The influence of the outcome of conservative periodontal therapy after intake of Nutrident Paro Pro during therapy - a placebo-controlled double-blind study

1.1.1 Background

A healthy periodontium is the basis for oral health and also for any further dental treatment. Furthermore, periodontitis can be associated with systemic diseases (Dommisch et al., 2015). Studies have shown that an anti-inflammatory diet led to a reduction of tooth loss by an average of 0.84 teeth (Kotsakis et al.2018). However, a consequent change of diet is guaranteed in only a few periodontitis patients. The supplementation of micronutrients in periodontitis patients represents a promising potential.

1.1.2 Periodontitis

The current classification of periodontitis was redefined at the World Workshop in Chicago 2018. To classify the extent of periodontitis, stages from I to IV, as well as grades A, B and C were defined.

1.1.3 Pathogenesis of Periodontitis

Increased deposition of bacteria on the tooth and root surface leads to an increase in the anaerobic bacteria spectrum. This results in an immune response of the periodontium by increasing the release of cytokines, prostaglandins and matrix metalloproteinases (MMP) (Page et al. 1997). This increases the inflammatory response of the body and leads to a state of chronic inflammation. A chronic or acute inflammation leads to an increase in the non mitochondrial oxygen consumption, resulting in an increase in free radicals, Reactive Oxygen Species (ROS) and other tissue-damaging substances. Free radicals destroy structures of micronutrients and supramolecular structures such as cell membranes. This leads to an increased demand for micronutrients in periodontitis patients (Enwonwu et al. 2007).

1.1.4 Clinical parameters for determining periodontitis

Every patient at the University Clinic of Dentistry, Vienna, Austria, is screened for periodontal disease at the initial admission by means of a periodontal basic examination (PGU) and classified accordingly. A PGU grade 3 or higher is referred to as periodontitis. After this screening, a precise periodontal diagnosis should be made and subsequently a conservative periodontal therapy should be initiated (Bruckmann et al. 2006). To determine the presence of periodontitis, a periodontal status is determined. In the case of periodontal status, the parameters probing depth, recession, BOP (bleeding on probing), plaque, mobility, furcation and sensitivity are assessed.

1.1.5 Clinical parameters to determine the severity of periodontitis

1.1.5.1 Interdental CAL

The CAL (Clinical Attachment Level) results from the probing depth + recession. When assessing the bony attachment loss, the bone loss is measured from the physiological upper margin in relation to the cemento-enamel junction. In periodontitis, stage I is classified with an attachment loss of 1-2 mm, stage II with 3-4 mm, and stages III and IV with 5 mm or more.

1.1.5.2 Radiological bone resorption

The radiological bone loss is the percentage difference between the original physiological state of the maxilla or mandible and the current bone status in the x-ray image. Stage I is classified with a reduction of less than 15%, stage II between 15-33% of the coronal third, stages III and IV with a loss up to the middle or apical third of the root.

1.1.5.3 Tooth loss

The number of teeth lost due to the pathology of periodontitis. Stage I and stage II show no tooth loss. Stage III shows a loss of less than or equal to 4 teeth and stage IV shows a loss of more than 4 teeth.

1.1.5.4 Local factors
The probing depth is the "distance from the sulcus floor to the upper edge of the gingiva." The maximum probing depth for stage I is 3-4 mm, for stage II 4-5 mm and both have horizontal bone resorption. In stage III the probing depth is greater than or equal to 6 mm and there is also vertical bone resorption and furcation involvement in class II or III. Stage IV also shows increased tooth mobility and other aggravating factors.

Current treatment concept and the role of Nutrident Paro Pro

The current treatment of periodontitis is based on removal of the biofilm and hard deposits on the root surfaces. For probing depths greater than 5 mm, additional systemic antibiotics can be added to the treatment.

Due to the multifactorial nature of periodontal diseases, different approaches to fight the inflammation seem to be reasonable. Currently, therapy focuses on restoring the balance of the microbial flora and eliminating the niches for periodontopathogenic germs. The immune response, which in the case of periodontitis is excessive and leads to tissue breakdown, could be influenced by nutrition.
However, no standardized dietary changes or dietary supplements are integrated in current treatments. These could help to minimize the inflammation and positively influence the success of the treatment.

1.3 Nutrident Paro Pro®
The product Nutrident Paro Pro® from Biogena contains certain micro-nutrients which should be taken twice a day. The preparation is taken from the beginning of the basic therapy and ends with the reevaluation date. Enclosed the ingredients are listed in descending order of quantity and checked for scientific coherence.

1.3.1 Cranberry extract (680mg)
The fruit cranberry (Vaccinium macrocarpon), originally from North America, has therapeutic potential in periodontitis. In vitro colonization by Fusobacterium nucleatum and Porphyromonas gingivalis has been shown to be prevented (Yamanaka-Okada et al. 2008; Labrecque et al. 2006). Cranberry preparations have an inhibitory effect on the MMP immune response (Bodet et al. 2007; La et al. 2009). Another property is the restriction of the proteolytic activity of the red complex (Prophyromonas gingivalis, Tannerella forsythia, Treponema denticola) (Mukherjee et al. 2014).
1.3.2 Vitamin C (200mg)
The healing of the connective tissue is influenced by the ability to form collagen synthesis. Proper folding and formation of collagen conformation is enabled by the enzymes prolyl hydroxylase and lysyl hydroxylase. Vitamin C is an important cofactor for the activity of these enzymes. In addition, vitamin C is an important cofactor for the activity of these enzymes and acts ans an antioxidant through neutralizing reactive oxygen species (ROS) which lead to cell destruction in the inflammatory zone (DePhillipo et al., 2018). This effect thus leads to a reduction of oxidative stress. Further studies showed that vitamin C can lead to osteoblast and fibroblast growth and proliferation (Harada et al., 1991).

1.3.4 Vitamin E (40mg)
Vitamin E, also known as a-tocopherol, protects the lipid membrane of cells from lipid peroxidation and is therefore one of the most effective lipophilic antioxidants (Torshabi et al., 2017). Vitamin E improves the level of anti-oxidative enzymes present in the blood and leads to a delay in the oxidative degeneration of lipids (Singh et al., 2014).

1.3.5 Zinc (7.5mg)
In addition to its positive effect on the remineralization of the teeth, zinc also plays an important role for the periodontium. An increased zinc supply leads to a reduction of the collagen fibre destroying matrix metalloproteinase-8 (MMP-8) enzyme. In addition, zinc in the form of zinc superoxide dismutase (SOD) can provide protection against free radicals and thus act as an antioxidant. Zinc can also lead to a reduction in plaque and gingivitis due to its increased presence (Lai et al., 2015).

1.3.6 Coenzyme Q10 (60mg)
Coenzyme Q10 (ubiquinone / ubiquinol) is a cell substance frequently present in the body which guarantees protection against free radicals due to its antioxidative effect. Due to its supporting effect in mitochondrial energy production, coenzyme Q10 is very important for the vitality of the cells. Coenzyme Q10 thus leads to an optimization of the ADP/ATP ratio and improves the mitochondrial membrane. According to studies, its antioxidant effect has a positive accompanying effect on the treatment of periodontitis. Coenzyme Q10 also has a regenerative effect on other antioxidants (Prakash et al., 2010).

1.3.7 Alpha lipoic acid (50mg)
Alpha lipoic acid is an antioxidant, which leads to an improvement of the nerve fibers through the increased release of glutathione.

1.3.8 Grape seed extract (20mg)
Grape seed extract contains oligomeric proanthocyanidins which have antioxidant activity.

1.3.9 Selenium
Selenium is considered to be an antioxidant, which leads to an inhibition of the immune response by inhibiting NF-kB. It can also cause an inhibition or how- deregulation of TNF-alpha levels (Duntas et al. 2009).

Objectives

1.4 Objective of the study
The aim of the study is to compare the product Nutrident Paro Pro® with a placebo preparation. The clinical parameters (BOP and probing depth) will be evaluated after 8-12 weeks. The results are compared with the parallel group.

1.5 Hypothesis
The use of Nutrident Paro Pro® during therapy leads to significantly better clinical results than the placebo preparation. The periodontal clinical criteria probing depth and BOP improve significantly.

2. Study design

At the initial clinical admission, patients undergo a basic periodontal examination (PGU) and, if a PGU grade greater than or equal to 3 is present, are referred to the Department of Conservative Dentistry and Periodontology. After the clinical diagnosis of stage III or IV periodontitis, the patients can be included in the study, after information and written consent. They will then be treated according to the Viennese Periodontal Treatment Concept. This includes, after establishing a periodontal status (= starting point for taking the preparation), supra- and subgingival cleaning of all tooth and root surfaces using sonic scalers and hand instruments under local anesthesia. At the same time, patients take either the placebo preparations or Nutrident Paro Pro® randomly for a period of 8-12 weeks. After a period of 8-12 weeks the re-evaluation takes place, in which the clinical parameters probing depth and BOP are determined. The end point of the study is the collection of the BOP parameters and the probing depth after the end of the conservative treatment of periodontitis. The lower the percentage of BOP and the lower the probing depth, the more successful the conservative periodontitis therapy was.

2.1 Therapies

2.1.1 Nutrident Paro Pro®
Nutrident Paro Pro® is given to patients in standard containers for use twice daily for 8-12 weeks.
RECOMMENDATION: Take 2 x 1 capsules daily with plenty of liquid

2.1.3 Placebo preparation

The placebo preparation, which is also provided by Biogena, contains methyl cellulose (a non-resorbable, indigestible dietary fiber) and should also be taken twice daily for 8-12 weeks. The verum does not differ externally from the packaging or the capsules.

2.2 Randomization

Randomization is carried out before the start of the study by an independent researcher (Univ.-Ass. Dr. Selma Husejnagic, MSc). A simple randomization will be carried out using a computer program (Rand function, Excel 2016 for Mac, Microsoft, Redmond, VA, USA). This code will be applied to the bottom of the two preparations by the manufacturing company. The treating physicians and patients are not given any decoding for the attached codes, thus double-blinding is guaranteed. The information about verum and placebo with the assignment to the participant is only available to the independent assistant (Univ.-Ass. Dr. Selma Husejnagic, MSc).

2.3 Admission criteria

- Periodontitis at least stage 3 - at least 18 years old

2.4 Exclusion criteria

- Pregnancy

2.5 Parameters collected during the re-evaluation

At the re-evaluation appointment, which is usually 8-12 weeks after the end of the last cleaning session, the probing depths (in mm) are measured, the mean value is calculated and then compared with the initial mean values of the probing depths. According to the Viennese periodontal treatment concept, the probing depths are recorded once a year after re-evaluation. Data from this annual and routine, i.e. not study related, so-called "recall with status" session are additionally used in the evaluation for the long-term success of the therapy. The probing depth, CAL, BOP and other clinical parameters such as mobility and furcation infestation are recorded.

2.6 Overview of the collected parameters

Probing depth (mm) X Bleeding on Probing (no/yes) X
Secondary target parameters
Recession (mm) X Mobility (grade 0-3) X Furcation participation (grade 0-3) X

Proximal space Plaque index (Lange et al., 1974)

Modified papilla bleeding index (0/1) (Saxer et al., 1975)

Risk & Benefit

Patients are only included in the study with their consent and the relevant admission criteria. The study participants do not notice any relevant increase in the time required for the course and duration of treatment. For study participants of the verum group, an existing deficiency of micronutrients can be eliminated by taking the preparations. In the unlikely event of any side effects, patients are advised to stop taking the preparation immediately. The unblinding can be carried out at any time on request from the staff member who is independent of the study (Univ.-Ass. Dr. Selma Husejnagic, MSc).

4. Data analysis

4.1. Power analysis

This randomized, placebo-controlled study is based on sample size estimation as we are not aware of any study using a multi-substance preparation such as Nutrident Paro Pro® with PPD as the primary target. Assuming a fairly normal distribution of the samples (independent T-test), the

calculations showed that at least 17 patients per group would achieve a power of 80% and a significance level of α=0.05 to detect a clinically significant difference in PPD of assumed 0.8 mm with a standard deviation of 0.8 mm between verum and placebo. To compensate for the possible loss of recruited patients during follow-up, we will increase the number of participants to a total of 42 patients with 21 patients in each group.

Null hypotheses and alternative hypotheses to the primary endpoint:
H0: Mean reduction of PPD in the verum group
= Mean reduction of PPD in the placebo group
H1: Mean reduction of the PPD of the Verum group
> Mean reduction of PPD in the placebo group

4.2 Statistical analysis
Descriptive statistics are used to represent the characteristics of the patients. The distribution of the data is assessed by visual inspection of the histograms and the Kolmogorov-Smirnov test. Normally distributed continuous data are presented as mean value with standard deviation, otherwise as median and range. Categorical variables are described as Proportions and Number.

To determine differences between treatment groups (Nutrident Paro Pro® and placebo), changes in PPD from baseline to after the 8-12-week intervention are analyzed in the re-evaluation/recall study (primary endpoint) using ANCOVA, with the treatment group as fixed effect and baseline PPD as covariant. The estimated mean difference is reported with 95% confidence interval.
Secondary exploratory endpoints are summarized and investigated using descriptive and inferential statistical (uni- and multivariate) methods. Parameters collected before and after the intervention (both 8-12 weeks after the end of the last cleaning session and at the time of the "recall with status" session) are used. Comparisons between patients randomly assigned to the Nutrident Paro Pro® or placebo group are made using independent T-tests (parametric) and Mann-Whitney U-tests (non-parametric) for continuous variables (Proximal Space Plaque Index (API), Papillary Bleeding Index (PBI); recession in mm) or Chi-square and accurate Fisher tests for categorical variables (bleeding on probing (BOP), furcation involvement: I, II, III; mobility: grade 1, 2, 3) examined. The differences between pre- and post-intervention of secondary outcomes are also investigated using paired T-tests (parametric) and Wilcoxon signed rank tests (non-parametric) for continuous variables (API; PBI; recession in mm) or McNemar tests for categorical variables (BOP; furcation involvement; mobility).

Statistical significance is indicated at a p-value of <0.05 (two-sided). All data are analyzed with the SPSS software version 21 or higher (PAWS Statistics 21; SPSS Inc, Chicago, IL) and analyzed with the statistics program R version 2.10.1.

The following data sets will be used for the analysis:

Safety set: All participants who were randomized and participated in the study for at least one month. The participants will be analysed according to the treatment they actually received.

Intention to treat (ITT) set: All participants who were randomized and who started the intervention regardless of protocol deviations. Participants are analyzed according to the treatment they were randomly assigned to.
As per protocol (APP) record: All participants who were randomized and who were treated without protocol deviations. The participants are analyzed as stated in the protocol. The effectiveness is analyzed based on the APP record.
Full analysis set (FAS): All participants who were randomized after APP and who completed the study (intake: 98%). Participants are analysed according to the verum group. FAS is used as the primary analysis set.

4.3 Data protection

The patients are given a code. The code for encryption is strictly separated from the encrypted data sets and is only kept at your trial centre.
Access to the non-encrypted data is available to the investigator and other study site personnel involved in the study or your medical care. The data is protected against unauthorized access. In

addition, authorized representatives of the sponsor of the Medical University of Vienna and representatives of domestic and/or foreign health authorities as well as the respective competent ethics commissions may inspect the non-encrypted data to the extent that this is necessary or required for the verification of the proper conduct of the clinical trial.
The data will only be forwarded in encrypted or anonymized form. Also for any publications only the encrypted or anonymized data will be used.
All persons who are given access to the encrypted and non-encrypted data are subject to the Basic Data Protection Regulation (DSGVO) and the Austrian adaptation regulations in the currently valid version when handling the data.
In the context of this study, no transfer of data to countries outside the EU is planned.

5. Ethical concerns

The therapeutic effects of various micronutrients have already been tested by their scientists. The nutrients contained in Nutrident Paro Pro® have no medically questionable levels, so that no health-damaging conditions are to be expected.

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