal territory changes.

A significant association between periodontal conditions and the concentration of triglycerides was determined in the blood. The Total blood cholesterol and LDL levels were significantly higher in patients with periodontitis of male sex, compared to patients with healthy periodontium and gingivitis. However, we cannot say that a significant gender-dependent variation has been identified, of the changes in lipid profile as a whole.

The noticed disagreement in various studies that relate to periodontal disease with hyperlipidemia may be caused in part to some factors such as:

a) A big number of involved variables
b) Assessment methods of the periodontitis
c) The measuring method of clinical indicators, such as bleeding from probing, clinical attachment level and depth of palpation and,
d) Of the paraclinical indicators, as well as the evaluation of immuno-inflammatory response, the production of specific antibodies consecutively to the aggression of bacterial lipopolysaccharides, of alveolar bone levels and vascular endothelial changes, when cardiovascular alterations occur. [223,234]

The periodontal disease is a bacterial infection induced by gram-negative bacteria by immuno-inflammatory response determined in conditions of infectious deterioration. Acute infections are known to interfere with lipid metabolism, and the increase of plasma triglycerides was observed in particular in the infection with gram-negative bacteria. These changes are thought to be mediated by cytokines, which can be produced in the inflammatory periodontal tissue in large quantities. Infectious with Chlamydia pneumonia and Helicobacter pylori, believed to be associated with an increased risk of cardiovascular disease, were soon proven to be correlated with increase of plasma levels of cholesterol and triglycerides. These findings support the hypothesis that chronic infections (including periodontitis) can alter the serum lipid profile augmenting therefore the risk of atherosclerosis (Reference).

Hyperlipemia is known for the consecutive determination of hyperactivity of white blood cells. The hyperactivity in leukocytes, and particularly of the monocytes, can afterwards induce, through accelerated production of free radicals (reactive oxygen species most often) periodontal inflammatory phenomena, frequently expressed in the adult population.

A suggestive aspect in this regard is the evidence of the potential for installation and development of the periodontal disease in the experiment animals, at which the diet was supplemented with food high in cholesterol. [111, 217]

In our study, we measured the plasma lipid level at 52 subjects with periodontal disease and we compared the results with those obtained from the control group with the 20 patients.

According to the data from the anamnesis, patients have presented in the medical history the absence of data relating to possible systemic flaws in their history. Total cholesterol, beta lipoprotein fraction (low density lipoprotein) and triglycerides were significantly higher in patients with periodontal disease with approximately 8% (p-0.03), 13% (p 0.003) and 39%, respectively (p 0.001), in comparison with the control group. Although the subjects with diabetes were excluded from the study, we found blood glucose levels significantly higher in the patients group compared to the control group (85 ± 25 versus 73 ± 17 mg/dl; p-0.02).

A significantly higher frequency of pathologic lipid profiles was also recorded in the patients group compared to control group. The results indicate that Hyperlipidemia may be associated with the periodontal disease in patients who are systemic free. These data do not allow us to conclude if periodontal disease results in facilitating the installation of hyperlipidemia status or if in fact, periodontal disease (and possibly cardiovascular complications) present Hyperlipidemia and possibly blood glucose values closer to the upper limit, as common risk factors. [76,218]

The periodontal disease is an infectious disease caused by a small number of bacteria, predominantly anaerobic gram-negative bacteria present on the surface of the teeth in the form of biofilm. Lipopolysaccharides and other microbial substances, by opening the access to subgingival tissues initiate and perpetuate inflammation, and mediated by the exaggerated production of increased levels of proinflammatory cytokines, lead to the destruction of the periodontal ligament and alveolar bone. Several studies have shown that subjects with periodontal disease may have a higher risk of cardiovascular disease, compared with subjects with healthy periodontium [86,114]. So far, the causality and the possible ways of establishing an association between periodontal disease...
and cardiovascular disease are not fully elucidated. The factors that place an individual in the risk group for the periodontal disease could place him also in the risk group for the cardiovascular disease, this meaning that the periodontal disease and the cardio-vascular disease might have common risk factors, such as smoking, diabetes, behavioural factors, age, male sex. In a number of case-control studies or cohort studies type, even if the adjusting of these factors in the multivariate analysis, the association was statistically significant. Thus, one can speculate that periodontal disease as a chronic infection could be related to cardio-vascular disease through mediators associated with infectious process and the hyperactivity of white blood cells or of the blood plagues, thus promoting the development of atherosclerosis. [9.25.46]

VI.6. CONCLUSIONS

1. In the present study we have conducted an assessment of plasma lipids in patients with and without impairment of periodontal territories.
2. The periodontal alteration degree in correlation to the plasma levels of cholesterol was also assessed.
3. Compared to the control group, subjects with periodontitis have had higher plasma levels of total cholesterol, LDL cholesterol and triglycerides, and a total higher incidence of pathological lipid profiles as well compared to subjects from the control group.
4. The frequency of hypercholesterolemia in patients with periodontal pathology was approximately double compared to the control group.
5. This was also valid for total cholesterol and for LDL cholesterol. Pathological levels of triglycerides have been more frequently registered in periodontitis compared to subjects from the control group, while no difference for good fraction of cholesterol was noticed.
6. However, there was a significant difference between mean values of both groups. The mean value of blood glucose was approximately 15% higher in patients compared to the group of individuals with no disorders in the periodontal territory.
7. An inter-determination between hyperlipemia and periodontal disease is possible.
8. The proatherogenic modifications of plasma lipids which have been noticed in patients with periodontal disease can represent the premises of subsequent development of cardiovascular diseases, and thus of a close association between periodontal and cardiovascular disease.
9. Our study confirms the existence of an association between periodontitis and lipid fractions alteration, an interaction probably mediated by activating and subsequently accelerating the inflammatory process induced by the presence of periodontal pathogens, or of their mediators.
10. Although modifications noticed in lipid metabolism are relevant for bi-directionality, we cannot establish exactly whether these are the substrate of intrinsic development or just the result of developing processes from the periodontal disease. These events are very likely mediated physiopathologically, at least in part, at the level of modifications induced by free radicals, products which are the response of the organism to aggression by pathogen microorganisms.
11. These hypotheses must be analysed by further studies, which would elucidate especially the mechanisms underlying the association between the cardiovascular disease and periodontitis.
VII. INTRODUCTION

The risk factors influence the therapeutic behaviour, especially in the aggressive forms of the disease. The risk factors also appear significantly in the disease prognosis, when it is involved in the periodontal territory. Understanding the mechanisms and degree of implication of genetic factors in modulating the immune-inflammatory response to bacterial charge from the periodontal disease (and generally in diseases with genetic support) could significantly influence the attitude of the practicing physician in making curative decisions [19,103,308].

Cluster variations which includes the three polymorphous genes of interleukin 1(IL-1) may alter the immune response of the body to the action of pathogenic periodontal factors and may significantly increase the risk of occurrence of severe forms of disease [103,308].

VII.2. AIM OF THE STUDY

The aim of this study was to assess the impact of specific periodontal aetiology therapy (by eliminating the infection at this level) on the serum markers of systemic inflammation (CRP, IL-6, IL-1) in patients with periodontal pathology with no systemic alteration. The effects of the therapy have been analysed from a clinical periodontal and biochemical point of view in order to validate the efficacy level of the periodontal treatment.

Moreover, we have considered the fact that some studies suggest that a series of genetically transmitted features, such as polymorphism of the genes which codifies the synthesis of proinflammatory cytokines, may accentuate the inflammatory response of the host to bacterial challenges and at the same time, exert some influences in regard to the sensitivity to alteration of periodontal territories.

VII.3. MATERIAL AND METHOD

In view of conducting this study we have selected:

- **Study group A** made of 29 patients, aged between 20-65 years old, assessed in requested assistance in the Periodontology Clinic – Faculty of Dental Medicine of UMF Iași, in which periodontal diagnosis included chronic marginal periodontitis of various degrees.
- **Study group B** with patients with aggressive periodontitis 23 patients
- **Study group C** with patients with no periodontitis 20 patients

Full medical examination has included: medical history, standard periodontal clinical parameters assessment and

- Laboratory tests, which included both assessment of routine serum markers, and interleukins level taken into study, and investigation of populations of peripheral mononuclear cells (density gradient separated from the patients’ blood)
SUMMARY

COMPLEX STUDIES ON THE ASSESSMENT OF RISK MARKERS IN PERIODONTAL PATHOLOGY

- IL-1 genotype analysis: The human resource involved in the present included only patients with AgP and the ones from the control group (group B and group C)
- A specific periodontal treatment phase has followed
- The patients have been re-examined at 2 and 6 months after finalising treatment when the same clinical, serological and immunological parameters have been re-assessed.

✓ INVESTIGATION OF CELLULAR PHENOTYPE BY FLOW CYTOMETRY

Gingival fluid was collected after bringing the patients in the dentistry, through the least aggressive method, avoiding mechanical irritations.

Paper stripes were introduced (Periopaper ProFlow Inc, Amityvile, NY) for 30 seconds in the gingival crevice, interproximal, at the level of areas with clinically relevant aspect (redness and local modifications of gingival tissue consistency).

From the gingival fluid solvated in PBS specimens were made of 50 µl each for FCM (flow-cytometry). The method used both for quantitative determinations of cytokines and for investigating the cellular phenotype was flow cytometry.

Investigation of the phenotype of mononuclear cells and granulocytes functionality in periphery was performed also by FCM, by surface marking with monoclonal antibodies conjugated with fluorochromes. Data acquisition was obtained at FACS (FACS Calibur, BD, 2 lasers).

✓ POLYMORPHISM ANALYSIS OF GENES IL-1A (-889) AND IL-1B (+3953)

Genomic analysis of DNA for polymorphism assessment of genes IL-1α (in the situs-889) and IL-1β (in the situs +3954) was performed by amplification of sequences by PCR (polymerase chain reaction), digestion with specific restriction enzymes and EF (electrophoresis) visualization.

DNA extractions on the integral blood was performed by optimisation of the extraction kit Wizard® Genomic DNA Purification Kit, Promega, operation version on 300µl integral blood.

Polymorphism genotyping IL-1α (-889) was achieved by optimisation of the RFLP method amplifying the area of interest by PCR, followed by restriction digestion of the material obtained with the help of the Ncol enzyme. The CRP products containing a cytidine nucleotide in the position -889 (allele 1) are digested by Ncol, generating smaller fragments of 83 and 16 pairs of bases. The size difference between the undigested fragment and the digested one is viewed by migration of fragments in the concentrated agarose gel. In the case of heterozygotic individuals, both alleles are present and both fragments can be observed.

Polymorphism genotyping IL-1β (+3954) was done by optimisation of the RFLP method amplification by polymerase chain reaction and restriction digestion of the material obtained with the help of the TaqI enzyme. The chosen primers amplify a 194 nucleotides fragment, situated between the positions +3858 and +4051 upstream the initiator codon of the gene IL-1B. The CRP products containing cytidine in the position +3954 (allele 1) are digested by TaqI, generating smaller fragments of 14, 85 and 97 pairs of bases. The size difference between the 97 pb fragment and the 85 pb one is viewed by migration of fragments in the concentrated agarose gel. In the case of heterozygote individuals, both alleles are present.
✓ INVESTIGATION OF THE CRP LEVEL, IL-1 AND IL-6

Serum samples useful for analysing these parameters have been collected by venous puncture 2 and 6 months after finalising the treatment.

The serum was obtained by centrifugation for 15 minutes at 2000 rpm within 1 hour of sampling and the samples have been stored at -70°C. The serum levels of CRP have been assessed by a high sensitive immunoturbidimetric automated system (Cobas Integra, Roche)

VII.4. RESULTS

Interpretation of the results of this study was focused on the quantitative evolution of the inflammatory markers during periodontal therapy and on the interpretation and assessment of the phenotype of the cells responsible for cytokine secretion as a response to aggression by periodontal pathogenicity elements as well. We have tried to concentrate the information obtained during research in the area of interest of the dentist, revealing that the presence of certain interleukin genotypes can signal improvement or aggravation of periodontal symptomatology.

We have also analysed and compared the results of genetic assessments, which are very useful in describing and predicting diagnosis and treatment in various periodontal pathologies. The results of the study aim at representing a significant indicator in diagnosing and treating periodontal diseases.

In this section we shall focus especially on the comparative results obtained before and after implementing periodontal treatment.

Treatment effects depending on the periodontal clinical results

The periodontal clinical results are presented in Table VII.1 and VII.2.

Table VII.1. Study group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36±8</td>
</tr>
<tr>
<td>Sex</td>
<td>Women 54%</td>
</tr>
<tr>
<td>Smoker</td>
<td>Currently smokers 42%</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.3±3.7</td>
</tr>
<tr>
<td>Periodontal diagnosis</td>
<td>Chronic 75%</td>
</tr>
</tbody>
</table>

Table VII. 2. Periodontal clinical parameters before and after treatment

<table>
<thead>
<tr>
<th></th>
<th>Initially Mean</th>
<th></th>
<th>SD</th>
<th>2 months Mean</th>
<th></th>
<th>SD</th>
<th>6 months Mean</th>
<th></th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of periodontal pockets*</td>
<td>77.08</td>
<td>23.23</td>
<td></td>
<td>27.82‡</td>
<td>16.36</td>
<td></td>
<td>22.91‡</td>
<td>15.04</td>
<td></td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>4.36</td>
<td>0.59</td>
<td></td>
<td>3.25‡</td>
<td>0.47</td>
<td></td>
<td>3.19‡</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Degree of recession (mm)</td>
<td>0.56</td>
<td>0.88</td>
<td></td>
<td>1.56‡</td>
<td>0.94</td>
<td></td>
<td>1.72‡</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>4.93</td>
<td>1.13</td>
<td></td>
<td>4.74‡</td>
<td>1.14</td>
<td></td>
<td>4.85‡</td>
<td>1.13</td>
<td></td>
</tr>
</tbody>
</table>
Oral hygiene has improved significantly at 2 and 6 months by about 20%.

The patients have shown a significant decrease in the number of periodontal pockets from 77±23 in the initial stage, to 28±16 at 2 months and respectively 23±15 at 6 months (p<0.0001, \( t \)-test).

Results indicate that most clinical improvements appear in the first 2 months, maintain during the 6 months study period. Other clinical parameters, such as main depth of the pocket and percentage of sites bleeding on probing have had a clear improvement at 2 months after treatment but have also continued during maintenance period.

It is interesting that these improvements have happened in the absence of significant decrease in the percentage of sites with visible plaque or gingival erythema. Clinical changes were similar to the ones reported in other studies too, which have described initial improvements followed by a stability period for the periodontal tissues.

**VII.4.1 Serum parameters level as an effect of periodontal treatment**

The mean level of CRP was 1.9 mg/l, while the mean level of IL-6 was 1.82 ng/l (1.45 ng/l IQ). There were no significant differences between the markers concentrations between various groups depending on age, sex and smoker status. Initial CRP concentrations have been associated significantly with age, body mass index, and CAL mean-attachment level.

The mean CRP level in the first stage was 1.9 mg/l with an IQ level of 3.6 mg/l. No differences were noticed in concentrations depending on age, sex, periodontal diagnosis (chronic or aggressive periodontitis) or smoking.

**Table. VII.3 Mean values of CRP, during study, at 2 months after initiating therapy**

<table>
<thead>
<tr>
<th>C-reactive protein</th>
<th>Initially</th>
<th>At 2 months</th>
<th>At 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 mg/l</td>
<td>1.8</td>
<td>1.3</td>
<td></td>
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</table>

A relevant decrease in Serum concentration of CRP was significant only at 6 months, the mean modifications of CRP concentration between the initial and final phase of treatment have been of 0.5 mg/l with a free distribution of 95% and an intermediary of 0.4-0.7 mg/l.
Due to heterogeneity of the response to the periodontal treatment (in terms of periodontal clinical parameters) and to the presence of well-established covariations such as age and body mass index, the data have continued to be analysed.

The group with the best response to periodontal therapy was characterized by persistence of less than 30 pockets of 5 mm or deeper.

Data indicates a significant interaction between the result of the treatment and the total levels of CRP (initial, at 2 months and at 6 months), and decrease of CRP level was more important in subjects with good periodontal results (Diagram VII.4).

A significant decrease in IL-6 concentration in serum was found at 2 and at 6 months after finalizing periodontal treatment (Wilcoxon test, $p=0.021, 0.006$ respectively).

CRP serum concentration has dropped significantly only after 6 months of monitoring (Wilcoxon test ($p<0.0001$)).

Mean modification of CRP concentration between the initial moment and at 6 months has been of 0.5 mg/l, with a free distribution of 95% of the confidence interval of 0.4-0.7 mg/l.

The difference in mean concentration of IL-6 was 0.18 ng/l (0.02-0.44 95% CI) between the initial moment and at 6 months and 0.22 ng/l (0.06-0.44 95% CI), between the base moment and at 6 months.

Diagram VII. 1. (A) Mean Serum levels of CRP at three periods of time. $^\dagger p<0.001$ $^\ddagger p=0.021$ $^\S p=0.006$ (Wilcoxon test by comparison to the initial level)

Table VII.5. Difference in mean values of inflammatory markers monitored at 2 and 6 months

<table>
<thead>
<tr>
<th>C-reactive protein</th>
<th>Initial value vs at 2 months</th>
<th>Initial value vs at 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± IQ</td>
<td>95%</td>
</tr>
<tr>
<td>PCR mg/l</td>
<td>0.1 ± 1.0</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

Table VII. 4. Mean differences of CRP at 2 and 6 months
SUMMARY

COMPLEX STUDIES ON THE ASSESSMENT OF RISK MARKERS IN PERIODONTAL PATHOLOGY

<table>
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<tr>
<th></th>
<th>CRP (mg/l)</th>
<th>IL-6 (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.1 ± 1.0</td>
<td>0.18 ± 1.0</td>
</tr>
<tr>
<td>2 months</td>
<td>0.4</td>
<td>0.22 ± 1.0</td>
</tr>
<tr>
<td>6 months</td>
<td>0.7</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*95% Confidence interval

†Δ = Difference of Serum systemic markers between visits

Diagram VII. 1(B) Mean Serum levels of IL-6 at three periods of time †p<0001 †p=0.021 §p=0.006 Wilcoxon test when compared to the initial level.

In our study the immediate purpose of periodontal therapy was to stop and eliminate inflammation and repair losses produced by the disease by modifications of measured pockets’ depth (PD), bleeding on probing (BOP), epithelial attachment level (CAL) after scaling and root planing (SRP) and by positively modifying the inflammatory markers values as well.

VII.4.2 Phenotypic and secretory analysis of peripheral blood mononuclear cells - PBMC

Phenotyping mononuclear cells from the PBMC cultures obtained by density gradient separation from the venous blood collected from the study groups was performed both from the culture sampled at 24 and at 48 hours after stimulation with bacterial lipopolysaccharide (LPS) isolated from periodontogenic agent (P gingivalis). It is known that LPS is one of the common virulent factors of periopathogenic bacteria, able to penetrate periodontal tissues with subsequent interaction at the level of the immune and non-immune cells of the host. This interaction leads to activation of cells with release of inflammatory mediators (cytokines), which are responsible, at least in part, of destroying periodontal tissues.

Our study has aimed at analysing the impact produced by the bacterial endotoxin on the phenotype and the function of peripheral mononuclear cells, and identifying the signalling ways activated by the bacterial antigen, by highlighting the intracellular proteins which are modulated especially at the level of macrophages.

In the following tables, the statistical individual analysis of mononuclear cells populations in PBMC cultures in the studied groups are presented.
**Table VII.6.** Statistical indicators of lymphocytes values $T^{+CD3+CD8+}$ in the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Lymphocytes $T^{+CD3+CD8+}$</th>
<th>Mean value Mean ±95%</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
<th>Midpoint</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS- CP</td>
<td>18.6</td>
<td>15.6</td>
<td>21.6</td>
<td>8.0</td>
<td>4.5</td>
<td>28.3</td>
<td>21.3</td>
</tr>
<tr>
<td>LPS- AGP</td>
<td>13.1</td>
<td>11.0</td>
<td>15.2</td>
<td>5.6</td>
<td>6.2</td>
<td>21.1</td>
<td>14.7</td>
</tr>
<tr>
<td>LPS+ CP</td>
<td>18.5</td>
<td>15.4</td>
<td>21.7</td>
<td>8.3</td>
<td>4.5</td>
<td>29.7</td>
<td>20.8</td>
</tr>
<tr>
<td>LPS+ AGP</td>
<td>13.3</td>
<td>11.2</td>
<td>15.3</td>
<td>5.5</td>
<td>6.4</td>
<td>21.2</td>
<td>14.9</td>
</tr>
<tr>
<td>Total 24 h</td>
<td>15.9</td>
<td>14.5</td>
<td>17.2</td>
<td>7.4</td>
<td>4.5</td>
<td>29.7</td>
<td>16.3</td>
</tr>
<tr>
<td>LPS- CP</td>
<td>17.0</td>
<td>13.9</td>
<td>20.0</td>
<td>8.2</td>
<td>3.4</td>
<td>28.5</td>
<td>17.7</td>
</tr>
<tr>
<td>LPS- AGP</td>
<td>13.1</td>
<td>11.0</td>
<td>15.2</td>
<td>5.6</td>
<td>5.7</td>
<td>20.5</td>
<td>14.5</td>
</tr>
<tr>
<td>LPS+ CP</td>
<td>17.4</td>
<td>14.4</td>
<td>20.5</td>
<td>8.2</td>
<td>4.3</td>
<td>29.4</td>
<td>17.9</td>
</tr>
<tr>
<td>LPS+ AGP</td>
<td>13.4</td>
<td>11.3</td>
<td>15.4</td>
<td>5.6</td>
<td>5.3</td>
<td>19.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Total 48 h</td>
<td>15.2</td>
<td>13.9</td>
<td>16.5</td>
<td>7.2</td>
<td>3.4</td>
<td>29.4</td>
<td>16.5</td>
</tr>
</tbody>
</table>

**Table VII.7.** Statistical indicators of lymphocytes values $T^{+CD3+CD4+}$ in the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Lymphocytes $T^{+CD3+CD4+}$</th>
<th>Mean value Mean ±95%</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
<th>Midpoint</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS- CP</td>
<td>32.8</td>
<td>30.2</td>
<td>35.3</td>
<td>6.8</td>
<td>20.3</td>
<td>40.1</td>
<td>34.8</td>
</tr>
<tr>
<td>LPS- AGP</td>
<td>22.3</td>
<td>19.4</td>
<td>25.2</td>
<td>7.8</td>
<td>11.6</td>
<td>33.4</td>
<td>19.3</td>
</tr>
<tr>
<td>LPS+ CP</td>
<td>31.6</td>
<td>29.2</td>
<td>33.9</td>
<td>6.2</td>
<td>22.8</td>
<td>38.2</td>
<td>34.7</td>
</tr>
<tr>
<td>LPS+ AGP</td>
<td>21.0</td>
<td>18.1</td>
<td>23.9</td>
<td>7.7</td>
<td>11.0</td>
<td>31.1</td>
<td>17.2</td>
</tr>
<tr>
<td>Total 24 h</td>
<td>26.9</td>
<td>25.3</td>
<td>28.5</td>
<td>8.9</td>
<td>11.0</td>
<td>40.1</td>
<td>28.6</td>
</tr>
<tr>
<td>LPS- CP</td>
<td>30.4</td>
<td>27.1</td>
<td>33.6</td>
<td>8.7</td>
<td>16.7</td>
<td>41.1</td>
<td>32.2</td>
</tr>
<tr>
<td>LPS- AGP</td>
<td>22.5</td>
<td>19.1</td>
<td>26.0</td>
<td>9.2</td>
<td>7.8</td>
<td>33.9</td>
<td>20.5</td>
</tr>
<tr>
<td>LPS+ CP</td>
<td>30.5</td>
<td>27.5</td>
<td>33.5</td>
<td>8.0</td>
<td>19.0</td>
<td>38.2</td>
<td>33.6</td>
</tr>
<tr>
<td>LPS+ AGP</td>
<td>22.3</td>
<td>19.0</td>
<td>25.5</td>
<td>8.6</td>
<td>7.5</td>
<td>32.2</td>
<td>23.2</td>
</tr>
<tr>
<td>Total 48 h</td>
<td>26.4</td>
<td>24.7</td>
<td>28.1</td>
<td>9.4</td>
<td>7.5</td>
<td>41.1</td>
<td>26.9</td>
</tr>
</tbody>
</table>
Table VII.8. Statistical indicators of MONOCYTES values in the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean MFI CD3+CD14</th>
<th>Mean value</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
<th>Midpoint</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-95%</td>
<td>+95%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS-</td>
<td>2.8</td>
<td>1.9</td>
<td>3.7</td>
<td>1.6</td>
<td>0.1</td>
<td>4.8</td>
<td>3.2</td>
</tr>
<tr>
<td>LPS-</td>
<td>2.9</td>
<td>2.5</td>
<td>3.2</td>
<td>0.6</td>
<td>1.8</td>
<td>3.4</td>
<td>3.0</td>
</tr>
<tr>
<td>LPS+</td>
<td>2.2</td>
<td>1.5</td>
<td>2.9</td>
<td>1.2</td>
<td>0.5</td>
<td>4.0</td>
<td>2.1</td>
</tr>
<tr>
<td>LPS+</td>
<td>2.5</td>
<td>2.0</td>
<td>3.0</td>
<td>0.9</td>
<td>1.0</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Total 24 h</td>
<td>2.6</td>
<td>2.3</td>
<td>2.9</td>
<td>1.2</td>
<td>0.1</td>
<td>4.8</td>
<td>2.9</td>
</tr>
<tr>
<td>LPS-</td>
<td>3.0</td>
<td>2.1</td>
<td>3.8</td>
<td>1.5</td>
<td>1.0</td>
<td>5.5</td>
<td>2.9</td>
</tr>
<tr>
<td>LPS-</td>
<td>5.5</td>
<td>5.1</td>
<td>5.8</td>
<td>0.7</td>
<td>4.7</td>
<td>6.6</td>
<td>5.2</td>
</tr>
<tr>
<td>LPS+</td>
<td>2.7</td>
<td>2.0</td>
<td>3.5</td>
<td>1.3</td>
<td>0.5</td>
<td>4.3</td>
<td>3.1</td>
</tr>
<tr>
<td>LPS+</td>
<td>3.4</td>
<td>3.1</td>
<td>3.6</td>
<td>0.4</td>
<td>2.7</td>
<td>3.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Total 48 h</td>
<td>3.6</td>
<td>3.2</td>
<td>4.0</td>
<td>1.5</td>
<td>0.5</td>
<td>6.6</td>
<td>3.4</td>
</tr>
</tbody>
</table>

LPS- : KW-H = 0.141463415, p = 0.7068; F = 0.0186225244, p = 0.8924
LPS+ : KW-H = 0.565853659, p = 0.4519; F = 0.4914040110, p = 0.4891
According to recordings by flow cytometry, we noticed that not only the production of mediators by the cells is different between the groups taken for study, but also the MFI (mean fluorescent intensity) which indicates the expression at the cell’s surface of the molecule of interest.

Table VII.9. Statistical indicators of IL-6 values in GCF in the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean IL - 6</th>
<th>Mean value</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
<th>Midpoint</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-95%</td>
<td>+95%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS+ CP</td>
<td>804.1</td>
<td>556.1</td>
<td>1052.2</td>
<td>530.0</td>
<td>60.0</td>
<td>1406.9</td>
<td>1045.0</td>
</tr>
<tr>
<td>LPS+ AG P</td>
<td>824.3</td>
<td>680.5</td>
<td>968.1</td>
<td>307.3</td>
<td>554.6</td>
<td>1386.9</td>
<td>669.7</td>
</tr>
<tr>
<td>LPS- CP</td>
<td>157.4</td>
<td>92.7</td>
<td>222.1</td>
<td>121.5</td>
<td>17.9</td>
<td>303.2</td>
<td>154.2</td>
</tr>
<tr>
<td>LPS- AG P</td>
<td>303.0</td>
<td>257.4</td>
<td>348.6</td>
<td>97.4</td>
<td>195.6</td>
<td>434.4</td>
<td>264.7</td>
</tr>
<tr>
<td>Total 24 h</td>
<td>541.4</td>
<td>442.6</td>
<td>640.2</td>
<td>432.2</td>
<td>17.9</td>
<td>1406.9</td>
<td>395.8</td>
</tr>
<tr>
<td>LPS+ CP</td>
<td>931.6</td>
<td>625.3</td>
<td>1237.9</td>
<td>654.5</td>
<td>65.4</td>
<td>1631.5</td>
<td>1045.0</td>
</tr>
<tr>
<td>LPS+ AG P</td>
<td>882.8</td>
<td>758.4</td>
<td>1007.2</td>
<td>265.8</td>
<td>621.5</td>
<td>1377.0</td>
<td>828.9</td>
</tr>
<tr>
<td>LPS- CP</td>
<td>199.6</td>
<td>117.8</td>
<td>281.4</td>
<td>153.6</td>
<td>30.5</td>
<td>397.6</td>
<td>185.1</td>
</tr>
<tr>
<td>LPS- AG P</td>
<td>324.5</td>
<td>273.5</td>
<td>375.6</td>
<td>109.1</td>
<td>237.6</td>
<td>511.9</td>
<td>253.9</td>
</tr>
<tr>
<td>Total 48 h</td>
<td>604.9</td>
<td>493.1</td>
<td>716.7</td>
<td>489.1</td>
<td>30.5</td>
<td>1631.5</td>
<td>397.6</td>
</tr>
</tbody>
</table>

Tables analysis reveals the influence of the IL-6 level of stimulation of the cellular cultures with LPS, a model which is shown both in the population with chronic and aggressive periodontitis, but at different levels of intensity.

VII.4.3 Interleukin genotype analysis

Nowadays, numerous researches have focused on identifying new biomarkers in periodontitis and discovering a possible relation between the genetic polymorphism of IL-1 and its use in proper diagnosis and prognosis of periodontal disease.

As regards the genetic polymorphism of Interleukin-1, our study has shown that there is a positive genotype made of the allele 2 (T/T), in 5.56% of health patients, in 72.22% of patients with aggressive periodontitis and in 38.89% of patients with chronic periodontitis.

The following sets of primers have been used, while clinical and paraclinical assessment of the periodontal status in groups of patients was performed by: ◊ assessment of the oro-dental hygiene
degree; ♦ colorimetric assessment of the mucous-bacterial plaque (PI) ♦ analysis of degree of inflammation – BOP bleeding index; ♦ periodontal probing and radiologic examination – orthopantomographies for assessing the clinical attachment loss and the degree of damage on the alveolar bone. ♦ Clinical attachment loss (CAL) was assessed by periodontal probing evaluating the deepness of pockets and degree of recession, and in 6 sites at the level of each tested tooth, the benchmark was the amelocemental junction, by comparison to the bottom of sulcus/pocket in millimetres.

As regards the frequency of the genotype (1.1) for IL-1α, it was registered at more than half of individuals from the control group (55%), and the genotype (2.2) was specific only for 10% of this group, while the heterozygote one (1.2) was 35%, compared to the group with aggressive periodontitis (AgP) where the frequency for the allele (1.1) was 34.78 %, for the allele (2.2) of 13 %, and for the allele (1.2) of 52.22% (table VII.10). Therefore, we can say that the genotypes frequency analysis for IL-1α has not revealed a statistically significant difference between the two groups taken into study (p= 0.18).

**Table VII.10.** Frequency of genotype IL-1α and alleles in control patients, compared to the ones with periodontal impairment of the AgP-type

<table>
<thead>
<tr>
<th>Study group</th>
<th>IL-1αAgP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>1.1</td>
<td>8</td>
<td>34.78</td>
</tr>
<tr>
<td>2.2</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>1.2</td>
<td>12</td>
<td>52.22</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>Allele 1</td>
<td>16</td>
<td>69.5</td>
</tr>
<tr>
<td>Allele 2</td>
<td>7</td>
<td>30.43</td>
</tr>
</tbody>
</table>

**Table VII.11.** The frequency of the genotype IL-1β and of the alleles in the AgP patients compared to the ones without periodontal impairment

<table>
<thead>
<tr>
<th>Study group</th>
<th>IL-1βAgP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>1.1</td>
<td>10</td>
<td>43.47</td>
</tr>
<tr>
<td>2.2</td>
<td>2</td>
<td>8.71</td>
</tr>
<tr>
<td>1.2</td>
<td>11</td>
<td>47.82</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>Allele 1</td>
<td>15</td>
<td>65.21</td>
</tr>
<tr>
<td>Allele 2</td>
<td>8</td>
<td>34.79</td>
</tr>
</tbody>
</table>
frequency indicates that the homozygote-type allele 1 for IL-1α was present in 50% of male subjects from the control group and in 10% of male subjects from the group with AgP (table VII.12). The type-2 allele for IL-1α was present in 12.5% of males from the control group and in 30% of males with AgP.

VII.5. DISCUSSIONS

Patients with periodontitis have higher levels of CRP, IL-1 and IL-6 compared to the healthy periodontal populations. But the role of some variables such as habitual or environmental factors cannot be ignored either; they can have a significant input in the case of these associations. The results of our study highlight the existence of a correlation between the seriousness of periodontitis and Serum concentrations of C-reactive protein, and systemic inflammation is very likely to mediate these effects in a dose-dependent manner.

A significant decrease in IL-6 in this study was noticed at 2 and 6 months after finalising periodontal therapy. A significant decrease in CRP was also revealed at 6 months of assessment. Our data does not offer perspectives on the possible reasons for this delay, but it is interesting that the specialty literature shows that in patients with cardiovascular diseases, this deviation of time was also reported. These effects can very likely be correlated with a possible relative inefficiency of the periodontal mechanical treatment in definitively eliminating local infectious events.

Variations in inflammatory response to periodontal pathogen agents are also connected to the individual genetic background. Although our study is limited in regard to the investigation availability by molecular biology studies of the gene’s polymorphism codifying IL-1 proinflammatory cytokines, and currently having only preliminary data, it reveals certain congruence between the Serum level of CRP and the incidence of specific genes’ polymorphism.

Recent studies have shown that some specific modifications of the IL-1A gene can have predictive value in evaluating the risk of serious evolution of the periodontal disease. These genetic variations could lead to a disequilibrium between inflammatory mediators and the influence of individual response to pathogens; Berger et al. have noticed in a population with significant atherosclerosis that the presence of allele 2 (3954) for IL-1B in some patients may significantly influence the (two-three-fold increase in) basic CRP Serum levels.

In the present study this specific genotype (IL-1A -889) has shown a significant association with the CRP serum levels.

The tendency to produce high quantities of IL-1 remains relatively consistent during lifetime in some patients. Thus, a study indicates that 67% of patients with severe forms in disease have shown positive genotype for IL-1. IL-1 positive patients show bleeding on brushing, and in IL-1 negative patients severe forms of disease generally appear after the age of 60, while in positive patients these forms appear 20 years earlier. Positive patients have severe forms of disease between 40 and 60 years, even if they are not smokers. Moreover, in positive patients, who continue smoking, the disease advances to teeth loss from the arcade. After surgical interventions performed on patients with periodontal pathologies, the negative ones recover in the ratio of 73%, while the positive ones may have a lower rate of recovery which usually does to go higher 21%.

For example, patients with chronic gingivitis, who do not maintain a rigorous control of the bacterial plaque, are in the situation of developing either lighter, or more serious forms of disease, only if they are either smokers, diabetics, or IL-1 positive. In this case, maintenance therapy is based on bacterial plaque control and elimination of risk factors. In certain situations a further test is
required in order to see which bacteria is involved in the infectious process. In reaching success in periodontal therapy, it is important to identify the risk of the patient for forms of periodontitis. If the patient does not present risk factors for serious forms of periodontal pathology, habitual control of bacterial plaque and of risk factors is sufficient; in more serious forms, this therapy is done with more carefulness, especially considering the management of associated factors.

But if results are late, in this case medical aggressive therapy shall be followed, with local solutions, combined with oral antibiotics. Certain studies have researched the association between the IL-1 genetic polymorphism and internal risk factors such as: rheumatoid arthritis, polyarthritis, coronary impairment, and bone inflammations as well. It has been shown that there is an exacerbated immune response to the action of inflammatory factors, including the key of genetic factors in this equation as well. It has been proven that genetic polymorphism plays an important role in human disorders, generating an excessive inflammatory response or a hyperinflammatory response (associated with the C-reactive protein and other systemic conditions).

Although serum samples have been collected and processed in the best possible way, they have not been sampled in the same period of the day. This could affect mainly the analysis of serum levels of IL-6. In fact, these cytokines have shown a diurnal effect. Obesity is also a major source of production of inflammatory mediators (e.g. adiponectin). We cannot exclude the fact that the lifestyle or changes in diet from the population sample included in the study could not represent variable factors to the extent to affect some data attesting the metabolic and inflammatory status, closely correlated to the status of periodontal disease. What can be noticed from the study is the fact that the genic IL-1 variation is associated with significant increases in C-reactive protein and other specific mediators of inflammation.

In the past years, a high number of epidemiological studies have shown that patients with periodontitis may have a high risk of cardiovascular diseases. The association between periodontitis and cardiovascular diseases remains however to be debated. Discussions have recently focused on divergent results obtained from various groups after analysing the material from the same study. Critics have highlighted the fact that both periodontitis and atherosclerosis collaborate from the point of view of risk factors, and that this association could be debatable even if it is established. Provided that the association has been well established, the critical problem concerning its nature in possible terms of causality shall remain central in order to understand the medical significance of periodontal infections.

Data registered by flow cytometry suggest that monocytes and macrophages can be included in the development mechanism of the periodontal disease and offer the scientific basis for the management of oral hygiene for the purpose of preventing establishment of inflammatory periodontal lesions.

Among the receptors mediating inflammatory response to the action of the components of bacterial wall of gram-negative bacterium, TLR2 and TLR4 are distinguished with a significant role in inducing cytokine production at the level of macrophages. Our study shows very high levels of IL-6 in stimulated cultures, while the highest values were registered in patients with aggressive periodontitis, and especially constantly higher in the 48 hours culture compared to the 24 hours one, suggesting the mean existence of a factor stimulation continuous cytokine production.

What would offer a clearer insight on the events and causes inter-determining would be the investigation of modifications induced by the live bacterium and surface bacterial products, in order to highlight possible quantitative (signal intensity) or qualitative (of the panel of secreted cytokines) modifications of the biological signal induced at the level of macrophages. [184]
Data registration within our study has clearly identified overlapping of an inflammatory systemic response associated with severe periodontitis, in systemic healthy individuals.

Numerous studies from the past years have revealed a close association between periodontitis and high level in inflammatory markers. Moreover, various genetic versions of some cytokines have been associated with susceptibility to establishment and subsequent development of this condition [268]. Currently, although no estimative number concerning the prevalence of AgP in the population of Romania is known, preliminary data that we have built in regard to this aspect in the present study shows that, at least for the area of Moldova, the estimative number could scan around the value of 1.8%, representing a mean value for the Balkan area.

Previous genetic studies on different populations have revealed relatively variable levels in Africans, Caucasians, Asians, Americans, and the distribution of alleles and genotypes for the group taken for study is identical to the one of the Caucasian population [144, 241]. However, although some authors bring arguments in favour of the existence of a very close association between aggressive periodontitis and polymorphism of the gene IL-1β[42], our results could not reveal a significant correlation between the polymorphism of the genes IL-1α and IL-1β in patients with AgP, in the groups taken for study.

Sexes analysis of the genic polymorphism, with assessment of alleles balance within the female and male population has revealed the existence of a correlation between AgP and polymorphism of the genes IL-1α (-889) and IL-1β (+3954) in male patients, probably indicating that males are more sensitive to genetic variations (this could be due to difference in prevalence of genic polymorphisms between males and females). In fact, studies performed by Gera and collaborators [129] certify the debates of association between the polymorphism of some genes and aggressive periodontitis, while possible associations are in close inter-dependence with race and sex of the subjects.

Scanning the literature from the past ten years in regard to the incidence and associations within periodontal aggressive disease, we could notice that most frequently the studies’ authors report a higher frequency of the disease in females than in males, which is also very likely due to higher rate of women of consulting the dentist in women, because they are more concerned about their physical aspect than men. Also, the differences (which probably disappear with age and lack of protection by estrogens) could be partly due to the early puberty in women as well as to the hormonal modifications during menstruation and to the degree of gravity, which could aggravate periodontal alteration. In regard to the more frequent association within male population of the polymorphism of IL-1 gene with other disorders (e.g. gastric) that currently exists in the literature, the evidence is insufficient to reach a conclusion concerning the association between the patient’s sex and impact of polymorphisms of genes on AgP.

The difficulty of association between the polymorphism of the genes IL-1 and AgP could be explained by the lack of a high expression of a single gene in this disease. There are probably other genes altering the genic expression and influencing the clinical aspect of the disease. Moreover, the coexistence of multiple polymorphisms could be very likely responsible for the severe evolution or aggravation of the disease.
VII.6. CONCLUSIONS

1. This study aims at assessing the effects of non-surgical periodontal therapy on the inflammatory markers. It has been shown that a control of periodontal infections determines a decrease in inflammation’s markers on a small population sample with severe and chronic forms of periodontal disorders. Considering the limits of this study, the data demonstrates an interrelation between general health and periodontal disease.

2. The mean C-reactive protein level in the first stage was 1.9 mg/l with an IQ level of 3.6 mg/l and no differences in concentrations depending on age, sex, periodontal diagnosis (chronic or aggressive periodontitis) were noticed.

3. A significant decrease in serum concentration of CRP was registered mainly at 6 months after initiating non-surgical therapy, while mean modifications of CRP concentration between the initial and final phase of the treatment was of 0.5 mg/l with a free distribution of 95% and an intermediary of 0.4-0.7 mg/l.

4. Data have indicated that there is a significant interaction between the results of the treatment and the general levels of CRP (basic, at 2 and at 6 months), and that this drop in CRP was significant in subjects who had the best results from the point of view of periodontal parameters.

5. The control of periodontitis, achieved without surgical treatment, has led to a decrease in the level of serum mediators of the C-reactive protein-type inflammation, and of cytokine-type soluble mediators.

6. The study has shown that an efficient control of periodontal infection has reduced serum concentration of inflammatory markers (CRP, IL-1, IL-6) in a relatively small population with alteration of the periodontal territory.

7. Data and results obtained reveal the existence of a causality connection between periodontitis and the inflammatory systemic state.

8. The significant Serum response has been associated with half of the population who responded well to non-surgical periodontal therapy.

9. Data registered by flow cytometry suggest that monocytes and macrophages can be included in the development mechanism of the periodontal disease and offer the scientific basis for the management of oral hygiene for the purpose of preventing establishment of inflammatory periodontal lesions.

10. The results of this study show that generalized severe periodontitis can interfere and interdetermine the systemic inflammatory status, which can have causality connotations, when the risk of subsequent development of cardiovascular events is assessed.

11. Thus, the activity of monocytes on the action of the antigenic stimulus registers responses with higher amplitudes in patients with aggressive periodontitis.

12. We believe that besides exploration of mononuclear cells, in order to fully explain the susceptibility to periodontal diseases, other cellular lines (epithelial, fibroblast or endothelial), which are known to have the ability to interact with the bacterial components through TLRs, consecutively producing cytokines, should also receive a special attention.

13. The study of the immune-inflammatory response induced by the bacterial plaque at the level of oral territories and also the expression of signalling mechanisms induced by the microbial agent at peripheral level, by determining the cytokines level from the PBMC cultures, stimulated or non-stimulated, in groups of patients with periodontal alteration, suggest that
the major source of pro-inflammatory mediators at this level is represented by monocytes. Moreover, these cells express consecutively with the stimulation with endotoxin, a particular phenotype, with preferential hypersecretion of certain cytokines.

14. Our results could not reveal a significant correlation between the polymorphism of the genes IL-1α and IL-1β in patients with AgP, in the groups taken for study. Considering the limits of this study, especially imposed by the relatively small dimension of the chosen sample, we cannot certainly assert that the polymorphism of the genes IL-1 is not generally associated with the periodontal disease, but, in regard of the group of patients with aggressive periodontitis, it has not revealed a statistically significant correlation;

15. On the other hand, sexes analysis of the genic polymorphism, with assessment of alleles balance within the female and male population has revealed the existence of a correlation between AgP and polymorphism of the genes IL-1α (-889) and IL-1β (+3954) in male patients, probably indicating that males are more sensitive to genetic variations (this could be due to difference in prevalence of genic polymorphisms between males and females).

16. Specific genes can vary within populations and ethnic groups and a real heterogeneity can be present in regard to the susceptibility to this disease, while the present study has not identified a clear association between the polymorphism of the genes IL-1α (-889) and IL-1β (+3954) and AgP, in the absence of other risk markers.

17. Instead, by broadening the study samples or in correlation with certain populations, the obtained results suggest the opportunity of some thorough studies which, to the extent of associating other factors as well, would contribute to defining the association between the prevalence of aggressive periodontitis correlated with the patient’s sex;

18. Moreover, it is still likely that in the future the genetic profile assessed by genes IL-1 would be used as evaluation marker of the risk of occurrence and progression of periodontal disease, especially in what concerns the association with chronic events at this level.
VIII.1. INTRODUCTION

Recent specialty literature suggests the fact that oral hygiene is the indicator of systemic health and supports the concept according to which periodontal disease is associated with systemic conditions.[1] This has led to the evolution of a new branch in periodontal pathology, called Periomedicine.

Cardiovascular diseases, which occupy the first place worldwide in the list of morbidity and mortality, are common among many adult populations, such as the ones with impairment in the periodontal area (chronic periodontitis). In this context, high levels of blood cholesterol (favourable for apparition of obesity), arterial hypertension and diabetes mellitus are widely known as risk factors for cardiovascular diseases.

VIII.2. AIM OF THE STUDY

The present study has been conducted in order to assess, from the perspective of alterations in the periodontal oral territory, the evolution of parameters certifying the lipid profile (the levels of total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL) and triglycerides) in subjects with and without periodontal disease. Moreover, the association between increased lipid profiles and periodontal disease has been assessed as well, in order to encourage the adoption of the best prevention measures for the purpose of reducing the risk of cardiovascular disease (CVD), by insuring a real periodontal management. Hypertriglyceridemia together with hypercholesterolemia are independent risk factors for the atherosclerotic disease. Among the serum parameters, the level of cholesterol fractions is also significant on this line, and the value of triglycerides is necessary to evaluate the LDL cholesterol fraction (LDLc).

VIII.3. MATERIAL AND METHOD

VIII.3.1 Creation of the study groups

We have analysed separately the evolution of markers to present the obtained results from a scientific point of view more efficiently. The groups of patients registered in this study, assessed in requested assistance in the Periodontology Clinic – Faculty of Dental Medicine of Iaşi and the Dentesse clinic – private practice Iaşi consisted of:

- **Study group**
  - 52 patients with periodontal disease (20 women and 32 men, aged between 18-65 years old, mean of 41.4±3.1)
- **The group was divided into 2 subgroups:**
  - The control group has included patients with periodontitis in which we have applied only specific measures of maintaining oral hygiene (professional brushing, administration of CHX 0.09%).
• The test group has included patients with periodontitis in which we have applied specific periodontal etiologic therapy.

VIII.3.2 Assessment of Serum levels of lipids

Blood sampling

Blood samples for biochemical test of the lipid levels have been collected for all subjects included in the study, after at least 12 hours of fast. The lipid level was tested by enzymatic colorimeter on a COBAS 6000-type biochemistry analyser.

The principle of dosing total blood cholesterol is based on an enzymatic, colorimetric method, while the cholesterol esters under the action of cholesterol esterase are split into free cholesterol and fatty acids. In the case of dosing the total quantity of Serum triglycerides, the assessment method uses a lipoprotein lipase (LPL) for fast and complete hydrolysis of triglycerides to glycerol, followed by its oxidation to dihydroxyacetone-phosphate and hydrogen peroxide. In identifying subjects with pathological values the following target levels have been used as limit value in conformity with the indication of the laboratory:

- total cholesterol <230 mg / dl,
- LDL-cholesterol <120 mg / dl,
- HDL-cholesterol >45 mg / dl,
- triglycerides <150 mg / dl,
- blood glucose <100 mg / dl.

All dental data have been expressed depending on the patient. Data are presented as mean values and standard deviation. The differences between mean values have proved to be significant using the t-student test for odd samples.

VIII.3.3 Periodontal examination

Periodontal examination

This was performed with a mouth plane mirror, explorer, Naber’s and William’s periodontal probes.

The assessed clinical parameters were:
1. Simplified index of oral hygiene (Greene and Vermillion 1964)
2. Periodontal pocket depth
3. Clinical attachment level
4. Degree of impairment of Glikman furcation
5. Teeth mobility based on Miller’s mobility index

Radiographic assessment

A panoramic radiography was performed which would offer an overall view of dental arcades, information about the dimension of alveolar events, about present teeth and contiguous anatomical structures, which served as a baseline for measuring the bone’s height.
VIII.4. RESULTS

VIII.4.1 Results of the overall data assessment
For this study, the group was similar to the one of the previous study. The evolution of markers was separately analysed to more effectively present the scientific results.

![Diagram VIII.1 Study group](image1)

![Diagram VIII.2 Control group](image2)

VIII.4.2 Periodontal and overall assessment results
All patients with less than three periodontal pockets, with a probing depth > 4 mm, were included in the study group.
According to the questionnaire, there were no major differences in social status. Most of the patients suffered from moderate periodontal disease, 47.2 ± 24.6% of sites had a probing depth of 4-5 mm and 8.6 ± 16.3% of the sites had a probing depth greater than 5 mm.

Average age distribution of the studied groups

![Diagram VIII.3](image3)

In case of the patients in the control group, the average age values were higher than those in the test group 40.3667 / 38.7.
The average body mass index distribution of the groups studied

Diagram VIII. 4 BMI (body mass index) in the studied groups

In the case of the patients in the test group, the average body mass index values were higher than those in the control group 22.8120 / 24.0230.

Diagram VIII. 5 Periodontal status

In some control cases, periodontal pockets of moderate depth were present (8.7 ± 8.5% of sites), but no deep periodontal pockets were present.

As expected, bleeding on probing was lower in subjects in the test group (5.1 ± 26.3%) than in the control subjects (35.0 ± 6.9%, p = 0.0001) after treatment.

Diagram VIII. 6 Bleeding when probing after treatment

A qualitative accumulation appraisal of plaque showed no significant difference between groups (50 ± 19% vs. 48 ± 23%)

Diagram VIII. 7 Bacteria plaque initially
**VIII.4.3 Results of the lipid profiles assessment**

After evaluating and making appropriate dosing of blood samples, fitting the values obtained after the values of normal and pathological categories, the differences in frequencies were tested for significance using chi-square test (Statview 5.0, SAS Inc.).

The values obtained have shown a close link between lipid profile lowering and periodontal status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group, average ± SD</th>
<th>Test group, average ± SD</th>
<th>t-student (P ≤ 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.3667 ± 6.5046</td>
<td>38.7000 ± 6.4656</td>
<td>0.324</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22.812 ± 3.154</td>
<td>24.023 ± 1.996</td>
<td>0.082</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>33.0000 ± 3.2203</td>
<td>32.9667 ± 4.0128</td>
<td>0.972</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>180.1133 ± 48</td>
<td>171.4883 ± 33.9837</td>
<td>±</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>136.3640 ± 95.8570</td>
<td>103.0487 ± 66.8766</td>
<td>±</td>
</tr>
<tr>
<td>HDL</td>
<td>45.8873 ± 10.4913</td>
<td>48.6653 ± 14.2200</td>
<td>0.246</td>
</tr>
<tr>
<td>LDL</td>
<td>103.0667 ± 46.4189</td>
<td>88.7400 ± 46.8956</td>
<td>0.239</td>
</tr>
<tr>
<td>VLDL</td>
<td>33.50000 ± 27.2599</td>
<td>37.7433 ± 30.9652</td>
<td>0.575</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>81.7667 ± 7.5370</td>
<td>82.1000 ± 7.4803</td>
<td>0.864</td>
</tr>
<tr>
<td>Chol_HDL (report)</td>
<td>4.09</td>
<td>3.66</td>
<td>0.182</td>
</tr>
<tr>
<td>LDL_HDL (report)</td>
<td>2.46</td>
<td>2.29</td>
<td>0.477</td>
</tr>
</tbody>
</table>

* SD – standard deviation; HDL – high-density lipoprotein; LDL – low density lipoprotein; VLDL – very low density lipoprotein.

Total cholesterol, LDL cholesterol (low density lipoprotein) and triglycerides were significantly higher in patients in the test group with about 8% (p, 0.03), 13% (p, 0.003) and 39% (p, 0.001), compared with the control group at one month after treatment.
Diagram VIII. 8
The assessment of the total cholesterol levels after the periodontal stage (at one month interval)

In the case of patients in the control group, the total cholesterol values were higher than those of subjects in the test group: 180.113 / 171.4883, **values assessed 1 month after treatment** (see Diagram VIII.12)

Diagram VIII. 9. Comparative evaluation of total cholesterol values, before and 1 month after treatment, in the two groups
Diagram VIII. 10 TG values assessed 1 month before treatment

Diagram VIII. 11 Comparative analysis of triglyceride levels in the two groups, measured at baseline and at 1 month after treatment

Following the conventional periodontal therapy, in patients in the control group (with periodontal disease), it has been observed a triglyceride level higher than the test group 103.0487 versus 136.3640.
Diagram VIII. 12 Values of alpha lipoprotein (HDL), assessed 1 month after treatment

In the case of patients in the test group, HDL values were higher than those in the control group 45.8873 / 48.6653.
**SUMMARY**

**COMPLEX STUDIES ON THE ASSESSMENT OF RISK MARKERS IN PERIODONTAL PATHOLOGY**

Diagram VIII. 14 Values of beta lipoprotein (LDL), assessed 1 month after treatment

In the case of patients in the control group, LDL values were higher than those in the test group after periodontal treatment 103.0667 / 88.7400.

Diagram VIII.15 Comparative analysis (at baseline and 1 month after treatment) of LDL level in the two groups

Diagram VIII.16 Prebeta lipoprotein values (VLDL), assessed 1 month after treatment
In the case of patients in the control group, VLDL values were higher than those in the test group 33.5000 / 37.7433.

Diagram VIII.17 Comparative analysis (at baseline and 1 month after treatment) of VLDL level in the two groups

In the case of patients in the test group, Chol_HDL values were higher than those in the control group 4.09 / 3.66.

Diagram VIII.18 The comparative analysis according to groups of the ColT/HDL ratio

In the case of patients in the test groups, LDL_HDL values were higher than those in the control group 2.46 / 2.29.

Diagram VIII.19 Comparative analysis on groups of the LDL / HDL ratio

Hypercholesterolemia, especially the elevated plasma levels of LDL cholesterol (low density lipoprotein), hypertriglyceridemia and diabetes mellitus are major factors of cardiovascular disease.
In contrast, high levels of HDL cholesterol (high density lipoprotein) associated with a decreased risk for cardiovascular disease.

The results of this study show the existence of a practically significant periodontal conditions worsened in subjects with hypercholesterolemia.

This study reveals the existence of a correlation between the degree of periodontal alteration and cholesterol plasma levels.

There were also significant differences between patients with periodontitis in test group and control patients, when frequencies of lipid pathological plasma concentrations were estimated.

The frequency of hypercholesterolemia in patients in the control group was about twice that of the test group. This was true for total cholesterol as well as proatherogenic cholesterol - LDL. Pathological levels of triglycerides were about 6.5 times more frequent in the control group (without periodontal treatment) compared with patients in the test group.

The recordings of the lipid profiles in subject included in the study in order to certify a possible association between periodontal territory affected and the risk of cardiovascular disorder, showed significantly higher levels in patients in the control group (approximately 8% and 13% compared with patients from the test group) of the mean values for total cholesterol and LDL cholesterol fraction. Also, plasma triglyceride levels were higher in patients with periodontitis territories than control subjects, not being registered significant differences in the value of protective, antiatherogenic lipoprotein.

**Diagram VIII. 20.** Average of records for ColT and TG correlated with the depth of periodontal pockets

Data from recent studies indicate that periodontal treatment seems to attenuate systemic inflammation and endothelial dysfunction (the first step in the process leading to atherosclerosis). Taken together, the data show a dose-dependent effect: better outcomes of periodontal treatment appear to be associated with significant changes in the system parameters. Periodontitis may contribute to systemic inflammation and to the risk of atherosclerosis installation at individuals otherwise healthy.

Most studies support the concept of association between periodontal disease and atherosclerosis (199). Although chronic inflammation clearly plays a role in the pathogenesis of
studied diseases, the changes in lipoprotein and lipoprotein metabolism appear to be equally important. There are few data in literature showing changes in the periodontal territory in variations of lipid metabolism, possibly correlated with cardiovascular risk. Thus, although very limited, there are some studies that support the motivation of investigating the modulation of lipid profile in patients with periodontitis together with the administration of specific treatments. Therefore, given the enormous costs associated with morbidity and mortality from atherosclerosis and cardiovascular diseases, our study, by the data recorded, confirms once again the need for scientifically installation of periodontal disease management, possibly delimiting the mechanisms governing the correlation between periodontitis and the risk of cardiovascular damage, or atherosclerosis.

Very likely, significant changes observed between groups of periodontitis patients and those in the control group, in terms of the values of triglyceride type lipids, it is explained by the interference of the inflammatory process certified in periodontitis and lipid metabolism, favoring the increasing of plasma level of those lipids, especially in dental infections with gram negative germs. The mechanisms for producing these distortions include local infection, with stimulation of pro-inflammatory mediators (such as interleukin - IL-1, IL-6, and TNF-alpha), and the inflammatory remote response to microbial toxins.

Cytokines produced in excess in multiple infections in the periodontium level are passing into the systemic circulation and subsequently determine the stimulation of other systemic mediators.

Knowing the data that supports normal metabolism of triglycerides, cholesterol and plasmatic lipoproteins, there can be estimated the normal level of disorder of such lipid fractions, produced subsequently to hyper-reactivity of leukocytes generating soluble chemical mediators. In this way, there are induced: alteration of lipid metabolism, increase of low-density lipoprotein - LDL and triglycerides, augmentation of liver lipoxygenase activity, lipolysis in adipose tissue. Subsequently, it is produced hyperlipidaemia and accumulation into the serum of fatty acids.

There are a few studies in the literature, attesting the existence of correlations between the level of triglycerides, cholesterol and severity of periodontal alterations, not being yet clearly established, the inter-conditioning way (if lipid metabolism is the cause or consequence of periodontitis) [116].

Local inflammatory production of cytokines (IL-1, TNF-α) and the effect on other systemic mediators induce apparently the alteration of lipid metabolism, increase of LDL and triglycerides, with the increasing of liver lipoxygenase and massive degradation of deposit triglycerides.

Moreover, the change in the LDL / HDL cholesterol ratio (HDL reduction and LDL increase) can be attributed, at least in part, also to the action of bacterial lipopolysaccharides. Subsequently to their action, the endothelial cell activation is possible by inflammatory cytokines, promoters of proatherogenic phenotype, leading to augmentation of proinflammatory factors expression and endothelial vasomotor skills, including reducing the biological activity of nitric oxide. Thus, the periodontal disease is one of the factors that may contribute to the development of atherosclerosis [149].

Interpretation of research studies in this area is complicated by the diversity of indices and metrics that are used to define periodontal disease or atherosclerosis. Therefore, there are still inconsistencies in both the quantitative and qualitative relationships between periodontal disease and lipid metabolism.

As has been stated, most studies have focused on inflammatory cytokines in relation to periodontal disease; however, in agreement with some data from literature, our study also confirms that specific periodontal therapy may lead to improvements in dyslipidaemia profile. Some reports indicate that the intensive periodontal therapy reduces total cholesterol and LDL-C, but has no effect
on HDL-C or triglyceride when tested at 6 months after treatment [177]. However, most studies demonstrate favourable changes in both LDL and HDL-C after periodontal therapy [155, 190, 191].

Although Losch et al. did not notice any changes in HDL-C or LDL-C, they presented a decline in LpPLA2 (lipoprotein associated phospholipase A2) consecutive to periodontal therapy [192]. In a large study of HDL metabolism, Pussinen et al. have shown that periodontal mechanical therapy plus antibiotics has led to an increase of HDL-C and an increase of HDL-2: HDL-3 ratio, a change which should promote reverse transport of cholesterol, and consequently lead to an improvement of atherosclerosis.

The same study also indicates an augmentation of HDL-associated phospholipids [115]. Moreover, the periodontal therapy also has a favourable impact on the oxidation of lipids and lipoproteins. Some studies attest modifications of the level of lipoperoxides and malondialdehydes, of the levels of oxidized lipoproteins (oxLDL), after the surgical manoeuvres (curettage and surfasage) [156, 157]. Correlated with these evidences supported in the specialty literature, our results also support the concept that periodontal treatment ameliorates the dyslipidaemia, not confirming only exclusively the association between the periodontal disease and atherosclerosis, but suggesting at the same time, the periodontal therapy as a strategy of the control potential of atherosclerosis.

The results of this study revealed a positive correlation between clinical parameters (depth of probing, level of attachment loss) and the paraclinical ones, with values of TG, ColT, LDL, and a negative correlation with HDL values. This shows that with the increasing of probing depth and local loss of attachment, the TG, ColT, LDL values are higher, while HDL levels decrease. Although slightly different from other studies (Chen L) [54], the results recorded in groups of periodontitis patients compared with those in the control group are in agreement with the study made by Katz [180], who hypothesized that there is a strong statistical association between the existence of periodontal pockets and plasmatic lipid levels, confirming a interrelation between periodontitis and hyperlipidaemia.

VIII.6. CONCLUSIONS

Although the treatments aiming at the reducing the periodontal infection and inflammation can reduce the level of predictive serum inflammatory biomarkers of cardiovascular disease and may improve the vascular responses, the clinical relevance of these surrogate changes in reducing the risks of myocardial infarction or ischemic stroke are not fully elucidated. However, clinicians and patients should be aware of this consistent association, but especially preventive potential benefits of periodontal interventions.

The values of parameters that reflect the patients’ lipid profile showed a link between the decrease of lipid profile and periodontal health status. In patients who received periodontal treatment and to which the periodontal status improved, we also noticed a decrease in plasma lipids concentrations.

We can therefore deduce that, in addition to the fact that lipid markers represent a risk factor for cardiovascular disease, they influence / are influenced indirectly also by the periodontal health status. From this study direction, there are several conclusions:

1. More and more epidemiological studies confirm the association between periodontal disease and atherosclerotic coronary artery disease, the two diseases having complex etiologies, genetic predisposition and many common risk factors;
2. Chronic periodontal infection may contribute to the process of atherogenesis, namely the development and evolution of this process in the arterial level;
3. Periodontal disease and cardiovascular disease share the inflammation. Local periodontal inflammation controls and modulates the systemic inflammation;
4. Blood samples from patients have been collected in order to investigate the lipid profile and analyse the influence of periodontal treatment.
5. All subjects gave their written consent, the study protocol being submitted to the approval of the Ethics Committee.
6. In the present study, we measured plasma levels of lipids in subjects with periodontitis who received etiological therapy and those who did not receive treatment.
7. Furthermore, we assessed the degree of periodontal alteration correlated with plasma levels of cholesterol.
8. Compared with the test group, the subjects in the control group showed higher plasma levels of total cholesterol, LDL cholesterol and triglycerides, and a greater frequency of altered lipid profiles;
9. Frequency of hypercholesterolemia in patients in the control group was approximately double from the test group; the records being similar at the measurement of total cholesterol and LDL proatherogenic lipoprotein;
10. Pro-atherogenic changes of plasmatic lipids and blood glucose that were observed in patients with periodontal disease may provide further evidence of a strong association between periodontal disease and cardiovascular disease;
11. Our study indicates that periodontal infection has a defined role in altering lipid metabolism, thus supporting the hypothesis of a relationship between periodontitis and hyperlipidaemia;
12. Patients with periodontitis showed significant increases in TG, Col T, and of LDL with the decreasing of HDL level compared to the control group. Furthermore, the results that show the value of periodontal clinical parameters also indicates a positive correlation between clinical markers of periodontal alteration (depth of probing, level of attachment loss) and the paraclinical ones, with values of TG, ColT, LDL, and a negative correlation with HDL values. Thus, with the increase of depth in probing and local loosing of attachment, the values of TG, ColT, LDL are higher, while HDL levels decrease;
13. In the case of gum inflammation not accompanied by clinical attachment loss, our data show the absence of any effect on serum lipid profiles.
14. However, it is still uncertain whether the observed changes in lipid metabolism are a cause or a consequence of periodontal disease.
15. In relation to clarifying the fine mechanisms governing the association between periodontal disease and possible cardiovascular complications, further studies are required to clarify the relationship between periodontitis and serum lipid levels, and to determine whether healthcare in the oral affected territories has the potential to significantly reduce serum lipid levels in people without systemic flaws.
GENERAL CONCLUSIONS

1. Based on the evidence showing that oral health is an indicator of systemic homeostasis, our study initially aimed at identifying possible correlations between clinical indicators of periodontal disease, in its various aspects, and systemic conditions, by evaluating changes occurred in immune-inflammatory response of the host, or changes in lipid profile. 

2. Our results support the involvement of risk markers in the pathogenesis of periodontal disease by the multi-faceted action of the group comprised in research through clinical, laboratory, statistical and biochemical investigation. 

3. The recorded data from groups of patients showed correlation of clinical indicators values as current diagnostic elements in periodontal disease, with systemic markers levels attesting modifications of lipid profiles. 

4. In the comparative analysis of inflammatory markers in different categories of patients with and without periodontal disease, there were registered significant differences between the groups regarding the values of total serum cholesterol, of blood glucose (p> 0.001). 

5. The inflammatory markers, C-reactive protein (CRP), ESR and fibrinogen have registered elevated values in the study group compared with the control group (CRP 5.02 ± 6.0 vs 1.56 ± 2.1 (p <0.01), ESR 10.6 ± 7.4 vs 6.3 ± 5.7 (p <0.001), fibrinogen 305.7 ± 54.1 vs 264.0 ± 73.7 (p <0.05) 

6. CRP has averages of 0.5 mg / l and in periodontitis localized, the data showed an average of 1.3 mg / l - value increased compared with the control group in aggressive periodontitis registering higher levels (an average of 1.45 mg / l). 

7. Another study aimed the evaluation of the levels of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides in the blood of people with periodontal disease and the healthy ones. Moreover, the frequency of associations between the modified lipid profiles and the periodontal disease were analysed, thus adopting preventive measures that would impose in such cases to prevent possible complications of cardiovascular field. 

8. It was identified a correlation of clinical indications with serum lipid profile (surrogate biomarker for periodontal and coronary disease) in patients with periodontitis. 

9. In addition to the specific treatment of periodontal disease, the comparative data show that subjects with periodontitis had higher plasma levels of total cholesterol, LDL cholesterol and triglycerides compared to individuals in the control group, the latter recording as well a lower frequency of altered lipid profiles; 

10. The frequency of hypercholesterolemia in patients was approximately double than that of the control group; a similar pattern being recorded not only for total cholesterol but also for LDL cholesterol. 

11. The pathological levels of triglycerides were about 6.5 times more frequent in periodontitis compared to subjects in the control group, while the HDL antiatherosclerotic lipoproteins did not show significant differences between groups. 

12. Although diabetes was one of the exclusion criteria in our study, we also determined the levels of fasting blood glucose. Indeed we did not observe pathological values in patients with periodontitis or subjects in the control group, although there was a significant difference
between the mean values in both groups. Mean blood glucose was approximately 15 % higher in patients than in the control group.

13. This study showed that there is a link between hyperlipidaemia and periodontal disease, most likely the mediation of the action of the latter being achieved via cytokines released into the blood, consecutive to inflammatory destructive oral alterations, which led to the installation of a catabolic status, characterized by a disturbance of lipid metabolism.

14. Pro-atherogenic changes of plasmatic lipids possibly correlated with blood glucose were observed in patients with periodontal disease may provide further evidence of the close association between periodontal disease and cardiovascular disease.

15. One of the directions of the study was also the assessment of the effects of non-surgical periodontal therapy on inflammatory markers, the records showing that a severe control of periodontal infections induce reduction of inflammation markers on a small sample population with severe and chronic forms of periodontal diseases.

16. The medium level of C-reactive protein in the first phase was 1.9 mg/l with an IQ level of 3.6 mg/l, not noticing any differences in concentrations regarding age, sex, periodontal diagnosis (chronic or aggressive periodontitis).

17. A significant decrease in serum concentration of CRP was observed predominantly at 6 months from the application of non-surgical therapy, the mean changes of CRP concentration of between initial and final phase of treatment being 0.5 mg/l with a free distribution of 95% and an intermediate of 0.4-0.7 mg/l.

18. The data indicated that there was a significant interaction between the treatment outcomes and the overall levels of CRP (basic, 2 and 6 months), and that this decrease in CRP was significant in subjects who had the best results in terms of periodontal parameters.

19. The control of periodontitis, achieved without surgical treatment resulted in reduction of serum mediators’ levels of inflammation of C-reactive protein type as well as soluble mediators of cytokines type.

20. The study demonstrated that an effective control of periodontal infection reduced serum concentration of inflammatory markers (CRP, IL-1, IL-6) in a relatively small population with alteration of periodontal territory.

21. Given the limitations of this study, the data and results obtained support the existence of a causal link between periodontitis and systemic inflammatory condition.

22. Our study also aimed to analyse the impact of bacterial endotoxin on the phenotype and function of peripheral blood mononuclear cells, with the identification of signalling pathways activated by bacterial antigen by showing intracellular proteins secreted in macrophages.

23. In parallel with the evaluation of the immune-inflammatory response of the host periodontally compromised, the identification of peripheral blood mononuclear cell phenotype in culture as well as a possible association between composite genotype of interleukin-1 (IL-1) and the prevalence of aggressive periodontitis is also one of the objectives of this study.

24. The study of immuno-inflammatory response induced by the bacterial plaque in the oral territory as well as the signalling mechanisms induced by the microbial agent in the periphery, by determining the level of cytokines in PBMC cultures, in groups of patients with periodontal alteration, suggests that the major source of proinflammatory mediators in this level is represented by monocytes.
25. The recorded data show that monocytes stimulated with bacterial endotoxin express a particular phenotype, with preferential hypersecretion of certain cytokines; there were observed higher amplitude responses in patients with aggressive periodontitis.

26. The results obtained in this study argue the major implication of monocytes in the mechanism of developing periodontal disease and provides scientific basis for the management of oral hygiene to prevent installation of inflammatory periodontal lesions.

27. The results of this study show that generalized severe periodontitis may interfere and inter-condition the systemic inflammatory status, which can acquire causal connotations, when assessing the risk of subsequent development of cardiovascular events.

28. To fully explain the susceptibility to periodontal disease, special attention should be however given to other cell lines (epithelial cells, fibroblasts) also able to interact with bacterial components.

29. While in literature there are studies that greatly assign the genetic predisposition from aggressive periodontitis to the polymorphism of IL-1 gene, our results could not reveal a significant correlation between the polymorphism of IL-1α and IL-1β genes in patients with AGP, in the groups studied.

30. Given the limitations of this study, imposed mainly by the small size of the sample chosen, we can only say that in the group of patients with aggressive periodontitis, the polymorphism of IL-1 genes did not reveal a statistically significant correlation.

31. It is not excluded that the genetic profile of IL-1 gene be used as a marker for assessing the risk of occurrence and progression in periodontal chronic events.

32. On the other hand, analysis by gender of gene polymorphism, with the assessment of alleles share in the female and male population revealed a correlation between AGP and polymorphism of IL-1α (-889) and IL-1β (3954) genes to male patients, indicating a greater sensitivity to their genetic variations.

33. Moreover, the present study did not identify an obvious association between the polymorphism of IL-1α and IL-1β genes studied and AGP, in the absence of other risk markers.

34. Instead, by broadening the sample of study or correlated in some populations, the results obtained suggest the opportunity of further studies that, to the extent that they associate other factors, they could help to defining the association between the prevalence of aggressive periodontitis correlated with the sex of the patients.

35. Following the same algorithm, another study measured lipid plasma levels in subjects with periodontitis who received etiological treatment-control group and those who received no treatment-control group, and assessed the degree of periodontal alteration correlated with plasma cholesterol levels and lipoprotein fractions.

36. Compared with the test group, the subjects with periodontitis from the control group had higher plasmatic levels of total cholesterol, LDL cholesterol and triglycerides, and also a higher prevalence of pathological lipid profiles.

37. We believe that once exceeded the limits imposed by the relatively small sample of patients, a careful evaluation of such records, could be the premises to lead to the identification of fine susceptibility mechanisms of individuals to aggressive forms of periodontal territory alteration.

38. The data recorded in this study confirm a relationship between the presence of infections in the mouth and disturbance of haemostatic mechanisms that influence the status of
inflammatory markers, also supporting the link between periodontal disease and altered levels of markers with biological risk, variable depending on the type of oral alteration.

**ORIGINALITY OF THE STUDY**

**CONTRIBUTIONS TO THE DEVELOPMENT OF THE AREA**

- The work falls into basic and applied scientific research trends, aiming the description of risk markers’ evolution during the periodontal disease.
- The innovations of this project lie in the multidisciplinary approach of periodontal disease and in the fact that it uses new techniques of investigation to validate the immuno-biological changes.
- The following were performed and interpreted in a correlative manner:
  - the clinical and biological analysis, with the assessment of changes appeared in the immuno-inflammatory response of the host, or metabolic changes reflected in the lipid profile in patients with alteration of periodontal territories with a variable severity degree;
  - the clinical and statistical evaluation on a personal data basis concerning the health and alteration of the periodontium in persons susceptible or affected by periodontal disease, of chronic and aggressive nature;
  - monitoring of non-surgical periodontal therapy effects on local inflammatory markers, the records showing that a severe control of periodontal infections induce the decrease of inflammation markers;
  - measurement and identification of lipid metabolism and lipoprotein changes consecutive to the non-surgical specific therapy in subjects with periodontitis (compared with those who did not receive treatment);
  - impact of bacterial endotoxin on phenotype and peripheral mononuclear cells function, identifying signalling pathways activated by bacterial antigen by showing intracellular proteins secreted in macrophages;
- The particular and original aspect of the thesis is mainly to assess the peripheral blood mononuclear cells phenotype in culture, as well as the relationship of association between the genotype of interleukin-1 (IL-1) and the prevalence of severe periodontitis forms.
- By their interdisciplinary nature, the research results will contribute to increasing knowledge in the field of genetic analysis and periodontology, aiming at the approach of the periodontal disease, according to the degree of severity, from the perspective of potential systemic complications.
SUMMARY

COMPLEX STUDIES ON THE ASSESSMENT OF RISK MARKERS IN PERIODONTAL PATHOLOGY

SELECTIVE BIBLIOGRAPHY


