Abstract

CLINICAL AND PARACLINICAL RESEARCHES REGARDING THE POSSIBILITIES OF SOME METHODS AND TECHNIQUES TO STIMULATE PERIODONTAL TISSUES

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2011
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INTRODUCTION

In Europe there are a few comprehensive and reliable studies related to national data on prevalence, extent and severity of periodontal diseases. The unfinished discussion and controversies regarding a common definition and common systems and indices to avoid different interpretations of clinical and epidemiological data and to allow an objective assessment of periodontal treatment needs (237).

The longitudinal studies show that progression rates for most periodontal pockets is reduced if a good hygiene is maintained by patients. However the initiation and progression of periodontal disease is widespread in adult population. The complete knowledge regarding these periodontal processes require a lot of clinical and paraclinical studies.

The host response to proper treatments depend by acute and chronic inflammation processes and by immune reactions. The new diagnostic tests based on biochemical and immunological biomarkers could offer new information regarding the activity status, the extension and severity of periodontal destruction and the potential response to treatment. The advantage of new diagnostic methods consists in non-invasive techniques or minimal invasive techniques used to collect samples of saliva or crevicular gingival fluid. These paraclinical tests can preview the success or potential for failure as well as diagnosis of periodontal lesion in incipient state.

Despite of proper technique and method, the classic ethiological therapy can fail in some cases. For these situation the additional method with antibacterial, antiinflammatory and biostimulatory effect are recommended to sustain and to improve the success rate of scaling or scaling associated with root planning.
V.1. The reason for theme choice

The reason for theme choice was determined by the request to highlight clinical and paraclinical aspects (biochemical tests, immunological tests) regarding efficiency of periodontal therapies based on the association between conventional ethiological therapy and therapeutical adjuvant procedures. The support offered by Disciplines Periodontology, Biochemistry and Immunology allowed correlation of clinical and paraclinical results.

V.2. Aim, objectives, methodology.

The objectives of my researches were as follows:
- Selection of literature data regarding etiopathogenic, clinical, paraclinical aspects linked by diverse categories of periodontal diseases, biochemical and immunological biomarkers and diverse categories of ethiological and additional periodontal therapies;
- Performing an epidemiological study based on clinical and radiographic data regarding prevalence, extent and severity of periodontal diseases in adult population as well as correlation between some parameters and periodontal disease;
- Monitorisation of clinical, biochemical and immunological parameters changes along conventional periodontal therapy (scaling; scaling and root planning);
- Assessment of the influence of additional therapies in the acceleration of periodontal healing/repair processes.
To realise the proposed aims the working protocol was as follows:
- clinical and radiographic assessment of a study group with age 15-65;
- performing of data base for statistical processing;
- measurement of clinical parameters (GI, PBI, PD, CAL) and paraclinical parameters (MMP8, IIβ1) at baseline;
- standardised therapeutical protocols;
- measurement of clinical parameters (GI, PBI, PD, CAL) and paraclinical parameters (MMP8, IIβ1) at 30 days posttreatment;
- Comparison and interpretation of results regarding the classical periodontal therapy (scaling, scaling/root planning) and additional therapeutical procedures (local antibiotherapy, laserotherapy, osonetherapy, local infiltration Gerovital H3).

Database for epidemiological study was collected between 2008-2010 in private practice and Faculty of Dental Medicine, UMF “Gr.T.Popă” Iasi. Database for researches linked by additional therapeutical procedures was collected between august-december 2010 in private practice. The biochemical and immunological studies were performed in collaboration with Disciplines of Biochemistry and Immunology, U.M.F.”Gr.T.Popă” Iasi.

The exclusion criteria of subjects were as follows:
- Moderate or severe chronic periodontitis;
- Minimum 2 periodontal pockets with vertical alveolisis and depth 4-6 mm;
- The absence of periodontal treatment in last 12 months;
- The absence of systemic antibiotherapy in last 6 months;
- The absence of systemic diseases which could influence periodontal status (diabetis, phosphat-
calcium metabolic disorders, liver disorders, immunological disorders);

- The absence of medication which could influence periodontal status (calcium antagonists, ciclosporine, phenitoine).

The inclusion criteria were as follows:
- Active periodontal pockets (indices PBI 3-4; GI 2-3);
- Periodontal pockets with depth 4-6 mm.

The patients were informed and gave their written consent to be included in study groups.

Every patient were submitted to clinical examen and radiographic examen. The paraclinical tests consisted in:
- measurement of MMP8 levels in GCF (baseline, 30 days posttreatment);
- measurement of IL-ß1 levels in GCF (baseline, 30 days posttreatment);

The clinical parameters were recorded also at baseline and 30 days posttreatment:
- (PBI);
- (GI);
- (CAL);
- (PD).

For epidemiological study the radiographic examen was used to diagnose periodontal status and to classify the alveolisis degree accordingly to Rateitschak (incipient, moderate, severe bone resorption).

The clinical examens included the measurements of periodontal pocket depth (PD), attachment loss (CAL). The assessments were repeated three times and mean value was recorded. The level of CAL was recorded as PD + RG (gingival recession).
The determination of MMP8 levels between baseline and final stage (30 days posttreatment) was performed to determine ability of additional therapeutical procedures to reduce levels of collagenases and to accelerate the initiation of periodontal healing processes. The determination of IL-β1 levels between baseline and final stage (30 days posttreatment) was performed to determine ability of additional therapeutical procedures to reduce the intensity of inflammatory and immunological reactions and to accelerate the initiation of periodontal healing processes.

The crevicular gingival fluid (GCF) was collected using paper points applied at 2-3 mm inside periodontal pocket, for 30 seconds. A proper isolation was performed to avoid contamination of paper point with saliva or blood. However when contamination was present the GCF collecting was repeated. The paper points were immersed in Eppendorf tubes containing a solution with pH 7,4. The Eppendorf tubes were introduced in refrigerator with -20 grd. Celsius until the laboratory tests were performed.

For determination of interleukine 1β levels, was performed ELISA method („Enzime Linked Immunosorbent Assey”). The MMP8 levels were determined using kit Quantikinine (Human MMP-8 Immunoassay, R&D System, USA) based on „quantitative sandwich enzyme immunoassay”. All values were corrected for dilution volume and GCF volume and presented as GCF concentration (ng/ml).

The changes of clinical parameters (GI, PBI, PD, CAL) were compared using statistical tests based on linear models with covariance structured matrices. The changes of mean values for paraclinical parameters (MMP8, IL1β) were assessed using statistical linear models.

The descriptive statistical analysis was performed using Microsoft Excel and analitical statistical analysis was performed using SPSS 16.0 (SPSS, Inc., SUA).
The study directions were as follows:
- Epidemiological study based on clinical-radiographic data and statistical tests;
- Clinical, biochemical and immunological study regarding periodontal therapy based on scaling;
- Clinical, biochemical and immunological study regarding periodontal therapy based on scaling and root planning;
- Clinical, biochemical and immunological study regarding periodontal therapy based on scaling associated with local antibiotherapy (metronidazole);
- Clinical, biochemical and immunological study regarding periodontal therapy based on scaling associated with laserotherapy;
- Clinical, biochemical and immunological study regarding periodontal therapy based on scaling associated with osonotherapy;
- Clinical, biochemical and immunological study regarding periodontal therapy based on scaling associated with local infiltration with Gerovital H3.

The epidemiological study was performed on 143 subjects with ages between 15-65 years.

The studies regarding role of classical therapy and additional therapeutical procedures in the initiation of repair/healing processes were performed on 65 subjects divided in study groups as follows:
- control group (5 subjects/10 periodontal pockets)- healthy periodontal status (levels of MMP8 and ILβ1 at baseline);
- Study group S (“scaling”) (10 subjects/ 20 periodontal pockets, chronic periodontitis)- one-appointment scaling;
- Study group SRP (“scaling.root planning”) (10 subjects/ 20 periodontal pockets, chronic periodontitis)-four appointments scaling/root planning;
- Study group S/MZ (“scaling/metronidasole”) (10 subjects/ 20 periodontal pockets, chronic periodontitis)- scaling and four-appointment local applications of metronidasole (Metrogyl Denta);
- Study group S/L (“scaling/laser”) (10 subjects/ 20 periodontal pockets, chronic periodontitis)- scaling and nine-appointments of laserotherapy (680 nm, DMC laser device);
- Study group S/OZ (“scaling/osone”) (10 subjects/ 20 periodontal pockets, chronic periodontitis)- scaling and four-appointments of osonotherapy (OZONYMED);
- Study group S/G (“scaling/Gerovital”) (10 subjects/ 20 periodontal pockets, chronic periodontitis)- scaling and nine-appointments of local infiltrations with Gerovital H3;

V.3. Data collecting and processing

The processing of reseraches data was performed using the following software:

- MS Office (graphs, tables);
- SPSS 16 (statistical tests).

The following statistical tests were used: t Test, test Kolmogorov-Smirnov, test Pearson, test Wilcoxon.

The descriptive statistical analysis was performed using Microsoft Excel and analitical statistical analysis was performed using SPSS 16.0 (SPSS, Inc., SUA).
VI.1. Introduction.

Epidemiology represents a central field of oral health and must be considered a major analysis tool for the planification of programs, therapeutical procedures and the assessment of their efficiency. Any epidemiological study is important in the light of the reduced amount of national data regarding the prevalence and extent of periodontal diseases.

VI.2. Aim.

Our study aimed to assess the prevalence, extent and severity of periodontal disease and to correlate them with specific parameters of study group with age 15-65.

VI.3. Materials and method.

The epidemiological study was performed on 143 patients (58 males, 85 females) with age 15-65 years (fig.1,2). The patients were selected in period march 2009-december 2010. The subjects were divided in the age groups as follows: 15-24 years (n=46); 25-34 years (n=38); 35-44 years (n=27); 45-54 years (n=12); 55-64 years (n=20). The distribution of periodontal lesions was correlated with sex (males, females), age group (15-24; 25-34; 35-44; 45-54; 55-65) and dental group (molar, bicusps, frontal teeth).

The values related to the structure of study group are presented in figures 1-2.
Every patient was examined using anamnesis, clinical examen and radiographic examen.

The clinical examen recorded parameters as follows: attachment loss, periodontal pockets depth (<5mm, >5mm), plaque indices (PI), gingival indices (GI-Silness-Loe), bleeding indices (PBI), CPITN indices.

The radiographic examen recorded the type and degree of alveolisis. The severity of periodontal disease was assessed using Rateitschak classification:

- Incipient periodontitis (alveolisis limited to 1/3 root length);
- Moderate periodontitis (alveolisis limited to 1/3-½ root length);
- Severe periodontitis (alveolisis extended to root apical third).
Accordingly to the clinical and radiographic examen, patients were classified in four categories (Carranza): 1- healthy periodontal status (S); 2- gingivitis (G); 3- chronic peridoontitis (CP), 4-rapid progressive periodontitis (localised, generalised) (PRP).

Data were presented as tables performed in Microsoft Excel and were statistically processed using software STATISTICA SPSS 6.0

**VI.4. Results and discussions.**

In figures 3-4 are presented radiographic aspects regarding alveolisis degree.

**Fig.3.** I.O., age 51. Chronic periodontitis (moderate and severe alveolisis)

![Fig.3. I.O., age 51. Chronic periodontitis (moderate and severe alveolisis)](image)

**Fig.4.** C.M., age 37. Localised severe periodontitis (severe alveolisis 1.3.-1.1.)

![Fig.4. C.M., age 37. Localised severe periodontitis (severe alveolisis 1.3.-1.1.)](image)

Figure 5 presents periodontal status in the entire study group (healthy subjects-S, gingivitis-G, chronic periodontitis-CP, rapid progressive periodontitis-PRP). The percents are as follows: 6% healthy subjects, 30% subjects with gingivitis (no periodontitis), 59.8% subjects with chronic periodontitis, 4.2% subjects with rapid progressive periodontitis subiecți (fig. 5).
Fig. 5. Periodontal status (study group)

The results for the CPITN indices (fig.6) were as follows: CPITN 0 - 6%, CPITN 1 - 8%, CPITN 2 - 22%, CPITN 3 - 47%, CPITN 4 - 17%. The results for the alveolisis degree (fig.7) were as follows: absent - 37%, incipient - 28%, moderate - 24%, severe - 11%.

Fig.6. CPITN indices (study group)

Fig.7. Alveolisis degree (study group)

In figures 8-9 are presented correlations regarding periodontal status, CPITN indices, alveolisis degree related to sex.
The results for periodontal status related to males were as follows: healthy- 5%; gingivitis- 31%; chronic periodontitis- 64%. The results for CPITN indices related to males were as follows: 0- 5%; 1- 10%; 2- 21%; 3- 43%; 4- 21%. The results for alveolisis degree related to males were as follows: absent- 36%; incipient- 26%; moderate- 26%; severe- 12%. The results for periodontal status related to females were as follows: healthy- 8%; gingivitis- 31%; chronic periodontitis- 54%, rapid progressive periodontitis-7%. The results for CPITN indices related to females were as follows: 0- 7%; 1- 7%; 2- 24%; 3- 48%; 4- 14%. The results for alveolisis degree related to females were as follows: absent- 38%; incipient- 29%; moderate- 22%; severe- 11%.

**Fig.8.** Distribution of clinical indices (males)

**Fig.8.a. Periodontal status**

**Fig.8.b. CPITN indices**
Fig. 8.c. Alveolisis degree

Fig. 9. Distribution of clinical indices (females)
Fig. 9.a. Periodontal status

Fig. 9.b. CPITN indices
Fig.9.c. Alveolisis degree

In figures 10.a-c. are presented the results related to the alveolisis distribution on dental groups (molar, bicusps, frontal teeth).

Fig.10.a. Relation alveolisis-molar group

Fig.10.b. Relation alveolisis-bicusp group
In the following graphs is presented the distribution of periodontal status related to age groups. The results are as follows: age group 15-24 is associated with 13% healthy subjects, 83% subjects with gingivitis, 4% subjects with chronic periodontitis; age group 25-34 is associated with 8% healthy subjects, 16% subjects with gingivitis, 8% subjects with rapid progressive periodontitis, 68% subjects with chronic periodontitis; age group 35-44 is associated with 3,7% subjects with rapid progressive periodontitis, 96,3% subjects with chronic periodontitis; age group 45-65 is associated with 100% percent of subjects with chronic periodontitis.

**Fig.11.a.** Periodontal status- age 15-24
In the following tables are presented a series of correlations between periodontal status, CPITN indices and alveolisis degree (tables 1-2).

**Table 1.** Periodontal status –correlation with sex and age

<table>
<thead>
<tr>
<th>Status (diagnostic)</th>
<th>Masculin</th>
<th>Feminin</th>
<th>15-24 ani</th>
<th>25-34 ani</th>
<th>35-44 ani</th>
<th>45-54 ani</th>
<th>55-64 ani</th>
</tr>
</thead>
<tbody>
<tr>
<td>sanatos</td>
<td></td>
<td></td>
<td>Col %</td>
<td>Col %</td>
<td>Col %</td>
<td>Col %</td>
<td>Col %</td>
</tr>
<tr>
<td>gingivita</td>
<td>5.2%</td>
<td>7.1%</td>
<td>13.0%</td>
<td>7.9%</td>
<td>.0%</td>
<td>.0%</td>
<td>.0%</td>
</tr>
<tr>
<td>PLP</td>
<td>31.0%</td>
<td>30.6%</td>
<td>82.6%</td>
<td>15.8%</td>
<td>.0%</td>
<td>.0%</td>
<td>.0%</td>
</tr>
<tr>
<td>PRP</td>
<td>63.8%</td>
<td>55.3%</td>
<td>.0%</td>
<td>68.4%</td>
<td>96.3%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

**Fig.11.b.** Periodontal status- age 25-34

**Fig.11.c.** Periodontal status- age 35-44
Table 2. Alveolisis degree- correlation with sex and age

<table>
<thead>
<tr>
<th>Grad de resorbție osoasă</th>
<th>Sex</th>
<th>15-24 ani</th>
<th>25-34 ani</th>
<th>35-44 ani</th>
<th>45-54 ani</th>
<th>55-64 ani</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Masculin</td>
<td>Column N %</td>
<td>Column N %</td>
<td>Column N %</td>
<td>Column N %</td>
<td>Column N %</td>
</tr>
<tr>
<td></td>
<td>Feminin</td>
<td>Column N %</td>
<td>Column N %</td>
<td>Column N %</td>
<td>Column N %</td>
<td>Column N %</td>
</tr>
<tr>
<td>absenta</td>
<td>36.2%</td>
<td>37.6%</td>
<td>95.7%</td>
<td>23.7%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>redușa</td>
<td>27.6%</td>
<td>28.2%</td>
<td>2.2%</td>
<td>55.3%</td>
<td>59.3%</td>
<td>8.3%</td>
</tr>
<tr>
<td>medie</td>
<td>24.1%</td>
<td>23.5%</td>
<td>0.0%</td>
<td>18.4%</td>
<td>33.3%</td>
<td>75.0%</td>
</tr>
<tr>
<td>severa</td>
<td>12.1%</td>
<td>10.6%</td>
<td>2.2%</td>
<td>2.6%</td>
<td>7.4%</td>
<td>16.7%</td>
</tr>
</tbody>
</table>

Data recorded were processed using statistical tests (Kruskal-Wallis, Kolmogorov-Smirnov, Pearson) to determine the existence of statistical differences between diverse categories of variables and the correlation between these variables and specific parameters of the investigated subjects.

Our study can be considered a cross-sectional study focused on the assessment of prevalence, extent and severity of periodontal diseases in a study group limited in number and specific to a certain region. However, this study can be useful to compare the characteristics of subjects related to sex, age groups, and dental groups. This study is also primary a study that uses descriptive analysis and presentation of recorded data. If repeated at regular time intervals can offer a view for the longitudinal evolution of periodontal disease or results of preventive and therapeutical procedures.
VI.5. CONCLUSIONS

- The study group presents 6% healthy subjects, 30% subjects with gingivitis, 59.8% subjects with chronic periodontitis, 4.2% subjects with rapid progressive periodontitis;
- Gingivitis is associated with age group 15-25;
- Rapid progressive periodontitis is associated with age group 25-35;
- Chronic periodontitis presents a prevalence of 96.3%, and 100% for age groups 35-44 ani, and 45-65;
- The statistical tests show that parameter sex is not correlated with presence of chronic periodontitis;
- The statistical tests show that prevalence of chronic periodontitis is correlated with age group and molar dental group.
CHAPT. VII. CLINICAL AND PARACLINICAL STUDY REGARDING ROLE OF LOCAL ANTIBIOTHERAPY IN STIMULATION OF ANTIBACTERIAL AND ANTIINFLAMMATORY EFFECTS OF CONVENTIONAL PERIODONTAL THERAPY

VII.1. Introduction.

The local antibiotherapy must not replace gold standard therapy (subgingival scaling associated with root planning), but must be considered as additional procedures in situations characterised by deep periodontal pockets or in cases of ethiological therapy failure (105).

VII.2. Aim.

This study aimed to assess role of local antibiotherapy (metronidasole) in the acceleration of healing processes as a result of its antibacterial action on anaerobic pathogens.

VII.3. Materials and method.

The research was performed on a study group of 30 subjects with chronic moderate and severe periodontitis, selected between August 2010 - December 2010. The age of patients ranged between 30-50 years. Subjects were classified in three study groups related to periodontal therapy. The results (clinical, paraclinical) were assessed after 30 days posttreatment:

- Study group S (“scaling”)/MZ (“metronidasol”) (10 subjects/20 periodontal pockets)- subgingival scaling + local antibiotherapy (metronidasole-Metrogyl Denta); protocol: 4 appointments (2 weekly), inside periodontal pockets;
- Study group S (“scaling”) (10 subjects/20 periodontal pockets)- subgingival scaling;
- Study group SRP (“scaling/rootplaning”) (10 subjects/20 periodontal pockets)- subgingival scaling/root planning.
The role of local antibiotherapy in the acceleration of healing periodontal processes was assessed using differences between clinical parameters at baseline and T2 (30 days) (decrease of periodontal pockets depth and loss of periodontal attachment, indices GI and PBI) and the decrease of levels MMP8, IIβ1 in GCF.

**Fig.12. METROGYL DENTA**

VII.4. Results and discussions.

In the following figures are presented clinical and radiographical aspects of some cases selected from study group S/MZ (scaling associated with local aplications of metronidasole)

**Fig.13.a. I.E., age 37,** Parodontita cronică marginală. Aspect radiografic.

**Fig.13.b. I.E., age 37.** Clinical aspect 1.2.(MV).

**Fig.13.c. I.E., age 37.** Clinical aspect 3.2. (MV).
In figures 14-17 are presented mean values in initial stage (T1) and final stage (T2) of the clinical parameters. In initial stage (T1) the mean values of clinical parameters for study group S/MZ (scaling associated with metronidasole applications) were as follows: GI- 2,75; PBI- 3,4; PD- 5,075mm, CAL- 6mm. Clinical parameters for study group SRP (scaling/root planning) were as follows: GI- 2,8; PBI- 3,45; PD- 5,175mm, CAL- 5,975mm. Clinical parameters for study group S were as follows: GI- 2,6; PBI- 3,25; PD- 4,95mm, CAL- 5,75mm. In final stage (T2) the mean values of clinical parameters for study group S/MZ were as follows: GI- 1,1; PBI- 1,4; PD- 4,725mm, CAL- 5,225mm. Clinical parameters for study group SRP were as follows: GI- 0,9; PBI- 1,3; PD- 4,65mm, CAL- 5,45mm. Clinical parameters for study group S were as follows: GI- 1,6; PBI- 2,1; PD- 4,8mm, CAL- 5,6mm.

**Fig.14.** Periodontal pockets depth (pretreatment; posttreatment)
Fig. 15. Attachment loss (pretreatment; posttreatment)

Fig. 16. PBI indices (pretreatment; posttreatment)

Fig. 17. GI indices (pretreatment; posttreatment)

Modifications of biochemical and immunological parameters (MMP8, Ilβ1)

Control group:
MMP8 - V.M. 25 ng/µl (+/-20)
Ilβ1 - V.M. 15 ng/µl (+/-15)

In initial stage (T1) mean values were as follows:
- S/MZ: MMP8 105.5 ng/µl; Ilβ1 264.25 pg/µl;
- SRP: MMP8 107 ng/µl; Ilβ1 267.25 pg/µl;
- S: MMP8 96ng/μl; Ilβ1 260,25pg/μl

In final stage (T2) mean values were as follows:
- S/MZ: MMP8 25,75ng/μl; Ilβ1 40,25pg/μl;
- SRP: MMP8 20,5ng/μl; Ilβ1 32,5pg/μl;
- S: MMP8 41,5ng/μl; Ilβ1 58,75pg/μl

The mean, minimal and maximal values for MMP8 and Ilβ1 (pretreatment, posttreatment) are presented in table 3.

**Table 3.** Paraclinical values for study groups S/MZ, SRP, S (pretreatment, posttreatment):

<table>
<thead>
<tr>
<th></th>
<th>S/MZ</th>
<th>SRP</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP8 (T1)</td>
<td>105,5ng/μl</td>
<td>107ng/μl</td>
<td>96ng/μl</td>
</tr>
<tr>
<td>MMP8 (T2)</td>
<td>25,75ng/μl</td>
<td>20,5ng/μl</td>
<td>41,5ng/μl</td>
</tr>
<tr>
<td>Ilβ1 (T1)</td>
<td>264,25pg/μl</td>
<td>267,25pg/μl</td>
<td>260,25pg/μl</td>
</tr>
<tr>
<td>Ilβ1 (T2)</td>
<td>40,25pg/μl</td>
<td>32,5pg/μl</td>
<td>58,75pg/μl</td>
</tr>
</tbody>
</table>

**Fig.18.** Inactive periodontal pockets (posttreatment)

The statistical analysis proves the existence of statistical significant differences between baseline (T1) and final stage (T2, related to clinical parameters (PD-periodontal pockets depth; CAL-attachment loss; gingival indices-GI; PBI-papilar bleeding indices) and paraclinical indices (MMP8, Ilβ1).
VII.5. CONCLUSIONS

- Local antibiotherapy (metronidazol), used as additional procedure of classical ethiological therapy (subgingival scaling) allows the improvement of clinical parameters (GI, PBI, PD, CAL);
- Paraclinical tests suggest role of metronidazole on the decreasing MMP8 and Ilβ1 levels in GCF;
- The association of conventional periodontal therapy (subgingival scaling) with local antibiotherapy conducts to the increase of antibacterial, antiinflammatory effects and to acceleration of tissue repair processes;
- The clinical and paraclinical results suggest that local antibiotherapy cannot replace gold standard therapy based on subgingival scaling/root planning (SRP); local application of metronidazol are recommended especially in deep periodontal pockets and failures of periodontal therapy.
VIII.1. Introduction.

The report presented by <Sixth European Workshop on Periodontology> (275) shows the heterogeneity of studies focused on laserotherapy in periodontology, responsible for the absence of definitive conclusions regarding the utility of lasers and optimal therapeutic protocols.

VIII.2. Aim. This study aimed to assess the role of laserotherapy in the acceleration of healing processes as a result of its anti-inflammatory and antibacterial action.

VIII.3. Materials and method.

The research was performed on a study group of 30 subjects with chronic moderate and severe periodontitis, selected between August 2010 - December 2010. The age of patients ranged between 30-50 years. Subjects were classified in three study groups related to periodontal therapy. The results (clinical, paraclinical) were assessed after 30 days posttreatment:

- Study group S (“scaling”)/MZ (“metronidazole”) (10 subjects/ 20 periodontal pockets)- subgingival scaling + laserotherapy (laser diode, DMC, 680nm); protocol: 9 appointments (3 weekly);
- Study group S (“scaling”) (10 subjects/ 20 periodontal pockets)- subgingival scaling;
- Study group SRP (“scaling/rootplaning”) (10 subjects/20 periodontal pockets)- subgingival scaling/root planning.
The role of laserotherapy in the acceleration of healing periodontal processes was assessed using differences between clinical parameters at baseline and T2 (30 days) (decrease of periodontal pockets depth and loss of periodontal attachment, indices GI and PBI) and the decrease of levels MMP8, IIβ1 in GCF.

**Fig.19.** Laser diode DMC (880 nm).

**VIII.4. Results and discussions.**
In the following figures are presented clinical and radiographical aspects of some cases selected from study group S/L (scaling associated with laserotherapy).

**Fig.20.** B.G., 36 ani., PMC. Clinical aspect 2.4. (MV). Clinical aspect 4.1. (DV).

**Fig.20.a.** Parameters for laser device
In figures 21-24 are presented mean values in initial stage (T1) and final stage (T2) of the clinical parameters. In initial stage (T1) the mean values of clinical parameters for study group S/L (scaling associated with laserotherapy) were as follows: GI- 2,7; PBI- 3,35; PD- 5,075mm, CAL-6mm. Clinical parameters for study group SRP (scaling/root planning) were as follows: GI- 2,8; PBI- 3,45; PD- 5,175mm, CAL-5,975mm. Clinical parameters for study group S were as follows: GI- 2,6; PBI- 3,25; PD- 4,95mm, CAL-5,75mm. In final stage (T2) the mean values of clinical parameters for study group S/L were as follows: GI- 1,25; PBI- 1,6; PD-4,675mm, CAL-5,65mm. Clinical parameters for study group SRP were as follows: GI- 0,9; PBI- 1,3; PD- 4,65mm, CAL-5,45mm.Clinical parameters for study group S were as follows: GI- 1,6; PBI- 2,1; PD- 4,8mm, CAL-5,6mm.
**Fig. 21.** Periodontal pockets depth (pretreatment; posttreatment)

**Fig. 22.** Attachment loss (pretreatment; posttreatment)

**Fig. 23.** PBI indices (pretreatment; posttreatment)

**Fig. 24.** GI indices (pretreatment; posttreatment)

*Modificări ale parametrilor biochimici (MMP8) şi imunologici (Ilβ1)*

*Lot martor:*
MMP8- V.M. 25 ng/μl (+/-20)
ILβ1- V.M. 15 pg/μl (+/-15)

In stadiul inițial (T1) valorile medii indicate de testele paraclinice au fost următoarele:
- lot S/L: MMP8 100,75ng/μl; ILβ1 261,75pg/μl;
- lot SRP: MMP8 107ng/μl; ILβ1 267,25 pg/μl;
- lot S: MMP8 96ng/μl; ILβ1 260,25pg/μl

In stadiul final (T2) valorile medii indicate de testele paraclinice au fost următoarele:
- lot S/L: MMP8 27,5ng/μl; ILβ1 42,5pg/μl;
- lot SRP: MMP8 20,5ng/μl; ILβ1 32,5pg/μl;
- lot S: MMP8 41,5ng/μl; ILβ1 58,75pg/μl

Valori minime, medii, maxime pentru mediatorii MMP8 și ILβ1 (pretratament, posttratament) pentru cele 3 loturi studiate sunt prezentate în tabel 4.

**Table 4.** Paraclinical values for study groups S/L, SRP, S (pretreatment, posttreatment):

<table>
<thead>
<tr>
<th></th>
<th>S/L</th>
<th>SRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP8 (T1)</td>
<td>100,75ng/μl</td>
<td>107ng/μl</td>
</tr>
<tr>
<td>MMP8 (T2)</td>
<td>27,5ng/μl</td>
<td>20,5ng/μl</td>
</tr>
<tr>
<td>ILβ1 (T1)</td>
<td>261,75pg/μl</td>
<td>267,25 pg/μl</td>
</tr>
<tr>
<td>ILβ1 (T2)</td>
<td>42,5pg/μl</td>
<td>32,5pg/μl</td>
</tr>
</tbody>
</table>

**Fig.25.** Inactive periodontal pockets

*The statistical analysis proves the existence of statistical significant differences between baseline (T1) and*
final stage (T2), related to clinical parameters (PD-periodontal pockets depth; CAL-attachment loss; gingival indices-GI; PBI-papilar bleeding indices) and paraclinical indices (MMP8, Ilβ1).

Literature data related to researches focused on laserotherapy in dentistry show very good results for improving clinical parameters on short term (1-3 months). However laserotherapy does not reduces bacterial recolonisation on long term (3-6 months). A major problem for the introduction of laserotherapy in the current practice is represented by the absence of a large number of longitudinal studies on long-term (6-12 months). The review of American Periodontology Academy showed that only 20 researches from 278 studies were longitudinal (55).

VIII.5. CONCLUSIONS

- Laserotherapy (DMC, 680nm), used as additional procedure of classical ethiological therapy (subgingival scaling) allows the improvement of clinical parameters (GI, PBI, PD, CAL);
- Paraclinical tests suggest role of laserotherapy on the decreasing MMP8 and Ilβ1 levels in GCF;
- The association of conventional periodontal therapy (subgingival scaling) with laserotherapy conducts to the increase of antibacterial, antiinflammatory effects and to acceleration of tissue repair processes;
- The clinical and paraclinical tests suggest that laserotherapy associated with subgingival scaling present inferior results comparing with gold standard therapy based on subgingival scaling/root planning (SRP).
IX.1. Introduction.
The use of osonotherapy could improve the clinical result as osone is used already in systemic therapy because of its antibacterial, antiinflammatory and regenerative effects.

IX.2. Aim. This study aimed to assess role of osonotherapy in the acceleration of healing processes as a result of it’s antiinflammatory and antibacterial action.

IX.3. Materials and method.
The research was performed on a study group of 30 subjects with chronic moderate and severe periodontitis, selected between August 2010- December 2010. The age of patients ranged between 30-50 years. Subjects were classified in three study groups related to periodontal therapy. The results (clinical, paraclinical) were assessed after 30 days posttreatment:

- Study group S (“scaling”)/OZ (“osone”) (10 subjects/ 20 periodontal pockets)- subgingival scaling +laserotherapy (laser diode, DMC, 680nm); protocol: 4 appointments (2 weekly);
- Study group S (“scaling”) (10 subjects/ 20 periodontal pockets)- subgingival scaling;
- Study group SRP (“scaling/rootplaning”) (10 subjects/20 periodontal pockets)- subgingival scaling/root planning.
The role of osonetherapy in the acceleration of healing periodontal processes was assessed using differences between clinical parameters at baseline and T2 (30 days) (decrease of periodontal pockets depth and loss of periodontal attachment, indices GI and PBI) and the decrease of levels MMP8, IIβ1 in GCF.

**Fig.26. OZONYMED**

IX.4. Results and discussions.
In the following figures are presented clinical and radiographical aspects of some cases selected from study group S/MZ (scaling associated with osonetherapy).

**Fig.27.a. T.R., age 50, CP. Clinical aspect**
In figures 28-31 are presented mean values in initial stage (T1) and final stage (T2) of the clinical parameters. In initial stage (T1) the mean values of clinical parameters for study group S/OZ (scaling associated with laserotherapy) were as follows: GI- 2,65; PBI- 3,3; PD- 5,075mm, CAL-5,875mm. Clinical parameters for study group SRP (scaling/root planning) were as follows: GI- 2,8; PBI- 3,45; PD- 5,175mm, CAL-5,975mm. Clinical parameters for study group S were as follows: GI- 2,6; PBI- 3,25; PD- 4,95mm, CAL-5,75mm. In final stage (T2) the mean values of clinical parameters for study group S/OZ were as follows: GI- 1,25; PBI- 1,6; PD-4,675mm, CAL-5,65mm. Clinical parameters for study group SRP were as follows: GI- 0,9; PBI- 1,3; PD- 4,65mm, CAL-5,45mm. Clinical parameters for study group S were as follows: GI- 1,6; PBI- 2,1; PD- 4,8mm, CAL-5,6mm.
**Fig. 28.** Periodontal pockets depth (pretreatment; posttreatment)

**Fig. 29.** Attachment loss (pretreatment; posttreatment)

**Fig. 30.** PBI indices (pretreatment; posttreatment)

**Fig. 31.** GI indices (pretreatment; posttreatment)
Modifications of biochemical and immunological parameters (MMP8, IIβ1)

Control group:
MMP8 - V.M. 25 ng/μl (+/-20)
IIβ1 - V.M. 15 ng/μl (+/-15)

In initial stage (T1) mean values were as follows:
- S/G: MMP8 103.5 ng/μl; IIβ1 264.5 pg/μl;
- SRP: MMP8 107 ng/μl; IIβ1 267.25 pg/μl;
- S: MMP8 96ng/μl; IIβ1 260.25 pg/μl

In final stage (T2) mean values were as follows:
- S/G: MMP8 33.0 ng/μl; IIβ1 50.5 pg/μl;
- SRP: MMP8 20.5 ng/μl; IIβ1 32.5 pg/μl;
- S: MMP8 41.5 ng/μl; IIβ1 58.75 pg/μl

The mean, minimal and maximal values for MMP8 and IIβ1 (pretreatment, posttreatment) are presented in table 5.

Table 5. Paraclinical values for study groups S/OZ, SRP, S (pretreatment, posttreatment):

<table>
<thead>
<tr>
<th></th>
<th>S/OZ</th>
<th>SRP</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP8 (T1)</td>
<td>103.5 ng/μl</td>
<td>107 ng/μl</td>
<td>96 ng/μl</td>
</tr>
<tr>
<td>MMP8 (T2)</td>
<td>33 ng/μl</td>
<td>20.5 ng/μl</td>
<td>41.5 ng/μl</td>
</tr>
<tr>
<td>IIβ1 (T1)</td>
<td>264.5 pg/μl</td>
<td>267.25 pg/μl</td>
<td>260.25 pg/μl</td>
</tr>
<tr>
<td>IIβ1 (T2)</td>
<td>50.5 pg/μl</td>
<td>32.5 pg/μl</td>
<td>58.75 pg/μl</td>
</tr>
</tbody>
</table>

Fig. 32. Inactive periodontal pockets (posttreatment)
The statistical analysis proves the existence of statistical significant differences between baseline (T1) and final stage (T2), related to clinical parameters (PD-periodontal pockets depth; CAL-attachment loss; gingival indices-GI; PBI-papilar bleeding indices) and paraclinical indices (MMP8, Ilβ1).

IX.5. CONCLUSIONS

- Osonotherapy (OZONYMED), used as additional procedure of classical ethiological therapy (subgingival scaling) allows the improvement of clinical parameters (GI, PBI, PD, CAL);
- Paraclinical tests suggest role of osonotherapy on the decreasing MMP8 and Ilβ1 levels in GCF;
- The association of conventional periodontal therapy (subgingival scaling) with osonotherapy conducts to the increase of antibacterial, antiinflammatory effects and to acceleration of tissue repair processes;
- The clinical and paraclinical tests suggest that osonotherapy associated with subgingival scaling present similar results comparing with gold standard therapy based on subgingival scaling/root planning (SRP).
CHAPT. X. CLINICAL, BIOCHEMICAL AND IMMUNOLOGICAL STUDY REGARDING ROLE OF GEROVITAL H3 IN THERAPY OF CHRONIC PERIODONTITIS

X.1. Introduction.

Procaine, principal component of Gerovital H3, was used for first time in periodontal therapy in 1967 in Germany (310). However the lack of interest from most dentists and absence of longitudinal studies stopped the introduction of these additional procedure in current practice.

X.2. Aim. This study aimed to assess role of GerovitalH3 in the acceleration of healing processes as a result of it’s antiinflammatory and antibacterial action.

X.3. Materials and method.

The research was performed on a study group of 30 subjects with chronic moderate and severe periodontitis, selected between august 2010- december 2010. The age of patients ranged between 30-50 years. Subjects were classified in three study groups related to periodontal therapy. The results (clinical, paraclinical) were assessed after 30 days posttreatment:

- Study group S (“scaling”)/G (“gerovital”) (10 subjects/ 20 periodontal pockets)- subgingival scaling +local infiltrations with GerovitalH3; protocol: 9 appointments (3 weekly);
- Study group S (“scaling”) (10 subjects/ 20 periodontal pockets)- subgingival scaling;
- Study group SRP (“scaling/rootplaning”) (10 subjects/20 periodontal pockets)- subgingival scaling/root planning.
X.4. Results and discussions.

In the following figures are presented clinical and radiographical aspects of some cases selected from study group S/MZ (scaling associated with local infiltrations with Gerovital H3).

Fig. 33.a-b. Gerovital H3

Fig. 34.a-b. Clinical aspect T1 (4.6.-DV; 3.6.-MV)
The role of local infiltrations with Gerovital H3 in the acceleration of healing periodontal processes was assessed using differences between clinical parameters at baseline and T2 (30 days) (decrease of periodontal pockets depth and loss of periodontal attachment, indices GI and PBI) and the decrease of levels MMP8, IIβ1 in GCF.

*In figures 35-38 are presented mean values in initial stage (T1) and final stage (T2) of the clinical parameters. In initial stage (T1) the mean values of clinical parameters for study group S/G (scaling associated with GerovitalH3 infiltrations) were as follows: GI 2,6; PBI 3,25; PD 4,975mm, CAL 5,775mm. Clinical parameters for study group SRP (scaling/root planning) were as follows: GI 2,8; PBI 3,45; PD 5,175mm, CAL 5,975mm. Clinical parameters for study group S were as follows: GI 2,6; PBI 3,25; PD 4,95mm, CAL 5,75mm. In final stage (T2) the mean values of clinical parameters for study group S/G were as follows: GI 1,4; PBI 1,75; PD 4,675mm, CAL 5,525mm. Clinical parameters for study group SRP were as follows: GI 0,9; PBI 1,3; PD 4,65mm, CAL 5,45mm. Clinical parameters for study group S were as follows: GI 1,6; PBI 2,1; PD 4,8mm, CAL 5,6mm.*
**Fig. 35.** Periodontal pockets depth (pretreatment; posttreatment)

![Periodontal pockets depth graph](image)

**Fig. 36.** Attachment loss (pretreatment; posttreatment)

![Attachment loss graph](image)

**Fig. 37.** PBI indices (pretreatment; posttreatment)

![PBI indices graph](image)

**Fig. 38.** GI indices (pretreatment; posttreatment)

![GI indices graph](image)
**Modifications of biochemical and immunological parameters (MMP8, IIβ1)**

Control group:

*MMP8* - V.M. 25 ng/μl (+/-20)

*ILβ1* - V.M. 15 ng/μl (+/-15)

**In initial stage (T1) mean values were as follows:**

- **S/G:** MMP8 102,25ng/μl; IIβ1 259,0pg/μl;
- **SRP:** MMP8 107ng/μl; IIβ1 267,25 pg/μl;
- **S:** MMP8 96ng/μl; IIβ1 260,25pg/μl

**In final stage (T2) mean values were as follows:**

- **S/G:** MMP8 36,75ng/μl; IIβ1 52,5pg/μl;
- **SRP:** MMP8 20,5ng/μl; IIβ1 32,5pg/μl;
- **S:** MMP8 41,5ng/μl; IIβ1 58,75pg/μl

The mean, minimal and maximal values for MMP8 and IIβ1 (pretreatment, posttreatment) are presented in table 6.

**Table 6.** Paraclinical values for study group S/G, SRP, S (pretreatment, posttreatment):

<table>
<thead>
<tr>
<th></th>
<th>S/G</th>
<th>SRP</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMP8 (T1)</strong></td>
<td>102,25ng/μl</td>
<td>107ng/μl</td>
<td>96ng/μl</td>
</tr>
<tr>
<td><strong>MMP8 (T2)</strong></td>
<td>36,75ng/μl</td>
<td>20,5ng/μl</td>
<td>41,5ng/μl</td>
</tr>
<tr>
<td><strong>IIβ1 (T1)</strong></td>
<td>259pg/μl</td>
<td>267,25 pg/μl</td>
<td>260,25pg/μl</td>
</tr>
<tr>
<td><strong>IIβ1 (T2)</strong></td>
<td>52,5pg/μl</td>
<td>32,5pg/μl</td>
<td>58,75pg/μl</td>
</tr>
</tbody>
</table>

**Fig.39.** Inactive periodontal pockets (posttreatment)
The statistical analysis proves the existence of statistical significant differences between baseline (T1) and final stage (T2), related to clinical parameters (PD-periodontal pockets depth; CAL-attachment loss; gingival indices-GI; PBI-papilar bleeding indices) and paraclinical indices (MMP8, Ilβ1).

The benefic effects of Gerovital H3 are linked to the ability to stimulate both local circulation and local metabolism, conducting to the acceleration of repair processes inside periodontal tissues.

X.5. CONCLUSIONS

- Gerovital H3, used as additional procedure of classical ethiological therapy (subgingival scaling) allows the improvement of clinical parameters (GI,PBI,PD, CAL);
- Paraclinical tests suggest role of GerovitalH3 on the decreasing MMP8 and Ilβ1 levels in GCF;
- The association of conventional periodontal therapy (subgingival scaling) with GerovitalH3 infiltrations conducts to the increase of antibacterial, antiinflammatory effects and to acceleration of tissue repair processes;
- The clinical and paraclinical tests suggest that local infiltrations with GerovitalH3 associated with subgingival scaling present inferior results comparing with gold standard therapy based on subgingival scaling/root planning (SRP).
FINAL CONCLUSIONS

- The additional periodontal therapy procedures (local antibiotherapy, laserotherapy, osonetherapy, local infiltration with Gerovital) stimulates the acceleration of repair and healing processes related to the decrease of clinical indices of inflammation and the decrease of biochemical and immunological level of GCF mediators (MMP8, IIβ1);
- The association of subgingival scaling with additional procedures can enhance the efficiency of ethiological therapy with clinical efects visible in maximum 30 days;
- The use of local antibiotherapy (metronidazole) associated to scaling presents inferior clinical and paraclinical results comparing with scaling/root planing therapy (SRP);
- The use of laserotherapy associated to scaling presents inferior clinical and paraclinical results comparing with scaling/root planing therapy (SRP);
- The use of osonetherapy associated to scaling presents similar clinical and paraclinical results comparing with scaling/root planing therapy (SRP);
- The use of local infiltration with Gerovital H3 associated to scaling presents, on short term, inferior clinical and paraclinical results comparing with scaling/root planing therapy (SRP);
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