Effects of Antimicrobial Peptides Application after Non-surgical Periodontal Therapy on Treatment of Stage III and Grade B Periodontitis

The Medical Ethics Committee of Beijing Stomatological Hospital Capital Medical University approved the clinical study protocol (CMUSH-IRB-KJ-PJ-2018-03)

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Part one:

The research purpose and project basis

1.Project basis

Chronic periodontitis is an infectious disease of periodontal support tissues caused by bacterial biofilm, which leads to inflammation and destruction of periodontal support tissues ultimately resulting in tooth loss. In 2018, EEP and AAP sorted out periodontitis into four classifications (Stage I to IV) based on several variables including clinical attachment loss, amount and percentage of bone loss, probing depth, presence and extent of angular bony defects and furcation involvement, tooth mobility, and tooth loss due to periodontitis, and three levels (grade A: low risk, grade B: moderate risk, grade C: high risk of progression) according to the rate of disease progression. In the clinic, patients with Stage III and Grade B periodontitis are difficult to gain desired outcomes on account of deep periodontal pockets, complicated anatomy, the removal of subgingival dental biofilms, and control of residual inflammation. A large number of studies have indicated that the effectiveness of local application of antibiotics as an adjunct to scaling and root planning (SRP), such as the antimicrobial and minocycline hydrochloride could affect bacterial metabolism and inhibit biofilm attachment particularly in terms of pocket depth reduction and attachment level gain. However, the use of wide-spectrum antibiotics may cause some inevitable side effects including drug resistance, pathogens and probiotics were eliminated leading to diversity of microbiota diminished, and toothstaining. To solve the problems of antibiotics in the clinic, antimicrobial peptides (AMPs) may be considered as an alternative to conventional antibiotics drugs. Many studies reported that AMPs have distinct advantages in specific selectivity for bacterial targets and minimal emergence of microbial resistance in comparison with antimicrobial agents. Gingival epithelial as a biological barrier has the characteristic of specificity in the natural immune system and could produce AMPs including the expression of β-defensins, hBD-1, hBD-2and hBD3[11], and a large number of α-defense and LL-37 exist in the neutrophils of gingival sulcus, which play an

important role in protecting gingival epithelial. However, the efficacy of antimicrobial peptide in the treatment of Stage III and Grade B periodontitis lack of more clinical studies.

2.Purpose of research

In this randomized clinical trial, we aimed to evaluate the effects of AMPs as an addition to SRP on clinical parameters and microbiological biofilms in patients with Stage III and Grade B periodontitis.

Part two:

Clinical trial protocol

1. Objects and source

Subjects were recruited from patients with Stage III and Grade B periodontitis from the periodontal department of Beijing Stomatological Hospital from Sep. 2022 to Oct. 2022.

2. Estimate of sample size

Multiple test indexes were involved in this experiment, and the main test index was the decrease value of the average probing depth, which was intended to be referred to. Existing literature studies have shown that the local application of minocycline hydrochloride ointment can reduce the average periodontal pocket depth by 1.5mm, and it is expected that biological antibacterial peptides can reduce the average periodontal pocket depth by about 2mm.Set α=0.05, test efficiency, that is, 1-β was 90%, and the ratio of two samples was 1:1. According to the statistical formula of sample size estimation of two independent samples: $n1=n2=2\left[\frac{(t\alpha + t\beta)s}{\delta}\right]^2$, the sample size of each group could be calculated as 14 cases. Assuming that the shedding rate was 20%, the required sample size of each group was 17 cases, and the total sample number was 51 cases.

3.Measures

Periodontal probing depth (PD) mm: from the gingival margin to the bottom of the periodontal pocket.

Attachment level (AL)（mm）: from the cementoenamel junction, crown margin or restoration to the bottom of the pocket.

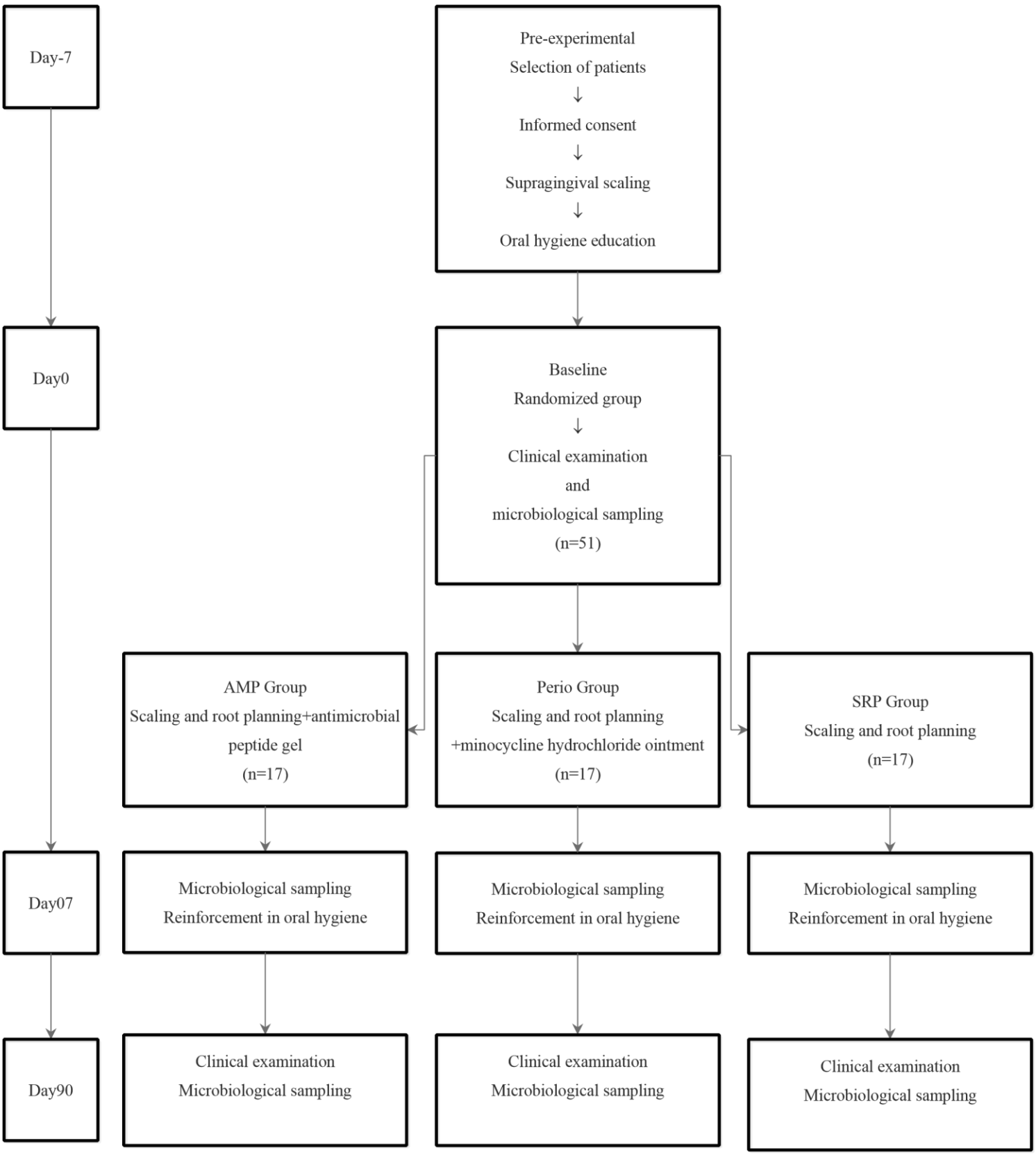
Bleeding index (BI): the rating method was assessed using the criteria of Mazza (Mazza, Newman, & Sims, 1981).

Microbiological sampling:(1) Sample site selecting: Four samples of subgingival plaque were collected separately from each subject: from the sites which following conditions: 5 mm≦ PD≦ 10 mm, PI < 1, Mov≦II°, samples were taken at the same sites at 7 and 90 days. (2) Plaque sampling: Dried samples teeth, remove the supragingival plaque with a probe, gently put the sterile Mini Grace scaler into the periodontal pocket, and take the subgingival plaque. The plaque sample was placed in a 1.5ml sterile centrifuge tube containing 1ml TE buffer (10mM Tris-HCl, 1mMEDTA; pH 8). (3) Samples storing: Specimens were collected and placed on ice immediately after sampling, transported to the laboratory within 15min and stored at −80°C.

Microbial DNA sequencing

Samples were conveyed on dry ice to the laboratory at Beijing Allwegene Tech, Ltd (Beijing, China) within 5 h. The microbial genomic DNA was isolated, amplified by PCR, and then the V3–V4 hypervariable regions of the 16S rRNA gene[18] were subjected to high-throughput sequencing using the Illumina Miseq PE300 sequencing platform (Illumina, Inc. CA, USA).

4.Clinical trial procedure



Part three:

Research object rights and adverse event treatment

1.Research object rights and adverse event treatment object rights:

a. All examinations and sample collection in this experiment were conducted by medical standard, and there was no trauma to the oral tissues and the whole body of the subjects.

b. All operations in this study were routine operations for the treatment of stage III grade B periodontitis, and there was no experimental treatment.

c. Minocycline hydrochloride and antibacterial polypeptide gel used in this research institute have been widely used in the treatment of periodontitis in our hospital to ensure their safety, and the costs of the two preparations are borne by the subject.

d. The subject's participation in the study is voluntary, and the subject may refuse to participate in the study or withdraw from the study at any time without any penalty or loss of his or her due interest.

e. To the extent permitted by applicable laws and/or regulations, records relating to the identification of subjects shall be kept confidential and shall not be made public. In the event of public publication of test results, the identity of subjects shall remain confidential

f. Since this study is also a regular procedure for the treatment of Stage III Grade B periodontitis, treatment for this disease can continue if any subjects withdraw from the study.

g. If there is any injury related to the test, the subject will be treated by a professional doctor and other alternative treatment options will be selected. The following are the possible injury events and treatment methods of the test.

h. Subject or his/her legal agent will be notified in a timely manner if information becomes available that may affect the subject's continued participation in the study.

adverse event treatment:

This treatment belongs to the routine treatment of stage III Grade B periodontitis. Possible adverse reactions during and after treatment and emergency treatment by doctors are as follows:

a. Anesthesia complications: subgingival curetting is a painless treatment, which requires local injection of anesthesia drugs before treatment. Possible complications of anesthesia include syncope, allergy, poisoning and hematoma. Emergency treatment methods are as follows:

Syncope: Prevention of syncope: Reducing the psychological and physical stress of the patient is essential to prevent syncope. Do a good job of ideological work before treatment, eliminate tension, avoid operating on an empty stomach

Management of syncope: 1) Stop medical procedures and remove foreign bodies in the mouth.2) Accurate monitoring of vital signs, focusing on monitoring patients' pulse (intensity and rhythm) and blood pressure: when the heart rate is lower than 60 beats per minute, the pulse is weak; When blood pressure is below 6.67 kPa (50 mmHg), it is generally difficult to reach a pulse.3) Patients with hypotension should change their body position to supine position and elevate their lower limbs; Loosen collar, oxygen; Ammonia stimulation increases ventilation.4) If symptoms do not improve, intravenous channels should be established quickly and Ringer's solution or normal saline should be injected at 250~500 mL per hour. If necessary, dopamine can be given to improve hypotension and atropine can improve bradycardia.5) Loss of consciousness is easy to lead to post lingual fall. When respiratory tract obstruction occurs, the lingual body and mandible should be pulled forward in time to open the respiratory tract.6) In case of cardiac or respiratory arrest, immediately perform cardiopulmonary resuscitation. Allergy: prevention of allergy: before anesthesia operation, ask patients in detail whether there is a history of local anesthetics allergic reaction, is the key to prevent allergic reaction, to ester local anesthetics allergic reaction and constitution of patients, are converted to amide drugs. If a patient has an adverse reaction to local anesthetics, it is important to consider whether the reaction is an allergic reaction and choose other local anesthetics or terminate the treatment.

Allergy treatment: For mild allergic reactions, desensitization drugs such as calcium, promethazine, cortisone hormone intramuscular injection or intravenous injection and oxygen inhalation can be given; If allergic reaction is suspected, start treatment as soon as possible. The general principles of treatment are as follows.1) Change the body position so that the patient is supine and the lower limbs are elevated.2) Maintain airway patency and oxygen intake. When the patient has difficulty breathing, open the respiratory tract and give oxygen to the mask; If laryngeal edema occurs, endotracheal intubation or tracheotomy should be performed. In case of emergency, cricothyroi membrane puncture can be performed first; Bronchial spasm can be used to relieve spasm.3) Rapid establishment of intravenous channels and correct administration. Poisoning: 1) When central nervous system excitation reaction occurs, all operations should be stopped immediately, oxygen inhalation should be given, venous access should be quickly established, and the patient should be comforted.2) When patients have loss of consciousness, strict airway management should be conducted to keep the airway unobstructed.3) When patients have convulsions, oxygen should be given to prevent respiratory obstruction caused by vomiting; Anticonvulsants such as diazepam may be given intravenously if ventilation is affected.4) When circulatory system failure occurs, lower limbs should be elevated, venous perfusion should be rapid, and vasopressor drugs should be given

Cardiac drugs such as dopamine, epinephrine, and isoproterenol.5) In case of respiratory and cardiac arrest, perform CPR immediately.

Hematoma: If local hematoma appears, immediately stop injection, pressure to stop bleeding, give ice. In order to avoid local infection and hematoma enlargement, antibiotics and hemostatic drugs are given as appropriate. Avoid taking aspirin orally. After 48 hours local hot compress or physiotherapy, promote the absorption and dissipation of hematoma.

b. Common adverse reactions after subgingival curetting include pain and bleeding. The pain and bleeding can be relieved spontaneously within 24H after treatment. If the

pain reaction is large, accompanied by systemic inflammatory reaction, systemic antiinflammatory drugs should be taken. The trial was terminated.

c. If other diseases in need of antibiotics occur during the test, in order to ensure the health of the test subjects, the test subjects can take antibiotics and terminate the test.

d. Allergy to minocycline hydrochloride or antibacterial polypeptide gel used in this study: To ensure the maximum safety of subjects, subjects should be observed in the hospital for half an hour after treatment. In case of suspected allergic symptoms, immediately rinse the periodontal pocket with normal saline until all the preparations are flushed out. In subjects who developed allergies, the study was terminated and mechanical therapy alone was selected to complete the treatment of stage III grade B periodontitis.

Procedures for withdrawing from the study

The trial is terminated when:

a. Subjects found not meeting the inclusion criteria after inclusion
b. In case of serious adverse events or diseases requiring antibiotic administration during the study, the patient may voluntarily terminate the study in order to ensure the health of the study participant
c. Subjects with poor compliance or inability to comply with test requirements
d. It is not appropriate to continue the test for medical reasons or subjects' interests
e. The subject asked to quit
f. The patient is pregnant
g. Patients who were combined with other drugs of the same type or used other treatments that affected the study during the trial
h. Participate in other clinical investigators during the trial
i. The researchers decided that the trial needed to be discontinued

Case definitions for withdrawal from the study:

All patients who fill in the informed consent form and are eligible to enter the trial have the right to withdraw from the clinical trial at any time, no matter when and for any

reason, as long as the subject does not complete the observation period specified in the protocol, referred to as shedding cases.

Treatment of shedding cases: After shedding patients, researchers should try their best to contact the patients, complete the assessment items that can be completed, and fill in the test conclusion form. For shedding due to adverse reactions, those who are determined by follow-up to be related to the treatment used in the study must be recorded in the case report form.

Records of shedding cases: For any shedding case, the investigator must fill in the pathology report with reasons for shedding. Generally, there are 6 reasons for shedding: adverse events, lack of efficacy, loss of follow-up, subject withdrawal, investigator discontinuation, and others.

2.Informed consent process

a. Before agreeing to participate in the study, subjects should carefully read the informed consent, which has been approved by the Ethics Committee.If the informed consent is renewed, the subject or his/her legal representative will receive a new, signed and dated update approved by the Ethics Committee. This consent form is in duplicate, with one copy for the investigator and one copy for the subject.
b. The informed consent process was carried out by Xie Yongmei, the researcher of this study, now working in the General Stomatology Department of Wangfujing Department of Beijing Stomatological Hospital.
c. The explanation process of informed consent shall be narrated by the researcher in plain language that the subjects can understand. Vulnerable groups and groups without full capacity for civil conduct are not recruited in this experiment, including children, pregnant women, people with mental illness and cognitive disabilities, terminally ill patients and prisoners.
d. In the process of informed consent, the researcher should give a detailed and clear explanation of the content that the subject does not understand or any question related to the experiment.
e. Subjects must be given sufficient time to consider whether to participate in the study.

f. Informed consent should be conducted in a quiet and separate environment to avoid pressure on the subject.

g. Neither the investigator nor the relevant personnel of the study can coerce or improperly influence the subjects to make the decision of whether to participate in the study.

h. The informed consent form shall be signed and dated by both the subject and the investigator performing the informed consent process.

i. In the process of informed consent, researchers need to explain the following issues to subjects:

(1) Purpose, duration, methods, costs, rights and inconvenience of subjects, expected benefits and risks of the study.

(2) Inform subjects of the different groups to which they may be assigned and the control group of the potential benefits and risks.

(3) Other current methods of diagnosis and treatment of this disease, and the possible benefits and risks of each method: Minocycline hydrochloride ointment and antibacterial polypeptide gel used in this treatment are sensitized. If the treatment plan of this test cannot be adopted, simple periodontal mechanical therapy can be chosen.

(4) Inform the subject that participation in the study is voluntary, that the subject may refuse to participate or propose the study at any stage of the study without discrimination or retaliation, and that his medical treatment and rights will not be affected.

(5) During the test, subjects will have access to relevant information at any time.

(6) The Ethics Committee and relevant regulatory authorities shall be granted direct access to the subject's original medical records for the purpose of verifying the procedures and/or data of the clinical trial, to the extent permitted by applicable laws and regulations and without violating the subject's privacy.

Part four:

Statistical analysis plan

All clinical indicator data were analyzed using IBM SPSS ver. 22.0, The data of normal distribution are represented by ($\bar{x}\pm s$), while the data of non-normal distribution are represented by $M\ (Q_{25},\ Q_{75})$. For the subjects' gender and age using chi-square analysis. First, the changes of PD, AL, BI at baseline and the three indicators after treatment were statistically analyzed, Secondly, PD reduction of anterior teeth, premolars, and molars after treatment were calculated. Finally, the reduced values of sites with different PD depths after treatment were analyzed. The above data satisfying the normal distribution were analyzed by ANOVA, and LSD was selected for comparison between two groups. Kruskal -- Wallis H test was adopted for the comparison between groups with nonnormal distribution. Tests with $p < 0.05$ were considered significant.

Microbial sequencing data were processed using QIIME[19] and MOTHUR[20] by Allwegene Tech, Ltd. (Beijing, China). To retain only high-quality sequences for the downstream analysis, sequences that were less than a 100bpin length after quality trimming, contained one or more ambiguous base-calls (N), or had < 90% quality scores > Q20 were eliminated. After trimming, high-quality sequences were aligned to the Ribosomal Database Project and were clustered into operational taxonomical units (OTU) using QIIME at 97%similarity levels. Before further analysis, singleton OTUs were removed.