COVER LETTER

04/09/2019

To whomsoever it may concern,

This is to certify that this is the bonafide title of our study- "The Effectiveness of Brushing and Flossing Sequence on Control of Plaque and Gingival Inflammation- A Randomized Controlled Clinical Trial in Klinik Pergigian, MMMC, Melaka." with a NCT number: NCT03989427.

We hereby assure that this was a bonafide study approved by Institutional Ethics and Research Committee of Melaka-Manipal Medical College (MMMC/FOD/AR/B6/E C-2019 (21). To the best of our knowledge all the information provided in this study is true and is being reported is being reported in compliance with the declaration of Helsinki for clinical trials.

NAME OF RESEARCHERS

Prof. Dr. Rajesh Hosadurga

Prof. Dr. Htoo Htoo Kyaw Soe

Sacha Miriam Augustus

Deevatharshini a/p Jayabalan

Jayahneiswary a/p Ganesan

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Operational Definitions

1. **Rustogi Modified Navy Plaque Index (RMNPI):**

This index divides buccal and lingual surfaces into nine areas (A to I) that are scored for the presence (score=1) or absence (score=0) of plaque.

Whole mouth=Areas A,B,C,D,E,F,G,H and I; Marginal areas A,B and C; Interdental D and F
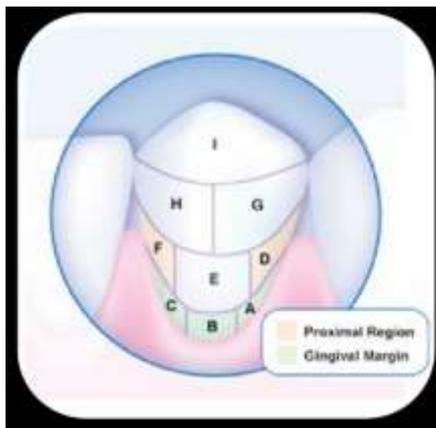
Presence of plaque: Score 1

Absence of plaque: Score 0

Buccal surface- 9 areas (A to I)

Lingual surface- 9 areas (A to I)

Interdental - D and F

RMNPI Score= $(Sum\ of\ all\ "1"\ marks\ )/(Total\ number\ of\ sites\ score)$

2. **BPI-Bleeding point index (bleeding point index, BPI; Lenox et al, 1973)**

Bleeding on probing is recorded and provides evaluation of gingival inflammation around each tooth in patient's mouth

Evaluates level of oral hygiene performance
- A periodontal probe is inserted 1 mm into the sulcus at the buccal, lingual,

mesial and distal surfaces

- After 20–30s scores will be recorded
- 0 – No bleeding
- 1 – Bleeding present

Percentage of BPI = $(Number\ of\ bleeding\ surface)/(total\ number\ of\ dental\ surfaces) \times 100$

The percentage of the number of bleeding surfaces will be calculated by:

$$\frac{Number\ of\ bleeding\ surface}{total\ number\ of\ dental\ surfaces} \times 100$$

Gingivitis- Inflamed gum tissue around their teeth, caused by bacteria found in dental plaque.[2] main types of gingivitis generalized and localized. Generalized has marginal gingivitis and diffuse gingivitis. Localized has marginal gingivitis, diffuse gingivitis and papillary gingivitis.

Dental plaque-Resilient clear to yellow-greyish substances.Primarily composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides .Considered to be a biofilm.Impossible to remove by rinsing or using sprays.

List of abbreviation

FB-Flossing and brushing

BF-Brushing and flossing

BPI-Bleeding Point Index

RMNPI-Rustogi modified navy plaque index

DJ- Deevatharshini Jayabalan

SA- Sacha Augustus

JG- Jayahneiswary Ganesan

DHE-Dental Health Education

MMMC- Melaka-Manipal Medical College.

Chapter 1: Introduction

Dental plaque removal is critical for preventing and treating periodontal disease. Toothbrush and toothpaste are the most reliable means of plaque control. Cleaning should be through and performed at regular intervals. Toothbrush has limited ability to reach interdental areas. Interdental areas are prone for plaque accumulation and gingival inflammation. Regular interdental cleaning results in less gingivitis. Most effective tool has been reported to be dental floss.

1.1 Research Background

Periodontal disease is the disease of the supporting dental tissues and the most common form is plaque-induced gingivitis.[1]

The removal of dental plaque is the most crucial action for preventing and treating periodontal disease.[2] Mechanical methods for plaque removal along with oral hygiene instructions are regarded as the first step in periodontal treatment. Toothbrush along with toothpaste are still the most reliable means of plaque control, provided cleaning is thorough and performed at regular intervals.[2]

Dentifrice is used as an adjunct to tooth brushing. The indication of use of dentifrices is mainly based on the presence of fluoride, antimicrobial agents that aim at further reducing the plaque formation or removal of previously established plaque. They have been used as plaque removal aids, especially because of their abrasive agents. In addition to fluoride and presence of antimicrobial agents, the actual adjunct role of dentifrices to mechanical removal of dental plaque is contradictory.[2]

Toothbrush has a limited ability to reach interdental areas. These areas are prone for plaque accumulation and gingival inflammation. Regular cleaning can reduce inflammation. Various products have been designed for accessing these sites such as dental flosses, interdental brushes and wooden dental sticks.[2] The most effective tool has been reported to be dental floss. It is effective in type 1 embrasure form. According to studies, the sequence of using toothbrush and dental floss may influence the removal of dental plaque and consequently reducing the bleeding on probing. Bleeding is one of the objective signs of periodontal disease. Therefore, it is possible

that the sequence of using a toothbrush and dental floss may influence the removal of dental plaque and consequently the BPI.[1]

1.1. Problem Statements

Regular oral hygiene using toothbrush and toothpaste are the most reliable for dental plaque control. But the cleaning should be thorough and performed at regular intervals. The reason why the plaque control is most often inadequate is due to limited ability of the toothbrush to reach interdental areas which are more prone for plaque accumulation and inflammation.[1]

According to a systematic review, flossing in addition to toothbrushing is better to reduce gingivitis compared to only tooth brushing. It is possible that while using the tooth brush and dental floss, the sequence of using toothbrush and dental floss may influence removal of dental plaque and gingival bleeding. Recent study by Mazhari et al in a plaque regrowth model has shown that flossing first is better than brushing first and then flossing. This has given rise to the conflict whether brushing first and then flossing is better or flossing first and then brushing is better. (reference)

1.2. Research Justification

Mechanical plaque removal has been directly related to prevention and treatment of periodontal disease. Interdental areas are the sites where plaque and inflammation are most pronounced. Dental floss is an effective tool to access these sites. Bleeding on probing is a sign of local inflammatory response related to destructive periodontitis. Tooth brushing and flossing significantly reduces gingivitis.[1] It is possible that the sequence of using toothbrush and dental floss may influence the removal of dental plaque and consequently the gingival bleeding. A study done using plaque regrowth model has reported that flossing first and then brushing is more effective. But, plaque regrowth model does not necessarily replicate the clinical situation. Cross-over clinical trials of at least two weeks duration in each phase not lasting less than two weeks with a wash out period of seven days (7 days) does provide more credible evidence. (put torkzaban article as reference). There are very few studies addressing this issue. No studies have been done among Malaysian population. Hence, this is be the first study assessing the effectiveness of sequence of flossing and brushing on gingival inflammation among dental students of MMMC..

1.3. Research Objectives

1.3.1 General Objective

To study the effectiveness of brushing and flossing sequence on plaque control and gingival inflammation

1.3.2 Specific Objectives

a) To evaluate the effectiveness of brushing first and flossing later sequence on plaque control
b) To evaluate the effectiveness of brushing first and flossing later sequence on gingival bleeding
c) To evaluate the effectiveness of Flossing first and brushing later sequence on plaque control
d) To evaluate the effectiveness of Flossing first and brushing later sequence on gingival bleeding
e) To compare the effectiveness of Brushing first and flossing later versus Flossing first brushing later

1.4. Research Questions

a) What is the effectiveness of brushing first and flossing later sequence on plaque control?
b) What is the effectiveness of brushing first and flossing later sequence on gingival bleeding?
c) What is the effectiveness of flossing first and brushing later sequence on plaque control?
d) What is the effectiveness of flossing first and brushing later sequence on gingival bleeding?
e) Which is better brushing first and flossing later or flossing first and brushing later?

1.5. Research Hypothesis (Null hypothesis)

The sequence of brushing and flossing does not influence plaque control and gingival inflammation.

Chapter 2: Literature Review

Author, Journal, Year	Material and methods	Conclusion
Mazhari F et al, J Periodontol. 2018	• Randomized, controlled, crossover, clinical trial • 25 **Iranian** dental students • Baseline - oral prophylaxis • Model - Plaque regrowth model • Phases - 2 • Phase 1- brush –floss group • Phase 2 – floss-brush group • Index - RMNPI • Wash out period- 2 weeks	The result shows – **Flossing first brushing later was better** than brushing first flossing later to remove interdental plaque

Author, Journal, Year	Material and methods	Conclusion
Torkzaban P et al, Oral Health Prev Dent 13. 2015	• Crossover design • 35 **Iranian** dental students • 2 phases • Phase 1 – flossing then brushing • Phase 2 – brushing then flossing • Index- PCR and PI • Washout period- one week	The results show – **Flossing followed by brushing provides more statistically significant** improvements over brushing followed by flossing with respect to plaque control

Author, Journal, Year	Material and methods	Conclusion
Worthington HV et al, Cochrane Database Syst Rev. 2019	• Databases searched: Cochrane Oral Health's Trials Register, the Cochrane Central Register of Controlled Trials (CENTRAL) (the Cochrane Library, MEDLINE Ovid Embase and CINAHL EBSCO	Using **floss in addition to tooth brushing** may reduce gingivitis or plaque, or both, more than tooth brushing alone.

Author, Journal, Year	Material and methods	Conclusion
Bagramian RA et al, American Journal of Dentistry, 2009	• The review was compilation of major epidemiological survey of 20 different countries • Assessed the prevalence of dental caries and periodontal disease	**Flossing along with regular tooth brushing** is beneficial in decreasing gingival inflammation. No mention of sequence was made.

Chapter 3: Theoretical Framework/Conceptual Framework

CONCEPTUAL FRAMEWORK

Effective oral hygiene

- Brushing technique*
- Flossing technique*
- Type of tooth brush*
- Type of floss

DENTAL PLAQUE REMOVAL

Decreased gingival inflammation

- Frequency of tooth brushing
- Medications
- Type of dentrifices
- Mouthwash
- Poor oral hygiene
- Diet

Sequence of flossing and brushing

Decreased gingivitis
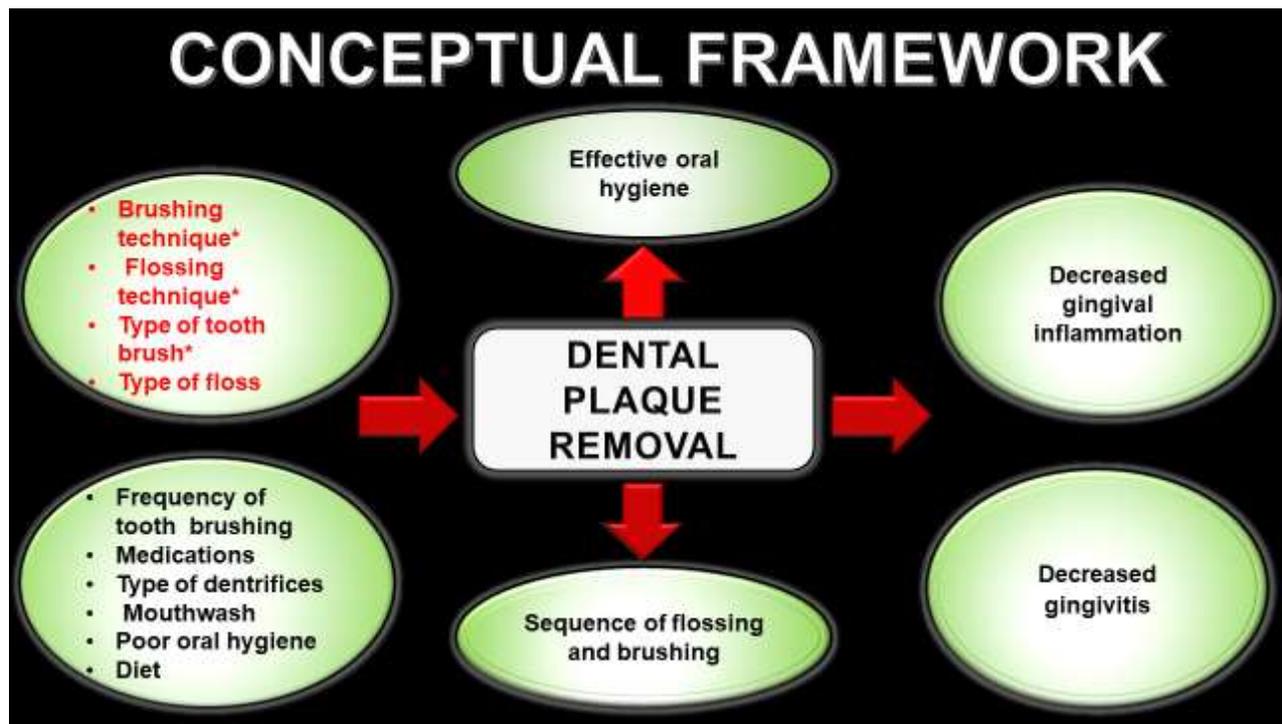
Chapter 4: Methodology

4.1 Study Design

4.1.1 Type of study

Single blind, cross-over randomized clinical trial

4.1.2 Sampling method

Non probability sampling and recruiting volunteers

4.1.3 Randomization

Computer generated randomization sequence (AB/BA)

4.2 Study Period

4.2.1 Reference Population

Dental students studying in MMMC, Melaka

4.2.2 Source Population

Phase II (Year 4 & 5) dental students studying in MMMC, Melaka

4.2.3 Sampling Frame

-Inclusion Criteria: Healthy students of either gender.

-Exclusion Criteria: Probing depth and attachment loss $\geq$ 3mm, use of antibiotics in the past 3 months, smoking, active orthodontic treatment, orthodontic retainers.

4.3 Sample Size Calculation

Since there is no previous study available with mean and SD, A pilot study was conducted on 30 participants.

4.4 Variables and Research Tools

Independent Variable: Sequence of brushing and flossing

Dependent Variable: Rustogi Modified Navy Plaque Index (RMNPI) scores and bleeding point index (BPI) Scores

Tools: Head cap, Face mask, Gloves, Mouth mirror, Oral prophylaxis paste, Oral prophylaxis cup/brush, Micromotor, Disclosing agent, William's probe.

Inter examiner reliability

Prior to the start of the study, the examiner was trained by an experienced periodontist until reaching informal agreement on the diagnostic criteria. After that, calibration with 10 participants was performed. Two assessments of the scoring criteria of the index was performed with 1-hour interval. When the strength of the kappa coefficient was 0.78, the data was recorded.

Experimental procedure

Clinical Trial was registered in ClinicalTrials.gov PRS on July 4th 2019 (ID: NCT03989427). Following selection of participants after applying inclusion and exclusion criteria, written informed consent was obtained. Participants received instructions about oral hygiene from DJ. They were subjected to a thorough dental prophylaxis for complete removal of dental plaque from JG. Then, dental plaque was stained with disclosing agent followed by a mouth rinsing with water for 1 min. All erupted teeth, except for third molars, were examined by a blind examiner (SA) at 6 sites per tooth (mesio-buccal, mid-buccal, distobuccal, mesio-lingual, mid-lingual, and distolingual surfaces) for RMNPI score and BPI score.

To ensure standardization, the participants were briefed on the type of toothbrush, toothpaste and dental floss to be used in the study. The amount of dentifrice used was equivalent to half-length of the toothbrush's head. Additionally, the Bass method of brushing and Spool method of flossing was taught to the participants during the baseline appointment. . The methods of toothbrushing and flossing were explained to the participants by a step-by-step video. The participants were instructed to brush for two minutes which corresponds to 1 min for each hemiarch, 30s for buccal surfaces and 30 s for lingual/palatine surfaces.

Cross-over design was used. The participants were studied in two phases with a 1-week wash-out interval. In the first phase of the study, 15 participants were asked to floss first and brush later for two weeks (method FB) and the remaining 15 participants were asked to brush first and floss later for two weeks (method BF). In the second phase, the participants were asked to do the opposite, i.e. floss after brushing, for another two weeks (method BF) and vice versa. In the wash-out period, the participants were asked to use their toothbrush and floss according to their usual routine. Dental prophylaxis was repeated at the beginning of the second phase in order to create the same baseline conditions as at the start of the first phase and study indices were measured again. For measuring RMNPI score, disclosing tablets were given to the participants to chew and rinse. The index score was calculated according to the guidelines.

Bleeding point index were determined at the buccal, lingual and proximal surfaces using the BPI. Periodontal probe was inserted 1 mm into the sulcus at the distal aspect of the tooth and moving it across the length of the sulcus to the mesial on the facial and lingual surfaces. Bleeding surfaces after 20–30 s was recorded. The percentage of the number of bleeding surfaces was calculated.

ADA specification of toothbrush: Soft-bristled, No. of bristle are 80-85 per tufts, No. of rows are 2- 4 rows per brush, Length: 25.4-31.8mm. Width: 7.9-9.5mm. Modified bass technique Dental floss: A thin waxed, fluoridated dental floss used to clean between the teeth[3], spool method

Dentrifice: toothpaste containing 1450 ppm fluoride. Brushed for three minutes with their assigned toothbrush with 1.5 g of toothpaste[3]

4.5 Statistical Analysis

We used Microsoft Excel for data entry and SPSS version 18 was used for data analysis. We calculated change score (Post – Pre) for outcome variables such as BPI scores and RMNPI scores. Descriptive statistics such as mean and standard deviation were calculated the outcome variables.

4.6 Data Collection and Flow Chart
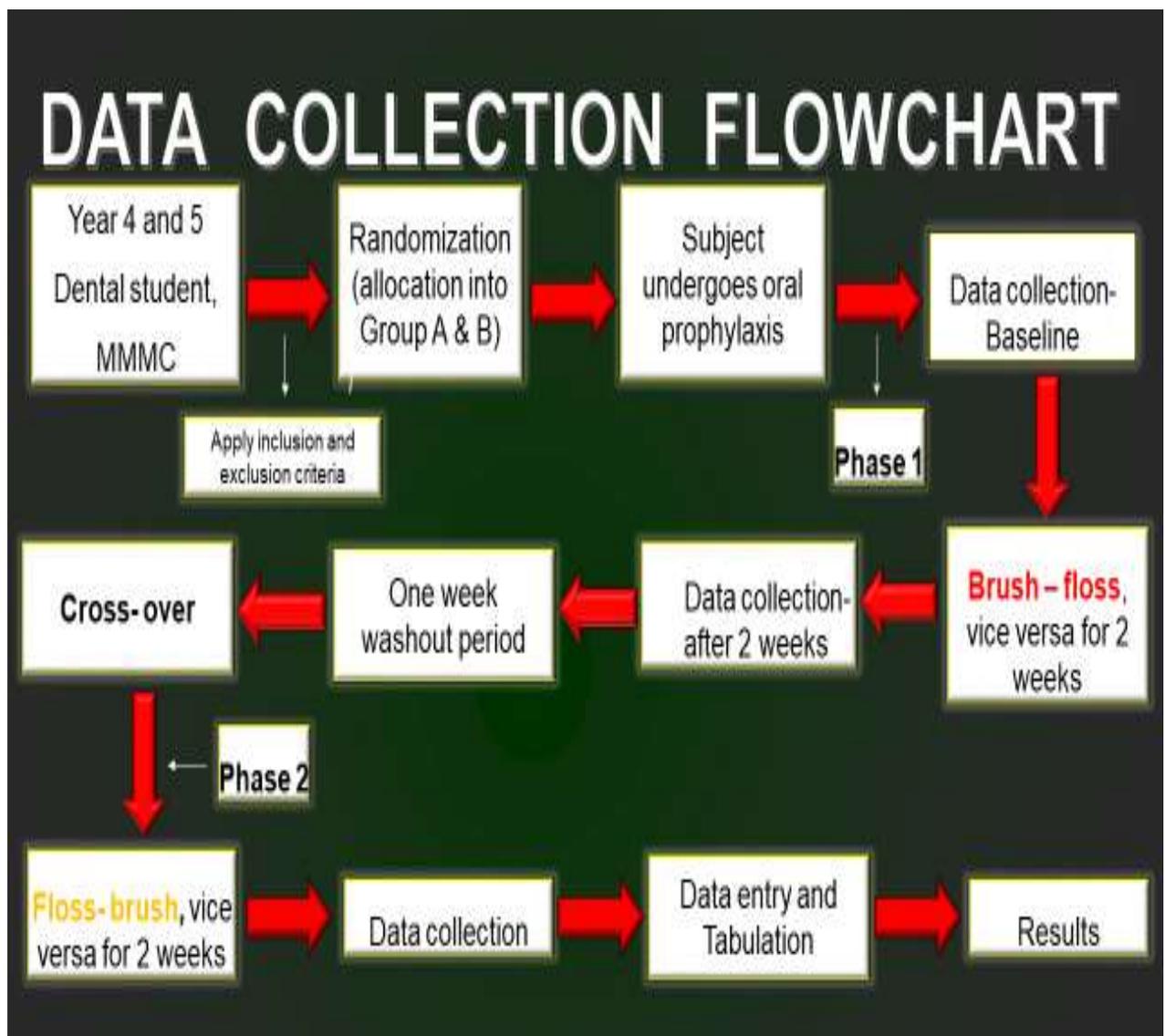
4.7 Ethical Consideration

a. Ethical approval was obtained from Institutional Ethics and Research Committee of Melaka-Manipal Medical College(MMMC/FOD/AR/B6/E C-2019 (21)

b. Written consent form was given to the participants before conducting the study and informed consent taken.

References

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