2.01.2022 RESEARCH PROTOCOL

Title of the Study:

Assessment of the Impact of the Probiotic Limosilactobacillus Reuteri Prodentis (DSM 17938 and ATCC PTA 5289) on Clinical and Microbiological Parameters in Orthodontically Treated Patients with Concomitant Gingivitis

1. Current State of Knowledge Insufficient oral hygiene promotes the development of plaque-induced gingivitis. According to current knowledge, the development of gingivitis is associated with nonspecific bacterial plaque, and in the early stage of inflammation a disturbance in the balance between the physiological and pathogenic microbiota of the oral cavity plays a significant role. Untreated gingivitis may lead to the development of periodontitis, a chronic inflammatory disease that is one of the major risk factors for tooth loss and is associated with numerous systemic conditions, such as coronary heart disease, myocardial infarction, ischemic stroke, and diabetes mellitus. Biofilm-induced gingivitis represents a significant public health problem, affecting diverse patient populations. Moreover, its presence may lead to complications in conservative, orthodontic, or prosthetic dental treatment. Treatment with fixed orthodontic appliances promotes dental plaque accumulation and hampers effective oral hygiene procedures, thereby increasing the risk of plaque-induced gingivitis. Gingival inflammation during fixed orthodontic treatment may result in marginal gingival enlargement, loss of connective tissue attachment, and alveolar bone resorption, which may ultimately compromise orthodontic treatment outcomes. Professional periodontal therapy combined with strict adherence to meticulous oral hygiene procedures ensures a reduction in dental plaque and a significant decrease in pathogenic microorganisms responsible for the development of gingival and periodontal diseases. This is particularly important in orthodontic patients. In addition to professional plaque and calculus removal and rigorous hygiene instructions, shortterm use of chlorhexidine mouthrinses is commonly recommended as adjunctive therapy in the treatment of gingivitis. An alternative to antiseptic mouthrinses may be probiotic preparations for local application, which exhibit anti-inflammatory properties and modulate the oral microbiome. One such preparation is GUM PerioBalance, a commercially available natural dietary supplement. Each lozenge contains at least 200 million viable probiotic bacteria Lactobacillus reuteri Prodentis. According to the manufacturer’s information leaflet, the probiotic strains contained in the product enhance natural defense mechanisms, exert anti-inflammatory effects, and inhibit the growth of pathogenic bacteria responsible for gingival diseases. The tablets are gluten-free, suitable for vegetarians, do not cause tooth discoloration, and have a fresh mint flavor. The planned study aims to determine whether the use of probiotic preparations may represent a valuable adjunct to standard gingivitis therapy and whether probiotic supplementation during orthodontic treatment may be beneficial for maintaining gingival health.

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References Laleman I et al. (2015). Probiotics in the dental practice: a review. Quintessence International, 46, 255–264. Teughels W et al. (2008). Probiotics and oral healthcare. Periodontology 2000, 48, 111–147. Alp S et al. (2018). Effects of probiotics on salivary Streptococcus mutans and Lactobacillus levels in orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopedics, 154, 517–523. Lucchese A et al. (2018). Changes in oral microbiota due to orthodontic appliances: a systematic review. Journal of Oral Microbiology, 10, 1476645.

2. Aim of the Study

The aim of this study is to evaluate the effect of a 12-week administration of a probiotic dietary supplement containing Lactobacillus reuteri Prodentis on: clinical periodontal parameters: Full-Mouth Plaque Index (FMPI), Bleeding on Probing (BOP), Probing Depth (PD), microbiological parameters of the dental biofilm: Fusobacterium nucleatum, Prevotella intermedia, Capnocytophaga gingivalis, Eubacterium nodatum, in adult patients undergoing fixed orthodontic treatment with concomitant gingivitis.

3. Methodology Study Design

A randomized interventional study with a control group. Study Population A total of 100 adult patients aged 18–59 years undergoing fixed orthodontic treatment and presenting with clinical signs of gingivitis (BOP >30%, PD ≤4 mm) will be enrolled. Patients will be recruited from individuals routinely treated at the Department of Dental Hygiene, Medical University of Warsaw, within the framework of healthcare services reimbursed by the National Health Fund (NFZ). Clinical Procedures At the baseline visit (T1), a comprehensive periodontal examination will be performed using a graduated periodontal probe (1-mm increments), including: FMPI – dichotomous plaque index (presence/absence) assessed on four surfaces per tooth (distal, buccal, mesial, lingual), BOP – dichotomous bleeding index (presence/absence) assessed at six sites per tooth (distobuccal, mid-buccal, mesiobuccal, mesiolingual, midlingual, distolingual), PD – probing depth measured at the same six sites per tooth. All patients will undergo ultrasonic scaling and will receive standardized oral hygiene instructions. Subsequently, participants will be randomly assigned to one of two groups: Test group (A) – 50 patients instructed to use probiotic lozenges containing

Lactobacillus reuteri Prodentis for 12 weeks, Control group (B) – 50 patients not receiving probiotic supplementation.

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Microbiological Assessment In the first 9 patients from the test group (A1) and 9 patients from the control group (B1), microbiological analysis will be performed. Prior to the periodontal examination and scaling, pooled dental biofilm samples will be collected from the mesial and distal approximal surfaces of teeth 15 and 35 using a sterile paper point inserted into the gingival sulcus for approximately 10 seconds with sterile tweezers. The collected samples will be transferred to transport tubes and sent to an analytical laboratory. Microbiological analysis will be performed using Real-Time PCR, ensuring high sensitivity and specificity, enabling accurate qualitative and quantitative determination of the selected bacterial species. Follow-up Visit After 12 weeks (T2), clinical and microbiological examinations will be repeated in the respective groups.

4. Eligibility Criteria

Inclusion Criteria:

- generally healthy patient

-age 18–59 years

-treatment with fixed orthodontic appliances

- plaque-induced gingivitis (BOP >10%, PD ≤4 mm).

Exclusion Criteria:

- hypersensitivity to any component of the probiotic preparation

-smoking

- use of probiotic supplements within the previous 30 days

-antibiotic or other antimicrobial therapy within the previous month

-use of chlorhexidine-containing mouthrinses within the previous 2 weeks

- age <18 or >59 years.

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5. Benefits, Risks, and Inconveniences Expected benefits:

Improvement in clinical periodontal parameters, including a reduction in BOP and PD, resolution of clinical signs of gingivitis (erythema, marginal gingival enlargement), and potential favorable changes in the qualitative and quantitative composition of the dental biofilm. Risks and inconveniences: Possible occurrence of hypersensitivity reactions to components of the dietary supplement.

6. Study Duration and Justification December 2021 – December 2025: clinical and microbiological data collection November 2025 – July 2026: data analysis and preparation of scientific publications and conference presentations Justification: The duration of the study is determined by the feasibility of recruiting an adequate number of eligible participants in each group in accordance with the adopted inclusion and exclusion criteria, as well as by the 12-week intervention protocol for each participant.