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The effect of adjunctive triple antibiotic paste in deep periodontal pocket management: a comprehensive investigation of clinical, immunological and microbiological outcomes through a randomised controlled trial

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Registration date 05/01/2025	**Overall study status** Completed	[ ] Statistical analysis plan [ ] Results
Last Edited 27/12/2024	**Condition category** Oral Health	[ ] Individual participant data [ ] Record updated in last year

Plain English summary of protocol

Background and study aims

Periodontitis is a long-lasting disease caused by the buildup of bacteria on the teeth. It leads to the gradual destruction of the structures that support the teeth, including the gums and the bone around the teeth. The main signs are loss of support for the teeth, which can be seen as the gums pulling away from the teeth and bone loss on X-rays, the presence of deep gum pockets around the teeth, and bleeding gums.

To treat periodontitis, the second step involves removing the bacteria and hardened plaque below the gum line. This can be done with additional treatments like locally applied or systemic antibiotics. However, using antibiotics regularly is not recommended because of health risks and the impact on public health. They might be used for specific cases, like severe periodontitis in young adults.

While antibiotics can be effective, they also come with risks like toxicity, allergic reactions, and antibiotic resistance. Their effectiveness depends on the patient following the dosage instructions and the antibiotics being properly absorbed and distributed in the body. Using locally applied antibiotics that release slowly is preferred because they require lower doses, have fewer side effects, are easier to use, and pose less risk of resistance.

Recently, a different antibiotic combination known as triple antibiotic paste (TAP) has been introduced. Therefore this study aims to explore the effectiveness of triple antibiotic paste in the management of deep periodontal (gum) pockets.

Who can participate?

Systematically healthy patients diagnosed with chronic periodontitis, aged between 30-65 years old, with deep periodontal pockets, who have not received antibiotic therapy within the last 3 months, have no known allergy to medications and have at least 10 teeth in each jaw

What does the study involve?
Patients will be randomly allocated into two groups: one receiving triple antibiotic paste (TAP) and the other receiving conventional periodontal therapy. Fluid will be taken using a sterile paper strip from the gingival (gum) crevice before treatment and 2 months after treatment for testing. Clinical assessments will be done at the same interval and long-term follow-up at 3 months.

What are the possible benefits and risks of participating?
Not provided at time of registration

Where is the study run from?
Spinel Clinic (Postgraduate Periodontic University Clinic) UiTM Sungai Buloh (Malaysia)

When is the study starting and how long is it expected to run for?
May 2024 to March 2026

Who is funding the study?
Investigator initiated and funded

Who is the main contact?
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Public, Scientific, Principal investigator

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Study information

Scientific Title
The effect of adjunctive triple antibiotic paste in deep periodontal pocket management: a comprehensive investigation of clinical, immunological and microbiological outcomes through a randomised controlled trial

Study objectives
1. Objective 1 :
H0: There is no significant difference in pocket depth, plaque reduction, bleeding on probing and clinical attachment level changes comparing non-surgical periodontal therapy (NSPT) with NSPT + triple antibiotic paste (TAP).
2. Objective 2:
H0: There is no significant difference in levels of IL-1β, IL-6, IL-10 and TNF-α post-treatment comparing NSPT with NSPT + TAP.
3. Objective 3:
H0: There is no significant difference in Aggregatibacter actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg) count post-treatment when comparing NSPT and NSPT + TAP.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 23/12/2024, Universiti Teknologi MARA Research Ethics Committee (Faculty of Dentistry, Sungai Buloh Campus, Selangor Branch, Jln Hospital,, Sungai Buloh, 47000, Malaysia; +60 (0)3-61266555; korporatpergigian@uitm.edu.my), ref: REC/12/2024 (PG/FB/41)

Primary study design
Interventional

Study design
Interventional double-blind randomized controlled trial

Study type(s)
Treatment, Efficacy

Health condition(s) or problem(s) studied
Periodontitis

Interventions
This research will be a randomized (clinical) controlled trial (RCT) to compare the effectiveness of Triple Antibiotic Paste (TAP) as adjunctive with a control group receiving conventional periodontal therapy; which is scaling and root surface debridement.

Patients will be randomly allocated into two groups: one receiving Triple Antibiotic Paste (TAP) and the other receiving conventional periodontal therapy. This study will be a double-blind design where both participants and investigators will be unaware of the assigned treatment until the completion of the study.

Randomization using computerized system, randomly distributed into two groups representing two therapeutic groups:
1. Scaling + root surface debridement + adjunctive Triple Antibiotic Paste (intervention group)
2. Scaling + root surface debridement + placebo (carrier only) (control group)

Treatment protocol allocations will be dispensed in numbered opaque, identical envelopes, which then will be given to a single dental assistant, who will be assigned in Spinel Clinic and will not participate in the study. The same dental assistant will unseal the envelope and mark the patient number and sites using the respective treatment container. The same dental assistant will delegate the patients according to the groups and send them to the assigned experienced periodontist for treatment. The periodontist will not be involved in other procedures of the study, except for delivery of the adjunctive, to avoid bias.

The procedure will begin with oral hygiene instructions during the first visit. Written informed consent will be acquired from patients. Initially, baseline clinical parameters will be taken by a standardized periodontist using UNC-15 periodontal probe. The parameters that will be measured are bleeding on probing (BOP), clinical attachment loss (CAL) and periodontal depth (PD) in six measurement points around each tooth, except the third molars.
Pocket >5 mm identified and impression taken using putty and probe inserted on site >5 mm (for reproducibility during clinical assessment).

Then, scaling and root surface debridement will be done under nerve block anaesthesia. Scaling will be carried out using an ultrasonic scaler and then Gracey curette (Gracey Curettes, Hu-Friedy). At the end of the visit, the triple antibiotic paste will be introduced to the patient assigned to the intervention group. At the end of the visit the patient will be sent to another Periodontist who is not blinded by the study. TAP will be placed for the intervention group and glycol only as a placebo for the control group. 0.2 ml of Triple Antibiotic Mix will be injected into the deep pocket >5 mm using a delivery syringe (Pradeep et al., 2013) for the intervention group and glycol only as a placebo for the control group.
The randomization group will only be known by this periodontist.

Gingival crevicular fluid (200 microL) will be taken using a sterile paper strip from the gingival crevice before treatment and 2 months post-treatment for microbiological and immunological testing. Clinical assessment will be done at the same interval and long-term follow-up at 3 months.

The clinicians involved in this research are Dr. Muhammad Hilmi bin Zainal Ariffin and the principal investigator, Dr. Nursafirah ‘Izzati binti Idrus. Dr. Muhammad Hilmi will handle subject allocation and the blinding of the TAP and control solutions. Dr. Nursafirah will conduct the clinical assessments, perform scaling and root surface debridement, and administer the TAP injection into the gingival sulcus. Intra-examiner calibration of the principal investigator will be completed prior to the start of the study.

The patients will also be instructed to avoid brushing, flossing, rinsing with mouthwash, or eating for at least 2 hours immediately after treatment. Patients will be provided with the same toothpaste and toothbrushes to be used during the experiment period to minimize the effects of other factors.

The patients will be informed about the probability of the need for extra follow-ups in case there is any adverse effect due to treatment that has been done. The patient will be contacted for any immediate adverse reaction within the first 24 hours. If there are no complications noticed, the subsequent visit for review will be one week and three months review. At the follow-up appointments (1 week and 3 months review), the above-mentioned parameters were recorded and only supragingival calculus was removed. Any residual periodontal pockets (pockets with PD = 4 mm + BOP, or PD ≤5 mm) were not re-instrumented. The patient will also be informed regarding 24-hour follow-up in case of allergy reaction post-treatment. Patients will be advised to fill in the Adverse Drug Reaction Monitoring form by the pharmacy in case of any adverse drug reaction. Patients will also be advised to visit the nearest Emergency Department in case of allergy reactions such as shortness of breath or rashes.

TAP Preparation:
Antibiotic mixing will be carried out using a mixture of ciprofloxacin, metronidazole and minocycline. The ratio for Ciprofloxacin: Metronidazole: Minocycline is 1: 1: 1 or 33%: 33%: 34%. As a carrier, Macrogol ointment and propylene glycol will be incorporated into the antibiotic mix to obtain 0.1-1.0 mg/ml concentration. In this research, 0.1 mg/ml concentration will be taken as a standard mix.

0.2 ml of prepared TAP will be injected into the gingival sulcus where a pocket >5 mm noted and taken for the study site.

Immunological Assessment
Immunological assessment will be done using Multiplex. Methods are designed according to Zhang et al., 2021.

Sample Collection
The examined area will be dried and isolated using cotton rolls. 200 microL gingival crevicular fluid (GCF) sample will be collected using sterile absorbent paper strips which will be placed at the gingival crevice until it reaches resistance for 30 seconds. Any blood-contaminated paper strips will be discarded. Paper strips will be placed in a 0.5ml Eppendorf tube containing 500µL of PBS.

Sample Storage
The sample will be placed in an ice box for transportation prior to storage. Immediately after collection, GCF samples will be stored at -80°C to preserve the integrity of the biomarkers until analysis.

Sample Preparation
GCF samples will be thawed on ice and will be centrifuged to remove any debris or cells following the manufacturer’s instructions. GCF will be transferred to clean tubes to avoid any particulates that may have settled at the bottom.

Assay Procedure
All reagents will be warmed to room temperature (20-25°C) before usage for the assay. GCF samples will be diluted appropriately based on the expected concentration range of the analytes and the sensitivity of the assay. 200 µL of assay buffer will be added into individual wells on the plate. 25 µL of the diluted samples and controls will be pipetted into the wells of the assay plate provided in the kit.

Analysation
TNF-α, interleukins-1β (IL-1β) , interleukins-6 (IL-6) and interleukins-10 (IL-10) in GCF will be examined through the Luminex fluorescent technique, utilizing Milliplex Magnetic Beads (Merck Millipore, BA, USA) in accordance with instructions from the manufacturer. The data obtained will be analysed with the Milliplex Analyst program.

Microbiological Assessment
Microbiological assessment will be done using the Relative qPCR technique. Quantitative PCR is commonly employed for the absolute quantification of gene expression, microorganism count, or copy number, as well as for studies of relative gene expression. The relative quantification method measures gene expression by comparing one sample to another, while absolute quantification uses a standard curve with known template concentrations for measurement. (Harshitha & Arunraj, 2021).

Collection of Samples
200 microL gingival crevicular fluid samples will be collected using sterile paper points from the GCF area by gently placing the sterile paper strip into the gingival crevice for 30 seconds to absorb the plaque and gingival crevicular fluid. Paper strips will be introduced into the gingival crevice until it reaches resistance. Any blood contamination on the paper strips will be discarded. After collection, the paper points containing GCF samples will be transferred into Eppendorf tubes containing reduced transport fluid (RTF) buffer. Labelling will be done to ensure samples are identified accurately.

Sample Storage
Samples will be transported to the freezer using an ice box. The sample will then be stored at -70°C until further analysis. This freezing step is being done to help preserve bacterial DNA integrity for qPCR analysis. Prior to qPCR analysis, the samples will be thawed by removing them from the freezer and allowing them to thaw at room temperature or in a refrigerator to avoid DNA degradation.

DNA Extraction
Genomic DNA extraction will be performed using a commercially available DNA extraction kit, such as Qiagen, following the manufacturer's instructions. DNA extraction will be done using 1 ml plaque sample. This step will ensure the efficient recovery of bacterial DNA from the plaque samples (Jebin et al., 2021).

qPCR Analysis
qPCR analysis will be conducted using the extracted DNA samples. Primers targeting specific virulence factors, such as the LtxA gene for A. actinomycetemcomitans and the rgpA gene (gingipain gene) for P. gingivalis, will be designed. Specific forward/reverse primer will be used for qPCR analysis to quantify the amount of these bacteria in the plaque samples in CT values. The samples will be analysed in duplicate in 20 µL containing 2 µL of template DNA, 10 µL of Green Master Mix and each specific forward/reverse primer (final concentration is 0.25 µL) (Miyazawa et al., 2020). qPCR data will be analysed to quantify the amount of bacterial DNA in the plaque samples. Chi-square will be used for the statistical analysis.

Intervention Type
Drug

Phase
Not Specified

Drug/device/biological/vaccine name(s)
Triple antibiotic paste

Primary outcome(s)
Measured at baseline, 8 weeks and 3 months postoperatively using UNC-15 periodontal probe:
1. Pocket depth
2. Plaque reduction
3. Bleeding on probing
4. Clinical attachment level

Key secondary outcome(s)
Measured using relative quantification at baseline and 8 weeks postoperatively:
1. Levels of IL-1β, IL-6, IL-10 and TNF-α measured using the Luminex fluorescent technique, utilizing Milliplex Magnetic Beads (Merck Millipore, BA, USA)
2. Aggregatibacter actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg) count using relative qPCR

Completion date
01/03/2026

Eligibility

Key inclusion criteria
1. Systematically healthy adult patients diagnosed with Chronic Periodontitis Stage III and IV Grade A/B/C, aged between 30-65 years old
2. Patient with deep periodontal pocket, at least two quadrants with pocket >5 mm
3. Patients who have not received antibiotic therapy within the last 3 months
4. Patients who have no known allergy to medications
5. Patients with at least 10 teeth in each jaw

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
30 Years

Upper age limit
65 Years

Sex
All

Key exclusion criteria

1. Patients with contraindications to TAP or specific antibiotic content such as patients with a history of seizures, myasthenia gravis, peripheral neuropathy, liver and kidney problems
2. Pregnant or lactating individuals
3. Patients on systemic antibiotics past 3 months
4. Smoking more than 10 cigarettes per day
5. Patients with a history of diabetes, cardiovascular disease and immune disorder
6. History of hypersensitivity to metronidazole, or minocycline, or ciprofloxacin
7. Patients on immunosuppressant in the last 6 months
8. Periodontal treatment done in the last 6 months

Date of first enrolment
03/01/2025

Date of final enrolment
31/12/2025

Locations

Countries of recruitment
Malaysia

Study participating centre
Universiti Teknologi MARA
Spinel Clinic, Faculty of Dentistry
Sungai Buloh Campus, Selangor Branch
Jln Hospital
Selangor
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Sponsor information

Organisation
Universiti Teknologi MARA

ROR
https://ror.org/05n8tts92

Funder(s)

Funder type
Other

Funder Name
Investigator initiated and funded

Results and Publications

Individual participant data (IPD) sharing plan
The data-sharing plans for the current study are unknown and will be made available at a later date

IPD sharing plan summary
Data sharing statement to be made available at a later date

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Participant information sheet			27/12/2024	No	Yes