

## Application of medical ozone in the complex treatment of the severe chronic marginal periodontitis

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### Abstract

**Background:** The microbial factor plays a major role in the pathogenesis of the periodontal disease. The presence of the periodontal bacteria was demonstrated during numerous researches. The susceptibility of the host in the initial stage of the periodontal disease is considered a mandatory condition.

**Material and methods:** For this study, there were 96 patients selected, men and women, aged between 41 and 73, diagnosed with severe marginal chronic periodontitis (severe CMP). The patients were divided into two lots – the control lot of 52 patients, who received a classical non-surgical periodontal treatment and the research lot of 44 patients, who received the same treatment complimented with systemic and topic ozone therapy.

**Results:** The recent researches have shown the other risk factors, such as the formation of free radicals, which are formed internally and externally. Another very important factor, which accumulates free radicals and creates an appropriate environment for the development of the inflammatory phenomena, is the disruption of the microcirculation, which leads to the development of the chronic tissue hypoxia. The interaction of the free radicals with oxygen leads to the creation of the reactive oxygen species (ROS). The excessive production of the ROS is associated with the development of conditions that involve a wide range of affections and degenerative diseases, including periodontitis. The reduction of the oxidative stress plays a critical role in the complex treatment of the periodontal disease. In the last years, the traditional nonsurgical methods for treating periodontitis have lost their effect, because of the number of cases, showing resistance to the antibiotics and increased allergic reactions, in addition to the high number of contraindications and the side effects of the medications. All these factors lead to a new approach and new researches of alternative methods of treatment.

**Conclusions:** The achieved result led to the rapid reduction of the inflammatory phenomena in the periodontal tissue ensuring a considerable decrease of the bacterial contamination in the periodontal sacks and a stimulation of the repairing processes, which led to the increasing of the health condition maintenance of the periodontal status when comparing it with the traditional treatment.

**Key words:** severe marginal periodontitis, medical ozone.

## Introduction

Marginal periodontitis continues to be a major health problem, because it is one of the most widespread and frequent disease of the human body at any life stage. That is why the main scope in the rehabilitation of the maxillary functions (mastication, phonation and aesthetics) is its prevention, the early stage diagnosis and the performance of a complete and complex treatment, through the integration of all dental specialities [1].

During the periodontal disease pathogenesis (PD), the main role is played by the microbial factor, the presence of the *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Treponema denticola*, *Bacteroides forsythus*, *Porphyromonas gingivalis* bacteria was determined by various researches [2].

During the first stage of the PD, the immune inflammatory response of the body plays an essential role, and the susceptibility of the host is a mandatory condition [3]. The reaction to the bacteria aggressiveness is unleashed by a specific mechanism, which is influenced by the genetic predisposition of each individual [4]. For many years, the immune genetic researches are trying to identify various associations between the PD appearance and the existence of any relevant genes. The identification of the genes is based on the analysis of researches over the PD genotype. Studies have demonstrated that the marginal periodontitis is associated with high levels of pro-inflammatory cytokines: interleukin 1 (IL-1) and the tumour necrosis factor - alpha (TNF - alfa), which are the key regulators within the immune response of the host, during the bacterial infections. IL-1 is also a major modulator of the extra cells matrix of the catabolism and of the bone resorption [5]. The researchers in the field claim that these specific genetic markers, which were associated with the IL-1 growth, are a powerful indicator of the susceptibility of the PD occurrence.

The recent researches have shown also the role of the risk factors, such as, the formation of the free radicals [6] which originate from the inside environment (phagocytosis, incomplete catabolism, energy production, etc.) and from the outside environment (stress [7], smoking, alcohol, polluted air, processed food, some types of medications, etc.). Another, also, very important risk factor is the periodontal chronic trauma [8]. At the same time, a predisposition role, for the inflammatory phenomena development, into the periodontics, is played by the microcirculation perturbation together with the development of the chronic tissue hypoxia; the role of the hypoxia in the periodontal pathogenesis condition was demonstrated by numerous studies [38]. The settlement and the progression of the hypoxia condition leads to the accumulation of free radicals, which determines the transformation of the aerobic cycles (mainly in the carbohydrates metabolism) in anaerobic cycles and, in consequence, the accumulation, especially, of the lactic and pyruvic acid, leading to the occurrence of the metabolic acidosis into the affected area. There is also an observation of a simultaneous pH decrease, which characterises the degree of hypoxia [39]. The rapid response to the occurrence of the inflammation, resulting from the respiratory enzymes, necessary for the respiratory processes,

suddenly decreased, and it can serve as a sign for the early manifestation of the necrosis changes inside the tissue. All the mentioned above factors create favourable conditions for the development of the pathogenic microbes [40].

The main element for the establishment of the diagnosis and for the PD treatment plan is the clinical and radiographical examination. In the last years, the PD treatment has registered success mainly because of the new surgical methods and techniques, the bio stimulator materials and the tissue regeneration, which allow the rehabilitation of the maxillary functions, but they do not eliminate completely the factors that are causing the appearance of the PD. Before not long ago, the scope of the periodontology was to treat and to maintain on the dental arch, by all means, the affected dental periodontal units. Nowadays, the tendency is to maintain and improve the bone tissue, due to unsuccessful periodontal treatments, where the severe atrophies of the alveolar crest make the use of dental implants very difficult or even impossible [9]. Therefore, alongside the surgical treatment, which is a therapeutical stage within the complex treatment of the PD, it is very important to discover and to apply systemic treatments, which could beneficially influence the PD evolution. Thus, in the last years, the traditional methods of the non-surgical periodontal treatment have lost their value. The main cause is considered the increased number of cases, which showed microbial resistance to antibiotics [10]; the frequent appearance of allergies, the high number of contraindications, and the side effects of medications - all these factors lead to a new approach and new researches of alternative methods of treatment.

In this context, ozone therapy deserves to be recognized as one of the simplest and most efficient method [11]. The action of the medical ozone on the human body is very diverse and multidirectional. The medical ozone has an antimicrobial, antioxidant, immunomodulatory [12], antihypoxic, disinfection, antiviral and antifungal effect, it stimulates the metabolic processes and improves the blood rheological proprieties [13]. If compared with the antibacterial therapy, the ozone therapy has a wider range of therapeutical action and it does not create microbial resistance. Also, it does not produce mutagenic or carcinogenic effects [14], more than that, if there is microflora resistant to antibiotics, the use of medical ozone in antibacterial therapy, leads to the neutralization of the resistance to antibiotics and to the intensification of the antibiotics effect [15].

The efficiency of the use of the medical ozone was demonstrated and proved in various systemic diseases, which mainly have inflammatory character, in both the field of surgery and therapy [16, 17].

The dental practice medical ozone is used only in combination with other treatment methods [18]. Therefore, the maxillofacial surgery is widely using ozonated solutions as antiseptic remedies for the care of local injuries and whole oral cavity (OC) [19, 20, 21]. This, as well, considerably improves the post-surgical convalescence and fastens the epithelialization of the operated wound. The ozone has a beneficial effect over the metabolism and the bone repairing process [22]. It has

been observed that the use of medical ozone in patients with chronic mandibular osteomyelitis normalises more rapidly and completely the unspecific resistance and the T-cell immunity, thus, accelerating the clinical recovery and reducing the incidence of complications [23]. The medical ozone is used, topically, during the treatment of periodontal affections, in the periodontal pockets (PPs), in the form of instillations, solutions or ozonated oils and has a real anti-inflammatory potential, established based on the objective criteria analysis of the diagnosis [24, 25].

Nevertheless, the specialized literature, basically, includes no data about the application of the medical ozone in the form of gas injections (infiltrations) in the submucosa region of the affected marginal periodontium and the data about the application of the semisolid ozonated oil are missing completely.

Therefore, the various use of the medical ozone in clinical periodontology needs a more thorough research.

### Material and methods

For this study, there were 96 patients selected, men and women, aged between 41 and 73, diagnosed with severe marginal chronic periodontitis (severe CMP). The patients were divided into two lots – the control lot of 52 patients, who received a classical non-surgical periodontal treatment and the research lot of 44 patients, who received the same treatment complimented with systemic and topic ozone therapy.

- The classical non-surgical treatment included: scaling and root planing treatment (SRP). In order to remove the supra- and subgingival plaque and tartar the ultrasonic NSK – MultiPad Varios170LUX was used instrumentally, through infiltration, under the local anaesthesia. The other parts of the tartar and plaque have been removed through Airflow, by using the NSK Prophy Mate prophylaxis bicarbonate device.

- The complementary treatment with medical ozone included: administration of systemic medical ozone in the form of major autohemotherapy (AHTM) and in the form of gas infiltrations (injections) as well as semisolid ozonated oil as topic applications.

All the patients from the research lot have followed:

Systemically:

- Six AHTM sessions, once in 3 days, with a concentration of 25 - 35 mg/ml O<sub>2</sub>-O<sub>3</sub>;

- Six sessions of submucosa infiltrations, once in 3 days, with a concentration of 5 - 10 mg/ml, 0,5 – 1 ml O<sub>2</sub>-O<sub>3</sub>.

Topically:

- Six sessions of semisolid ozonated oil applications on the dental arch.

For the generation of medical ozone was used the universal medical ozone generator HERRMANN Medozon (Germany).

In order to pathogenically prove the use of medical ozone in the complex treatment of the severe CMP, the present study has performed the following:

1. Clinical examination of patients, in which periodontal status was recorded: CPITN Index - the Community Periodontal Index of Treatment Needs identifies the periodontal disease, its severity, as well as, the need of treatment. The

papillary bleeding index (PBI) - Saxer, Mählemann (1975) - is an indicator of the severity of gingival inflammation and allows the individual monitoring of the condition of the periodontium through the evaluation of the papillary bleeding intensity during the examination. The recording of the ZMK periodontal charting form, (School of Dental Medicine, Bern University), allows for the overview of the periodontal status.

2. Radiographic examination of the periodontal modification. For this purpose, ortopantomography was used by applying the cone beam computed tomography device VATECH Pax-Flex 3D. After the treatment, the radiographic analysis indicated the lack of bone tissue regeneration for both of the lots.

3. Laboratory examination which include:

- Identification of germs associated with periodontitis - micro-IDent® plus 11 test, based on polymerize chain reaction (PCR) and represents a high specificity for the identification of the eleven periodontal pathogens bacteria and the establishment of their relative quantity: *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* and *Treponema denticola*, *Peptostreptococcus micros*, *Fusobacterium nucleatum/periodonticum*, *Eikenella corrodens*, *Campylobacter rectus*, *Eubacterium nodatum* and *Capnocytophaga* spp.

- Evaluating the serum level of the oxidative stress marker - superoxide dismutase (SOD), which was analysed based on blood analysis by using the photometric (enzyme) method.

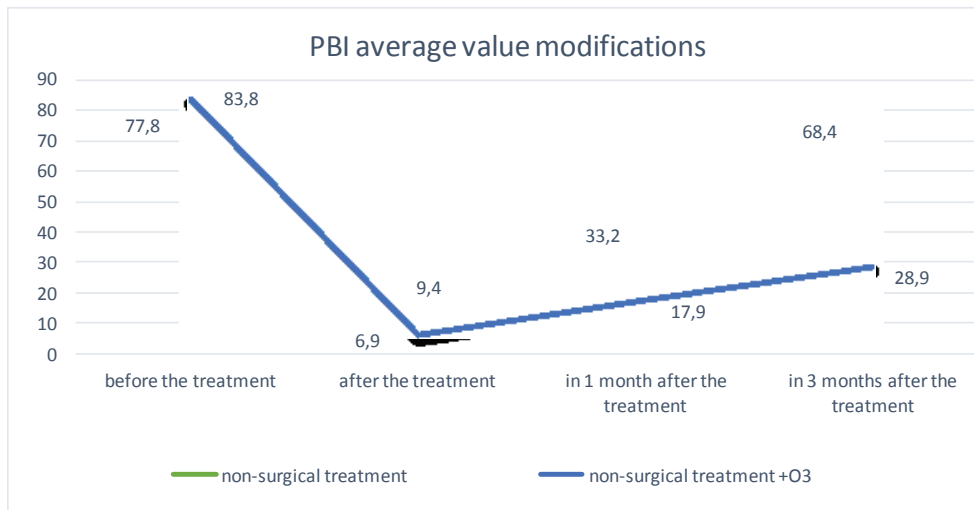
4. Functional examination, during which the blood microcirculation within periodontal tissue was evaluated, by using the Laser-Doppler (LDF) flowmetry method and the laser LAKK-02, SPE «LAZMA» (Russia) analyser. The status of the blood perfusion in the periodontal tissue was evaluated based on the microcirculation level (M) and on the microcirculation effectiveness index (IEM).

The examination of patients with a severe CMP was dynamically performed before the treatment, after the treatment, after 1 month, 3 months and 6 months after the treatment.

### Results and discussion

The clinical picture of the evolution of periodontal disease in both groups of study, before the treatment, did not show significant differences ( $P > 0,05$ ). Patients very well tolerated both methods of treatment. The findings of this study showed that patients which received additional treatment with medical ozone, applied systematically and topically, have demonstrated improvement of all clinical parameters, which had maintained during 3 months, then, however returning to initial status within 6 months, with the exception of the average of the total quantity of germs, which returned to the initial status within 3 months. At the same time, the average value of the serum level marker (SOD) has maintained as normal after 6 months.

Taking into account the fact that the infectious factor plays a very important role in the aetiology of the periodontal inflammatory affections, the best method of treatment should include elimination of this factor [26]. Thus, special attention was paid to the control of the oral hygiene status.



**Fig. 1. The comparative analysis of the PBI index average value, between the lots, before the treatment, after the treatment and after 1, and after 3 months after the treatment.**

The dynamics of the clinical indices have shown that the complementation of the non-surgical classical treatment with ozone therapy applied systematically and topically, has contributed to a more rapid improvement and longer lasting of the oral hygiene condition in comparison with the non-surgical classical treatment. Moreover, the rapid decrease of the inflammatory phenomena in the periodontal tissues, has allowed the patients with severe CMP to have a better hygiene of the OC, without being afraid of inflicting pain or bleeding during teeth brushing.

The comparative analysis of the papillary bleeding index (PBI) average value, between the lots, has shown the following:

Within the control lot, the average value of PBI was reduced after the treatment from 78 % (<0.01) - to 9 % (<0.001); an improvement which was maintained during one month period, followed by a gradual increase, and after 3 months, coming up, almost to the initial values.

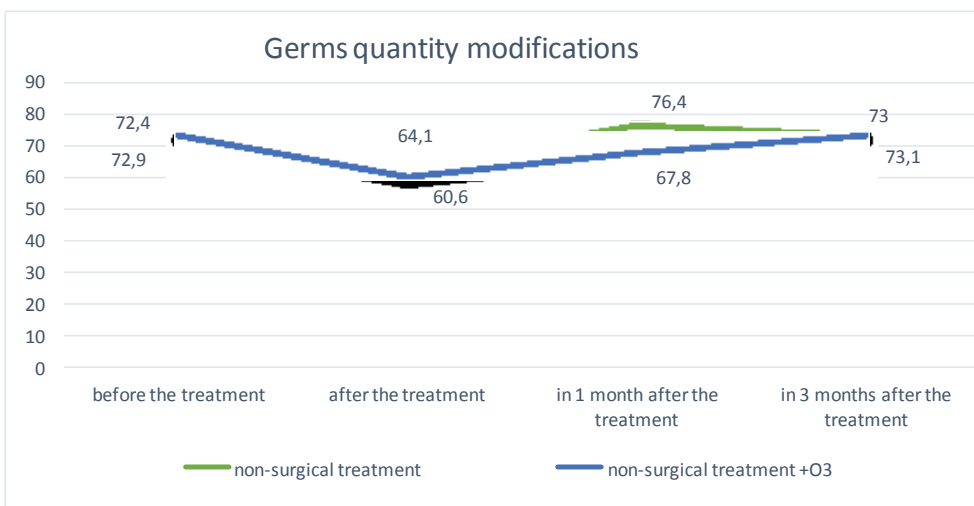
Within the research lot, where patients received additional treatment with medical ozone the average value has shown a

higher reduction of PBI, after the treatment, from 84 % (<0.01) to 7 % (<0.001), and an improvement which was maintained for 3 months period followed by a gradual increase, and within 6 months, coming almost to the initial values (fig. 1).

From a bacteriological point of view and taking into account the microbiological research findings on how medical ozone affects bacteria [11, 17, 20, 27], the present study has the scope of researching to what extent the medical ozone has an effect over the periodontal pathogenies bacteria in patients with severe CMP.

In this regard, the micro-IDent® plus 11 test was chosen, used for the identification of the periodontal pathogenic bacteria, based on polymerise chain reaction (PCR) technique and represents a high specificity for the identification of eleven periodontal pathogenic bacteria, and allow the establishment of their relative quantity. Also, this test is more sensitive than the bacterial culture because it identifies the germs according to DNA, regardless of their viability [28].

The comparative analysis of the findings demonstrated a reduction of total quantity of germs:



**Fig. 2. The comparative analysis of the total quantity of germs associated with periodontitis between lots, before the treatment, after the treatment and after 1 and 3 months after the treatment.**

Within the control lot by 11% ( $P < 0, 01$ ) after the treatment, and 1 month after the treatment increasing by 5% ( $P < 0, 01$ ) compared with the initial value.

Within the research lot treated with medical ozone with 17% ( $P < 0, 01$ ) after the treatment, and in 1 month after the treatment was still decreased with 7% ( $P < 0,001$ ) compared with the initial values.

On the other hand, there has not been registered any significant difference between the lots in 3 months, after the treatment ( $P > 0, 05$ ) (fig. 2).

The findings showed the efficiency of complementation of the classical periodontal non-surgical treatment with medical ozone, applied systematically and topically. However, the improvement has lasted for 1 month only, followed by a recolonization of the periodontal pathogenic bacteria, favoured by the PPs depth, which has come, after 3 months, to initial values. A confirmed result of the fact that a wider than 6 mm depth represents an important limitation factor to the adequate cleaning of the root surfaces and the complete elimination of the soft and hard sediments from PPs [29].

As a result, in the case of the patients with severe CMP, the complex treatment of the PD may require also the surgical management of the PPs, which would enable the elimination of this factor.

Another objective of this study was the comparative evaluation of the serum level values of the oxidative stress marker - superoxide dismutase (SOD). Together with the glutathione peroxidase and the catalase, the SOD is found inside and outside the cells membranes and they form the primary internal anti-oxidant defence system of the body and plays an important role in the oxidative stress reduction, involved in the development of a wide range of degenerative conditions, which may endanger life [30].

Statistically, the accumulated data, before the treatment, have not shown any significant difference between the two lots

( $P > 0, 05$ ), the average values of the serum level of the SOD marker were normal only within 11%, increased within 81% and decreased within 7% from the total number of patients. After the treatment within the control lot, there has not been registered any evident change of the average value of SOD serum level. On the one hand, within the research lot, after the complementary treatment with medical ozone, there has been registered a significant decrease of the average values of SOD serum level, in patients with high level of SOD marker up to normal values. On the other hand, in patients with decreased values of SOD marker, there has been registered a significant increase of the average value of the serum level of SOD marker, up to normal values. Therefore, the patients, having a normal serum level of the SOD marker, increased from 11% to 84% ( $P < 0,001$ ) after the treatment and up to 95% ( $P < 0,001$ ) 1 month after the treatment, coming up to 100% ( $P < 0,001$ ) 3 months after the treatment and having maintained its value to normal 6 months, after treatment (fig. 3).

The outstanding results demonstrated the modulation effect of the serum level of the superoxide dismutase, induced by the systemic ozone therapy. The modulation of SOD and of catalase by the medical ozone was observed during other clinical studies and probably involves the modulation of the genes expression [31, 32]. The overall number of these modifications is the metabolic adaptation, which promotes the integration of the homeostatic reactions at different levels and the disorders reduction of the body's self-regulation [33].

Another very important element in the development of the PD is the disruption of the blood microcirculation within the periodontal tissues [35]. During the ozone therapy, both plasma and the erythrocytes are saturated with oxygen [36].

The present study evaluated the medical ozone effect on blood microcirculation within the periodontal tissues in patients with severe CMP by using the Laser-Doppler (LDF) flowmetry method. LDF is a non-invasive, highly informa-

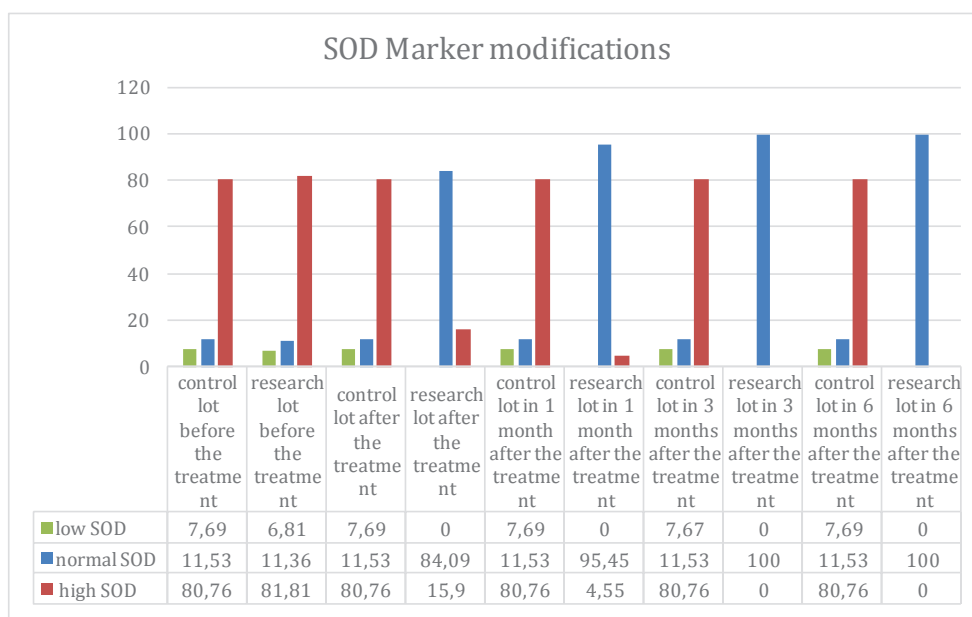


Fig. 3. Comparative analysis of the average value of the serum level of the SOD marker, between the lots, before and after the treatment, after 1 month, 3 months and 6 months after the treatment.

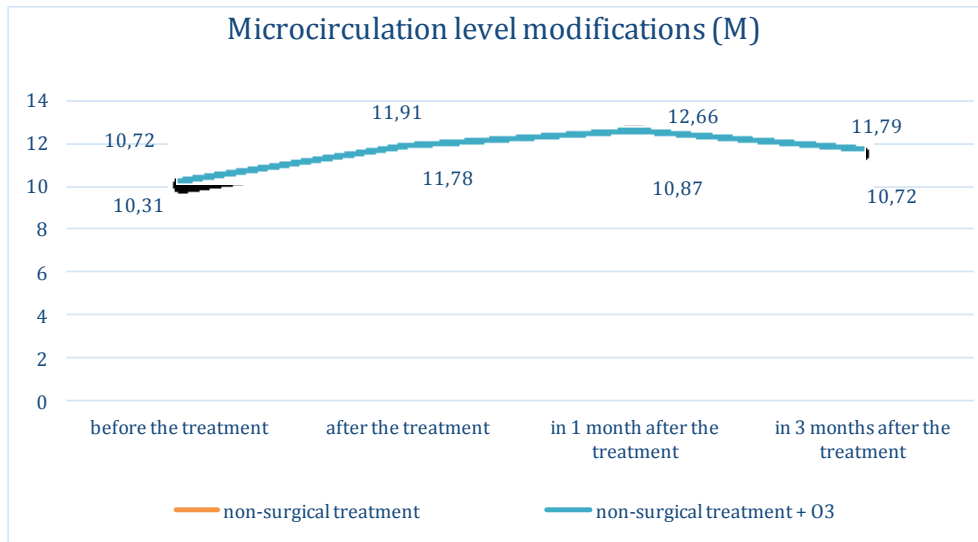


Fig. 4. Comparative analysis of the average value of the capillary blood microcirculation level (M) within the periodontal tissue, between the lots, before the treatment, after the treatment, from 1 and 3 months after the treatment.

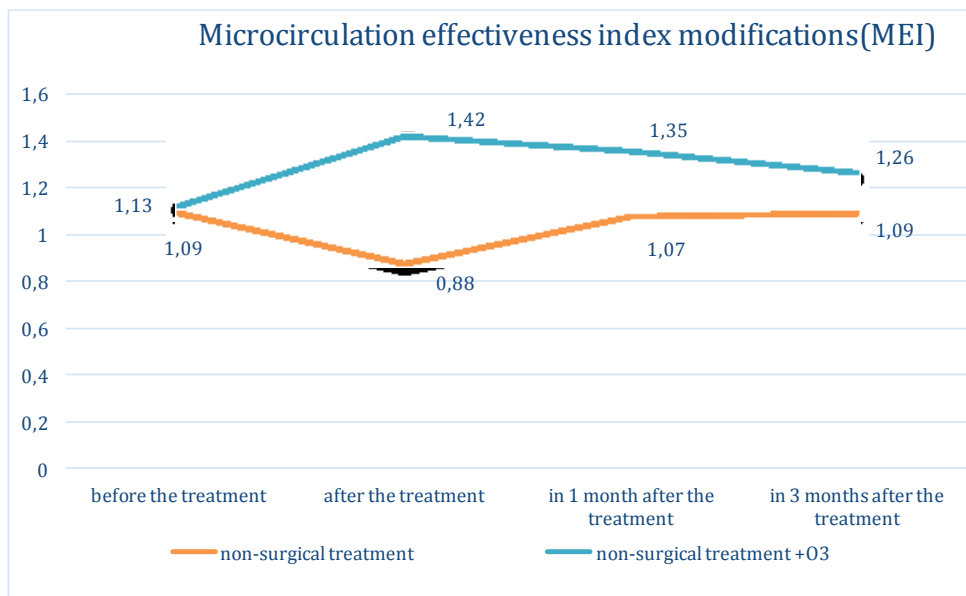


Fig. 5. Comparative analysis of the average value of the capillary blood microcirculation effectiveness index (MEI) within the periodontal tissue, between the lots, before the treatment, after the treatment, from 1 and 3 months after the treatment.

tional and sufficiently sensitive method, used for diagnosis, having a high resolution, for assessing the degree of blood circulation modification, within the tissues, which allows the prominence of the blood flow volume, at the capillary level, the primary symptoms of the affected capillary network, the evaluation of methods and the effectiveness of the chosen therapy [37].

Statistically, the analysis of the microcirculation level values (M) before the treatment did not show, a significant difference, between the two lots ( $P < 0,05$ ).

Within the control lot, the average value M increased, after the treatment by 10% and after 1 month of treatment, it was increased by 1%, returning almost entirely to the initial values. Respectively, in the research lot, which was treated with medical ozone, the average M values increased after the

treatment by 15% and continued to grow, in 1 month after the treatment, up to 23 %; and in 3 months, the values were still increased by 14%, returning within 6 months closely to initial values (fig. 4).

Statistically, the analysis of the microcirculation effectiveness index (MEI) before the treatment did not show a significant difference between the two lots ( $P > 0,05$ ).

Within the control lot, the MEI values decreased, after the treatment, by 21% and after 1 month from the treatment, the values were decreased by 1%, returning close to initial values. Within the research lot, treated with the medical ozone, the MEI values increased, after the treatment by 27%, and were increased after 1 month after the treatment by 19%, after 3 months by 11%, returning within 6 months closely to initial values (fig. 5).

The spectral dissolution of the LDF - grams allowed the analysis of the flowmotion components of the blood flow. The factors, which are determining the passive mechanism for the modulation of the blood flow within the microcirculation system and specifically the increased maximum amplitude of the high frequency oscillations of the blood flow (AHF), and the decreased maximum amplitude of the pulse oscillations of the blood flow (ACF), lead to the decrease of the MEI average value.

The MEI decrease within the control lot, characterises the development of the vein congestion in the microcirculatory bed of the periodontal tissue due to the minimum traumatic exposure of the periodontium after the classical non-surgical treatment and due to blood flow increase M as well as difficulty of the blood reflux, show the existence of severe hemodynamic disorders at the microcirculation level, within the periodontium in patients with severe CMP. After one month from the treatment, the gradual decrease of the blood flow level led to the return to initial M values and MEI within the control lot.

On the other hand, the research lot treated with medical ozone demonstrated a continuing increase of the average M and MEI values. The MEI values that were obtained immediately after the treatment ( $1.42 \pm 0.02$ ) corresponded to the normal values of the healthy intact periodontium [34]. These values gradually decreased, returning within 6 months after the treatment up to the initial values. This result shows the temporary recovery of the microhemodynamics at the periodontal tissue level.

### Conclusions

1. The obtained result led to the rapid reduction of the inflammatory phenomena within the periodontal tissue, ensuring a considerable decrease of bacterial contamination within the periodontal pockets and a stimulation of the recovery processes, which led to the increase of the maintenance period of periodontal health condition, compared with the classical treatment.

2. The comparative analysis of the results showed a reduction of the total quantity of germs and demonstrated the effectiveness of the complementary treatment with ozone therapy applied systemically and topically compared to the classical non-surgical periodontal treatment. Nevertheless, the achieved improvement was followed by a recolonization of the periodontal pathogens bacteria, favoured by the periodontal pockets depth, which in 3 months, came up to the initial values. As a result, the periodontal pockets depth, wider than 6 mm, represents an important limitation factor impairing their adequate cleaning of the root surfaces and thus the elimination of this factor may require surgical management.

3. The increased serum level of the superoxide dismutase showed that the excessive production of the reactive oxygen, involved in the development of certain conditions and degenerative diseases, is associated with severe marginal chronic periodontitis. The dynamic analysis of the serum level of the superoxide dismutase marker showed that the systemic administration of medical ozone represents a key element of

the antioxidant therapy and demonstrated an extraordinary effectiveness of the medical ozone. The modulation of the serum level of the superoxide dismutase reduced the oxidative stress, involved in the pathogenesis of severe marginal chronic periodontitis and led to the balancing of the redox homeostasis.

4. The analysis of LDF-grams has pointed to the existence of the severe hemodynamic disorders at the microcirculation level of the periodontal tissue, within patients with severe marginal chronic periodontitis. The systemic and topic application of the medical ozone led to an increase of the average values of the microcirculation level (M) and of the microcirculation effectiveness index (MEI), which characterised the microhemodynamics recovery at the periodontal tissue level. These values have gradually decreased, returning at 6 months after the treatment, almost to the initial values.

5. The obtained therapeutic result has proved the effectiveness of complementing the classical non-surgical periodontal treatment in patients with severe marginal chronic periodontitis with medical ozone applied systemically and topically, in comparison with the classical treatment.

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