THE UNIVERSITY OF WESTERN AUSTRALIA

Research Proposal

Clinical and microbiological evaluation of one-stage full mouth disinfection in conjunction with systemically administered azithromycin: a Randomised Controlled Clinical Trial

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BRIEF CURRICULUM VITAE

This research is conducted under the lead and supervision of **A/Professor Alessandro Quaranta** and **Dr Julio Rincon** at the University of Western Australia.

ALESSANDRO QUARANTA is the Discipline Lead and Program Convenor, of the discipline of Periodontics and Implantology at the School of Dentistry at the University of Western Australia. He is a specialist in Periodontics. Dr Quaranta is the leader of the Research Program in Oral Implantology and Periodontics at Oral Health Centre WA

A/Professor Quaranta has broad research and clinical experience in Periodontology and Implantology. He has been involved in the research of Implant Dentistry and Periodontology for more than 10 years and actively conducted research in the broad fields of non-surgical periodontal therapy, periodontal medicine, peri implant tissues, post extraction implants, bone to implant surfaces and implant immediate loading.

His main research topics focus on non-surgical treatment of periodontal diseases, biological complications and microbiology of peri implant tissues, implant surface decontamination procedures, clinical trials and systemic reviews in implant dentistry. Over recent years, his research has contributed to the progression of the scientific knowledge in Periodontology and implant Dentistry. His enthusiasm in research resulted in a total of 139 publications, 254 citations. Sixty of his publications were published as full length articles in scientific journals (indexed on databases such as PubMed and Scopus).

In addition, Dr Quaranta works with a relatively large number of collaborators based in Italy ,Newzealand and the United States of America (USA).

JULIO RINCON A/Professor Dr Julio C Rincon, is a Senior Lecturer at the University of Western Australia. He has keen interest in research and has published numerous articles and reports in the microbiology and regeneration of the periodontium and use of antibiotics in treatment of chronic periodontitis. With many years of clinical experience, Dr Rincon has supervised Postgraduate Research students at University of Western Australia since 2008. He is also a member of the Research program in Oral Implantology and Periodontics at Oral Health Centre , WA

EMAN ELHASSAN is a current postgraduate student in the Clinical Doctorate Program (DClinDENT Periodontics) at the University of Western Australia, with 8 years of clinical experience in general Dentistry in various countries including Australia, Dubai and Sudan. She has undertaken a number of courses with Dental Board of German Implantology on surgical placement and restoration of Dental Implants. She is also an Honorary Research Associate at the International Research Collaborative- Oral Health and Equity (University of Western Australia). Having completed her ADC exams in 2013, she has worked in private practice in Perth for two years before commencing her studies at the university of Western Australia. Dr Elhassan has also passed Part1 of the MJDF(membership of the joint dental Faculties- UK) examinations. Dr Elhassan is also a member of the Research Program in Oral Implantology and Periodontics at OHCWA

PROJECT TITLE

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II - INTRODUCTION

Periodontitis is an inflammatory disease that results in the destruction of teeth-supporting tissues. It is a result of the imbalance between the wide range of microorganisms, the host response and some essential modifying factors. [1]The primary etiology of periodontal disease is bacterial biofilm (dental plaque) which consists of bacterial communities embedded in a glycoprotein matrix. Despite the fact that dental plaque could contain over 900 species of bacteria, only a limited number of species are considered true periodontopathogens. These include *Bacteroides forsythus, Porphyromonas gingivalis and Treponema denticola, F. nucleatum subspecies, P. intermedia and P. nigrescens, Peptostreptococcus micros and Campylobacter rectus, Campylobacter showae, Campylobacter gracilis, Eubacterium. nodatum and Streptococcus constellatus)* and *Aggregatibacter actinomycetemcomitans*[2].
The primary therapy for periodontal disease is plaque control, which is accomplished by plaque control measures (brushing, flossing, and other cleansing aids) and mechanical debridement (scaling and root planning: SRP) using machine driven and hand instruments. Sometimes additional periodontal surgical treatments are performed to gain further improvement in clinical parameters, such as probing depth (PD), periodontal attachment level (PAL), gingival index (GI), bleeding on probing (BOP) and gingival crevicular fluid (GCF). All these changes are related to a microbial shift of the periodontal flora to a state of periodontal health. [3]

The conventional form of staged nonsurgical periodontal therapy (SRP) has been shown to result in clinical improvements [4] However it has been suggested that it carries the risk for recontamination of already-treated areas from untreated sites still harbouring large amounts of periodontal pathogens. [5] Based on this hypothesis , Quirynen introduced the protocol of one-stage full mouth disinfection (OSFMD) in order prevent the intra-oral transmission of periodontal pathogens from periodontal pockets to recently instrumented and healing periodontal sites.[6]
The one stage full mouth disinfection (OSFMD) protocol is based on a one-stage (24-hour) scaling and root planning procedure divided into two sessions with concomitant use of chlorhexidine (CHX) gel subgingival irrigation and daily CHX mouthwash for a period of two weeks[6]

There has been controversy regarding the advantages of OSFMD. However, in recent years a number randomised controlled studies have demonstrated the additional benefits in clinical and microbiological outcomes of this protocol over the conventional staged nonsurgical scaling and root planning in the treatment of periodontal infections.[7, 8] It must be noted that other systemic reviews found that, although the differences between OSFMD and conventional staged debridement were statistically significant, they were small and probably clinically insignificant. Therefore, it can be concluded that OSFMD is equal to conventional staged debridement .[9]

Non-surgical Periodontal therapy has various limitations, such as difficulties in accessing deep pockets, furcations and concavities [4] and inability to eliminate microbial pathogens located in dentin tubules, lacunae and concavities[10]. To overcome these limitations, researchers have suggested several protocols including systemic antimicrobial administration. This strategy assumes that a higher amount of bacteria species can be eliminated or suppressed during periodontal therapy leading to a better microbial intraoral environment and improved host response.[11] Systemic antibiotics were proven to be uniformly beneficial in providing an improvement in attachment level when used as adjuncts to scaling and root planning (SRP) and were consistently favourable for both subjects affected by chronic and aggressive periodontitis. [11]

Among the different types of antimicrobials, azithromycin is of particular interest. Azithromycin is a macrolide bacteriostatic agent that inhibits bacterial protein synthesis. In addition to its antibacterial role in suppressing periodontopathogens, it is thought to have two more actions: anti-inflammatory and assistance in healing through persistence at low levels in fibroblasts and macrophages [12]. Azithromycin (AZ) has produced better clinical outcomes when used as an adjuvant to conventional staged nonsurgical scaling and root planning[12]. Its use as adjuvant to Full mouth scaling and root planing has also demonstrated superior clinical and microbiological results [13, 14] suggesting that systemic antimicrobials further reduce the intraoral bacterial counts. Furthermore, it

was reported that OSFMD frequently induces post-operative pyrexia, probably due to the large quantity of bacteria entering the blood stream. This event was not observed in studies where azithromycin was administered as an adjuvant therapy to nonsurgical periodontal treatment .[13]

III- HYPOTHESIS /RESEARCH QUESTION

Test null hypothesis: there is no difference in pocket depth reduction, between one stage full mouth disinfection with and without the use of adjuvant systemic antimicrobial therapy (Azithromycin) against the alternative hypothesis of a difference.

IV- AIM

The purpose of this study will be to evaluate the use of systemically administered azithromycin as an adjuvant to OSFD (one stage full mouth disinfection) in the treatment of chronic periodontitis through clinical and microbiological periodontal parameters at baseline, 90 and 180 days post therapy.

V- SIGNIFICANCE OF THE RESEARCH

- Although general consensus favours the use of systemic antibiotics in conjunction with conventional staged debridement therapy in treatment of advanced periodontal diseases, there are limited studies where systemic antimicrobials were used in conjunction with one stage full mouth disinfection. To the best of our knowledge, there are no studies that evaluated clinically or microbiologically the use of azithromycin as an adjuvant to the OSFMD. This trial could help provide evidence based guidelines for the use of azithromycin in conjunction with OSFMD in treatment of patients with chronic periodontitis.

VI- RESEARCH PROJECT DETAILS

The research will be undertaken at the University of Western Australia, school of Dentistry. Research grant applications will be submitted to the Australia Dental Research (ADRF) and Australian Periodontology Research Foundation (APRF) grants. The principles outlined in the Declaration of Helsinki (as modified at the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013) on clinical research involving human subjects will be adhered to. Approval from the University of Western Australia Human Research Ethics Committee will be sought. The trial will be reported according to the CONSORT statement for improving the quality of reports parallel group ramdomised trials.

Materials and Methods

- A single centre, randomized, placebo controlled, parallel-design, double-masked trial of 6 months duration
- The Research will consist of 2 arms (one randomly allocated group will receive OSFMD with placebo, the other randomly allocated arm will receive OSFMD treatment with Azithromycin)

The comparisons will be between:

One Stage Full Mouth Disinfection protocol + placebo

- One Stage Full Mouth Disinfection protocol + systemic antimicrobial (Azithromycin, 5oomg x 3)

The main parameters that we will be assessed are the following :

- Difference in pocket depth (PPD) reduction (primary outcome)
- Differences in Probing attachment Level (PAL)
- Differences in bleeding on probing (BoP)
- Microbiological changes attributed to treatment

- Assessment of oral health related quality of life changes after non surgical periodontal treatment in chronic periodontitis

Sample size calculation

The Sample size of 20 patients in each group has been calculated by a biostatistician according to the estimation of sample size at first visit (baseline) and 25 weeks post therapy, group sample sizes of **16 and 16** achieve 91% power to detect a difference of 0.9 between the null hypothesis that both group means are 1.6 and the alternative hypothesis that the mean of group 2 is 0.8 with known group standard deviations of 0.9 and 0.5, with a significance level (alpha) of 0.05 using a two-sided two-sample t-test.

Patient Recruitment and Randomisation

- Multiple screening sessions will be conducted to recruit patients from the waiting list of the Discipline of Periodontics and Implantology at the Oral Health Centre ,WA

  The following **inclusion criteria** will be adopted:

  1) Healthy patients (ASA I and II classification)
  2) Presence of ≥ 12 scorable teeth (not including third molars and teeth with orthodontic appliances, bridges, crowns, or implants)
  3) Diagnosis of chronic periodontitis with the presence of at least four teeth with a probing depth (PPD) ≥ 5 mm, periodontal attachment loss (AL) ≥ 3 mm, and radiographic evidence of bone loss
  4) Participant Age: 25 to 70 years; Both genders

  The **exclusion criteria** will be the following:

  - Uncontrolled systemic illnesses (i.e. diabetes mellitus, cancer, human immunodeficiency syndrome, bone metabolic diseases or disorders that compromise wound healing, radiation, or immunosuppressive therapy)
  - Pregnancy or lactation
  - Confirmed or suspected intolerance to azithromycin, macrolides or Chlorhexidine mouthwash
  - Smoking history will be recorded, but smoking will not be an exclusion criterion
  - Regular use of antibiotics or anti-inflammatory drugs or use within 3 months preceding the start of the study
  - Regular use (twice a day) of mouthwashes or regular use within 3 months before study entry
  - Periodontal therapy including dental scaling and root planing procedures in the 12 months preceding the start of the study
  - Antibiotic prophylaxis required for periodontal clinical examination, Antibiotic allergy

  Written explanation of the trial, objectives, possible side effects and consent will be provided to each participant. Participants will sign a consent form and will be randomly assigned to test and control groups according to a computer-generated list

Examiner Calibration

Examiners responsible for clinical evaluations will be properly trained for calibration and masked to the intervention group For calibration procedure, measurements of PD and PAL will be recorded and repeated within 1-week intervals for 10 patients (at all sites) randomly selected from both groups to calculate Kappa.

Clinical Outcome Parameters

The following clinical outcome parameters will be measured by a blind examiner at baseline, 3 months and 6 months after the completion of non surgical therapy and will be all reported in a dedicated periodontal chart:

- **Six measurements of PPD and PAL** will be performed per tooth on all teeth (except third molars)
- **Recession** (REC) will be expressed in mm as positive if the gingival margin is located apical, negative if located coronal to the cemento-enamel junction
- **Plaque index:** the presence or absence of plaque will be assessed by running a probe across four sites in the entire dentition [15]
- **Bleeding on probing** (BoP): presence or absence of BoP within 10 seconds will be measured at 4 sites on each tooth
- Full Mouth Plaque and Bleeding on Probing scores (FMPS, FMBS) will be calculated as a percentage of sites positive to plaque out the total number of available sites
- Every patient will be subjected to a radiographic status and a panoramic X-ray exam depending on the severity of the periodontal condition

Clinical Protocol:

A total of 7 clinical appointments will be carried out during the study:

1) **Appointment 1, OHI (Oral Hygiene Instructions) and Supragingival scaling and polishing :** OHI on brushing techniques and use of interdental devices will be provided. Supragingival scaling will be carried out by the operator involved in the OSFMD procedure.
2) **Appointment 2, Periodontal charting (Baseline)**
   A full-mouth periodontal clinical examination and collection of microbiologic samples will be performed at the baseline, 90 and 180 days after the OSFMD procedure by a blinded examiner. All the parameters will be reported in a dedicated periodontal chart.

- PPD and PAL values will be collected on six sites (mesial, mesiobuccal, mesiolingual, distobuccal, distolingual and lingual) per each tooth in all teeth (except third molars) using a calibrated periodontal probe
- FMPS, FMBS, REC, clinical mobility and furcation involvement will be measured
- Subgingival samples for microbiological analysis will be collected and transferred to the specialised laboratory according to the laboratory instructions
  Oral hygiene instructions will be repeated and re-enforced

3) **Appointment 3, OSFMD Session 1**
   All the subjects will undergo the same non surgical periodontal therapy and clinical steps with the exception of the prescription of systemic antibiotic or placebo:

- Body temperature will be checked using a digital thermometer before appointment 1st and second appointment
- OHI will be re – enforced again

- **OSFMD Protocol**: At the beginning and at the end of the instrumentation session, subgingival irrigation with 0.5% CHX gel, tongue brushing with 0.5% CHX gel for 1 minute, rinse with 0,2% CHX mouthwash for 30 seconds (including gargling during the last 10 seconds) will be performed.
- Non surgical instrumentation will be carried out on one side of the dentition by an operator (postgraduate student) not involved in the diagnostic procedures as follows:
- Manual area specific curets (SG7-897; SG11-1293; SG13-1498; SAS7- 897; SAS11-1293; SAS13-149, Hu-Friedy®, Chicago, USA) and mechanical tips (Air Flow Master Piezon® A, P and PS debridement tips, EMS Switzerland) will be used to instrument the entire dentition of one side. (followed by other side in 24 hours)
- 0.2% CHX mouthwash (2ml twice a day) for a total of 2 weeks
- Participants will be given by a chemist, numbered anonymous medication pack containing the antibiotic/placebo to use for 3 consecutive days (1 tab / day starting from the day of Appointment 3)
- Written instructions and a diary sheet will be provided

4) Appointment 4: OSFMD Session 2

- All the clinical steps of the OSFMD protocol previously described (Appointment 3) will be performed by the same operator on the other side of the dentition. Participants will be informed to continue the use of the antibiotic/placebo package as previously instructed .Body temperature will be recorded before session.

5) Appointment 5, 2 weeks following the completion of OSFMD

- OHI will be reviewed and participants will be asked to return the empty medication packages and the diary sheet
- Discolouration will be assessed and supragingival removal of plaque including polishing will be done if necessary. Body temperature will be recorded .
- Participants will be asked to fill a survey sheet about adverse effects and experience

6) Appointment 6, 90 days following the completion of OSFMD

- OHI will be re- enforced
- Microbiological samples will be collected with same procedure performed during Appointment 2
- Microbial samples will be transferred to Laboratory for analysis
- Complete Clinical Periodontal assessment will be repeated by the same blinded examiner involved in clinical assessment during Appointment 2
- Participants will be asked to answer a survey sheet about adverse effects and experience

7) Appointment 7: 6 months following the completion of OSFMD

- Complete Clinical Periodontal assessment will be repeated by the blinded examiner as previously described.

Medications

- All the systemic medications (capsules) will be prepared by an approved compounding chemist
- Azithromycin (500 mg, 3 capsules each) and identical placebo packs will be provided to the participants accordingly to the randomisation lists for control and test groups
- Dosage: Participants will take 3 capsules (1 per day for consecutive 3 days) of either test or control medication
- The treatment groups will be concealed from the patient, clinical examiner, the therapist, and statistician.

- The participants allocations will be revealed to the investigators only after the completion of statistical analysis

Treatment compliance:

- Compliance will be evaluated on the basis of the remaining amount of mouthwash and capsules at the 4th visit and by assessing the participants compliance sheet

Microbiological Assessment:

- The microbiological sampling and analysis will be done at baseline and at 90 days post treatment
- Bacterial samples will be collected in each participant with sterile paper points from the four sites displaying the deepest periodontal probing depths
- Each selected tooth will be isolated with sterile cotton rolls and the supragingival plaque removed with sterile curets. Paper points will be inserted in the periodontal sites and immediately stored in a mini tube)
- The samples will be shipped to a laboratory specialised in analysing periodontopathogenic bacteria
- The Laboratory will be blinded to participants allocation
- Quantification of the total number of bacterial cells and levels of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, and Prevotella intermedia will be carried out by quantitative real-time polymerase Chain reaction (qPCR)

Confidential /Sensitive Information:

- Clinical record forms (CRFS) will be completed at the time of data collection by all participants included in the study. The CRFs will be stored in a secure locked place at the Oral Health Centre, UWA.

- Once the results are obtained, microbiological samples will be discarded appropriately according to the lab protocol of discarding hazardous material , using professional waste disposal companies

Statistical Component:

Data analysis will be carried out according to a pre-established analysis plan. The procedure will be performed by the principal investigator (postgraduate student) under the supervision of a biostatistician with expertise in dentistry and blinded to the group allocation. A comparison of the baseline PPD values between the two groups at baseline, 3 and 6 months after therapy will be presented as primary outcome.

Comparison of the Microbiological results (baseline and 3 months), PAL values, FMPS and FMBS scores at (baseline, 3 and 6 months) will be used as secondary outcomes.

Duration of the Research Study:

Human Research Ethics Review Application at UWA: 4- 6 months from application submission

Australian Register of Therapeutic Goods: Not required

Anticipated Commencement date: 1 Jan 2017

Anticipated Completion of Participants Recruitment: 3 months form commencement date- Dec 2016

Anticipated Completion of Data collection from all Participants: December 2017

Anticipated Publication Date: 2018

VII- Budget

DATA PROCESSING: External hard drive Statistical analysis Applied statistics' consulting fees for University of Western Australia staff is at an hourly rate of $180 +18 GST - The statistical analysis for this project should take 10 hours ($198 x 8h)	**100$(In kind)** **1584 $ (A)**
EQUIPMENT AND APPARATUS	
CONSUMABLE SUPPLIES **Micro biological Sampling/collection Kits (PCR quantification of Samples)** - One sample Kit costs 62$ - Each participant requires 2 sample kits - 40 participants x 2= 80 sample kits required **FedEx/ Shipping costs of Microbiological collection Kits** - Express courier shipping will be required to send the microbiological sampling collection kits to Australia - 2 batches of 40 collection kits will be shipped back to Germany for analysis using priority shipping **Azithromycin capsules (500mg x3) /Placebo (500mg x3)** - Cost of preparation of Azithromycin and placebo in capsules by specialist chemist **Chlorohexidine mouth wash 2%** - Each participant requires 2 bottles of chlorohexidine 2% mouthwash (2 home use) - 40 participants require 80 bottles of mouthwash - Estimated cost of one bottle chlorohexidine 2% mouth wash 16.6$ **Chlorohexidine 0.5% gel** - Each participant will require one tube of chlorohexidine gel 0.5% - Estimated cost of gel is 10$ for 40 participants **Digital Thermometer** - digital ear thermometer (50$) x1	**4960$ (A)** **400$ (A)** **800$ (A)** **1328($)(A)** **400$(A)** **50$ (In kind)**
TRAVEL	
OTHER **Stationary/printing costs / consent sheets/survey sheet/ Advice sheet**	**100$(In kind)**
TOTAL	**9722$**
GRAND TOTAL ***NB: Figures MUST AGREE with item 12 on Page 8***	**9722$**

VIII- References:

1. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal-disease - current concepts. Journal of Periodontology 1992;63:322-331.
2. Socransky S, Haffajee A, Cugini M, Smith C, Kent Jr R. Microbial compexes in subgingival plaque. J Clin Periodontol 1998;25:134-144.
3. Listgarten MA. Structure of the microbial flora associated with periodontal health and disease in man. A light and electron microscopic study. Journal of periodontology 1976;47:1.
4. Badersten A, Nilvéus R, Egelberg J. Effect of nonsurgical periodontal therapy. Journal of Clinical Periodontology 1981;8:57-72.
5. Quirynen M, De Soete M, Dierickx K, van Steenberghe D. The intra-oral translocation of periodontopathogens jeopardises the outcome of periodontal therapy - A review of the literature. Journal of Clinical Periodontology 2001;28:499-507.
6. Quirynen M, Bollen CML, Vandekerckhove BNA, Dekeyser C, Papaioannou W, Eyssen H. Full-vs partial-mouth disinfection in the treatment of periodontal infections - short-term clinical and microbiological observations. Journal of Dental Research 1995;74:1459-1467.
7. Teughels W, Dekeyser C, Van Essche M, Quirynen M. One - stage, full - mouth disinfection: fiction or reality? Periodontology 2000 2009;50:39-51.
8. Fonseca DC, Cortelli JR, Cortelli SC*, et al.* Clinical and Microbiologic Evaluation of Scaling and Root Planing per Quadrant and One-Stage Full-Mouth Disinfection Associated With Azithromycin or Chlorhexidine: A Clinical Randomized Controlled Trial. Journal of periodontology 2015;86:1340.
9. Lang NP, Tan WC, Kraehenmann MA, Zwahlen M. A systematic review of the effects of full-mouth debridement with and without antiseptics in patients with chronic periodontitis. Journal of Clinical Periodontology 2008;35:8-21.
10. Mombelli A, Cionca N, Almaghlouth A. Does adjunctive antimicrobial therapy reduce the perceived need for periodontal surgery? Periodontology 2000 2011;55:205.
11. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. Annals of periodontology / the American Academy of Periodontology 2003;8:115.
12. Hirsch R, Deng H, Laohachai MN. Azithromycin in periodontal treatment: more than an antibiotic. Journal of Periodontal Research 2012;47:137-148.
13. Yashima A, Gomi K, Maeda N, Arai T. One-Stage Full-Mouth Versus Partial-Mouth Scaling and Root Planing During the Effective Half-Life of Systemically Administered Azithromycin. Journal of Periodontology 2009;80:1406-1413.
14. Gomi K, Yashima A, Nagano T, Kanazashi M, Maeda N, Arai T. Effects of full-mouth scaling and root planing in conjunction with systemically administered azithromycin. Journal of periodontology 2007;78:422.
15. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. Journal of periodontology 1972;43:38.