STUDY PROTOCOL

THE IMPACT OF LOCAL VS. SYSTEMIC ADJUVANT ANTIBIOTICS DURING NON-SURGICAL PERIODONTITIS THERAPY ON CLINICAL PARAMETERS, BACTERIAL COUNT AND CYTOKINE LEVELS – A RANDOMIZED CLINICAL TRIAL

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Background and objectives

Periodontitis is a chronic inflammatory disease that leads to progressive destruction of periodontal tissues and tooth-supporting structures, primarily driven by microbial dysbiosis and an altered host immune response (1,2). The excessive accumulation of immunomodulatory cells due to inadequate immune regulation contributes to alveolar bone loss (3). Among key inflammatory mediators, tumor necrosis factor-alpha (TNF-α) plays a crucial role in initiating immune responses against periodontal pathogens and promoting bone resorption (4-6). Similarly, interleukin-17 (IL-17) has been linked to both immune defense and pathological bone loss through neutrophil recruitment and activation of osteoclasts (7,8).

Non-surgical periodontal therapy (NSPT) is the first-line treatment aimed at reducing bacterial burden and controlling inflammation through mechanical debridement (9). Although effective, NSPT alone may not be sufficient for optimal outcomes, leading to the adjunctive use of systemic antibiotics, particularly amoxicillin, and metronidazole, which have demonstrated significant clinical benefits in younger individuals with generalized stage III periodontitis (10-12). The combination of these antibiotics has been associated with favorable shifts in the subgingival microbiome and long-term clinical improvements (13). However, while in vitro studies suggest metronidazole can inhibit TNF-α production, clinical evidence remains inconsistent (14,15). Additionally, local antibiotic delivery has shown mixed results, with some studies reporting clinical benefits while others limit its use to maintenance therapy or persistent deep pockets (16-18).

Given the conflicting evidence, the primary objective of this study is to compare the clinical outcomes of systemic versus locally delivered adjuvant antibiotics during NSPT at baseline and after six months. The secondary objective is to evaluate their effects on total bacterial load and the relative expression levels of key pro-inflammatory mediators such as TNF-α and IL-17.

Material and methods

Study design and participants

This randomized clinical study will include 38 patients diagnosed with periodontitis stage III. The investigation will be conducted at the Department of Periodontology and Oral Medicine, at the School of Dental Medicine, University of Belgrade. The laboratory part of the research will be carried out at the Department of Human Genetics, at the School of Dental Medicine, University of Belgrade. This research initiation is approved by the Ethical Committee of the School of Dental Medicine, University of Belgrade (No 36/6). Patients will be divided into two groups. The test group will consist of 19 patients who will receive a local antibiotic (LA group), while the control group will consist of 19 patients who will be prescribed systemic antibiotic therapy (SA group). The division of patients into groups will be done using randomization envelopes after the diagnostic procedure.

Participants will be eligible for inclusion if they meet the following inclusion criteria:

- Individuals aged 18–40 years
- Willingness to participate in the research, comply with all study protocols, and provide signed informed consent
- Diagnosis of active periodontitis stage III according to the latest classification (1)
- Non-smokers or light smokers (≤10 cigarettes/day)

Exclusion Criteria:

- Presence of systemic diseases affecting periodontal or bone metabolism (e.g., uncontrolled diabetes, cancer, immunodeficiency, metabolic bone diseases)
- Ongoing treatment with immunosuppressive, anti-resorptive, or anti-inflammatory medications
- Known penicillin allergy
- History of periodontal therapy within the last six months
- Use of local or systemic antimicrobials in the past six months
- Use of oral anti-plaque mouthwash within one month before the study
- Alcohol or drug abuse
- Pregnancy or lactation

Data collection and sampling

Upon clinical examination, clinical periodontal parameters such as pocket probing depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), and plaque index (PI) will be recorded for each patient around every tooth. Prior to treatment, subgingival crevicular fluid will be sampled with paper points from periodontal pockets for laboratory analysis.

Sampling will be performed as follows: first, a relatively dry working field will be established using paper water rollers and air jets. Then, three paper points (number 30 – marked in blue) will be applied for 30 seconds in the periodontal pocket area of the selected premolar/molar tooth with a depth of more than 5 mm. The procedure will be repeated immediately in the same region to obtain duplicate samples. After that, the paper points will be placed in Eppendorf tubes and immediately transported to the basic research laboratory, where they will be stored at a temperature of −80 degrees Celsius for subsequent microbiological and molecular analyses.

Non-surgical periodontal therapy and antibiotic administration

After sampling, all patients will undergo a non-surgical phase of periodontal therapy (NSPT) following a Full-Mouth Disinfection protocol (19). Six months after the NSPT, at follow-up, the detection of clinical parameters and the sampling procedure will be repeated.

In the LA group, a combination of piperacillin and tazobactam in gel form (Gelcide ®, Italmed MedTechDental, Florence, Italy) designed for subgingival use will be applied locally. The local antibiotic will be administered into the periodontal pockets using a syringe and a flexible blunt needle, 24 hours after NSPT. The administration will be done by quadrants, following the principles of a dry work field, and after application, the dry field will be maintained for five minutes. After this procedure, the patient will be instructed not to rinse the oral cavity for 15 minutes.

For systemic use in the SA group, patients will receive a combination of Amoxicillin (Amoxicillin ®, 500 mg, three times a day, for seven days) and Metronidazole (Orvagyl ®, 400 mg, three times a day, for seven days) for oral use after NSPT is completed (11).

Laboratory Analyses

Total bacterial count (TBC) and relative expression levels (REL) of proinflammatory cytokines TNF-α and IL-17 will be detected using quantitative real-time polymerase chain reaction (qPCR) in a basic research laboratory at the Department of Human Genetics, at the School of Dental Medicine, University of Belgrade (20, 21).

Laboratory analyses

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Statistical analyses

All statistical analyses will be performed on an intention-to-treat basis, with no expected dropouts during the observational period. Data analysis will be conducted using GraphPad Prism 9.0 (GraphPad, San Diego, CA, USA) and Statistical Package for Social Science (SPSS, version 26.0; SPSS Inc., Chicago, IL, USA). For numerical data, mean values, medians, standard deviations (SD), and ranges will be used for descriptive statistics.

The Kolmogorov–Smirnov normality test will be applied to examine the distribution of outcome variables. Depending on the distribution, paired Student's t-test or Wilcoxon signed-rank test will be used to compare baseline and six-month follow-up measurements between treatment groups. Repeated measures ANOVA with Bonferroni correction or Friedman and Wilcoxon signed-rank test will be used for within-group comparisons. Statistical significance will be set at $p < 0.05$.

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ISO 9001 BUREAU VERITAS Certification

ETHIC COMMITEE

N° 36/6

11-03-2020

On the ground of request of Assist. Prof. Iva Milinkovic, Ethic Committee of the School of Dental Medicine University of Belgrade, on the day of February 13th 2020, hereby signs the following

AGREEMENT

We hereby agree with the methodology of research suggested by Assist. Prof. Iva Milinkovic within the following study

Adjunctive effect of local and systemic antimicrobials in non surgical treatment of periodontitis grade II and III

Translation of the seal:
University of Belgrade
School oh dental medicine
1948, Belgrade

President of the Ethic Committee

Assoc. Prof. dr Natasa Nikolic Jakoba
(signature)

Belgrade, March 10th 2020.