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 in compliance with the declaration of Helsinki for clinical trials.

NAME OF RESEARCHERS
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4.4 Variables and Research Tools

4.5 Statistical Analysis

CHAPTER 5: RESULTS

5.1 Descriptive Statistic/Sociodemographic Profiles

5.2 Table/Graphs

5.3 Inferential Statistic answering the objectives
**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

   \[
   \text{RMNPI Score} = \frac{\text{Sum of all } "1" \text{ marks}}{\text{Total number of sites score}}
   \]

2. **BPI- Bleeding point index (bleeding point index, BPI; Lenox et al,**
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 = \( \frac{\text{Number of bleeding surface}}{\text{total number of dental surfaces}} \times 100 \)

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

\[
\frac{\text{Number of bleeding surface}}{\text{total number of dental surfaces}} \times 100
\]

**List of abbreviation**

FB-Flossing and brushing
BF-Brushing and flossing
BPI-Bleeding point Index
RMNPI-Rustogi modified navy plaque index
DJ- Deevatharshini Jayabalanc
SA- Sacha Augustus
JG- Jayahneiswary Ganesan
DHE-Dental Health Education
MMMC- Melaka-Manipal Medical College.
Statistical Analysis Plan

Participant Flow Overview
Protocol Enrolment: 30
Total Started in Participant Flow: 30
Total arms :2

Arm1: Brushing first flossing later (BF)
Arm2: Flossing first Brushing later (FB)
Number of participants per arm :15 each

Variables and Research Tools-
Independent variables: age , sex, ethnicity, Academic year , Treatment order
Dependent variables are : BPI scores and RMNPI scores.

Research tools: We will be using Microsoft Excel for data entry and SPSS version 18 for data analysis. We will calculate change score (Post – Pre) for outcome variables like BPI and RMNPI scores. Descriptive statistics such as mean and standard deviation were calculated the outcome variables

Baseline characteristics of the population;
1. Age- continuous data in years
2. Sex: Male or female in percentage
3. Ethnicity- Malay, Chinese, Indians and others in percentage
4. Academic Year- Number of participants’ year wise.
5. Study specific measurements- change score of BPI and RMNPI index.

Inferential statistics: ANOVA
Results

Sociodemographic Profile

Fig1 : Gender
Fig 2: Ethnicity

Fig 3: Academic Year
Table 1: Descriptive data

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Mean Difference (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brush-Floss</td>
<td>-0.015 (0.015)</td>
</tr>
<tr>
<td>Floss-Brush</td>
<td></td>
</tr>
</tbody>
</table>

Three-way mixed ANOVA

To determine the effect of "Intervention" on "BPI score " is said to be moderated by "order (BF-FB or FB-BF)" and "academic year" combined.

Table 2.1: Interaction between intervention, academic year and order of intervention on BPI score

<table>
<thead>
<tr>
<th>Interaction</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention<em>Academic year</em>Order of intervention</td>
<td>0.061</td>
</tr>
<tr>
<td>Intervention*Academic year</td>
<td>0.064</td>
</tr>
<tr>
<td>Intervention*Order of intervention</td>
<td>0.127</td>
</tr>
<tr>
<td>Academic year*Order of intervention</td>
<td>0.938</td>
</tr>
</tbody>
</table>

Table 2.2: BPI change score (Post – Pre) between two Brushing Flossing and Flossing Brushing

<table>
<thead>
<tr>
<th>Mean (SE)</th>
<th>Mean difference (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brushing Flossing</td>
<td>0.396 (0.410)</td>
<td>1.424 (0.221, 2.628)</td>
</tr>
<tr>
<td>Flossing Brushing</td>
<td>-1.028 (0.392)</td>
<td></td>
</tr>
</tbody>
</table>
There was no significant three-way interaction between intervention, academic year and order of intervention. Therefore, we assessed if there was two-way interaction. There were no significant two-way interaction between intervention and order of intervention, academic year and order of intervention, and intervention and academic year.

There was significant difference of BOP between brushing flossing and flossing brushing (P=0.022). Mean change score (Post – Pre) of BOP in brushing flossing was 0.396 while it was -1.028 in flossing brushing.

**Plaque score (RMNPI)**

Table 3.1: Interaction between intervention, academic year and order of intervention on plaque score (RMNPI)

<table>
<thead>
<tr>
<th>Interaction</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention<em>Academic year</em>Order of intervention</td>
<td>0.282</td>
</tr>
<tr>
<td>Intervention*Academic year</td>
<td>0.050</td>
</tr>
<tr>
<td>Intervention*Order of intervention</td>
<td>0.497</td>
</tr>
<tr>
<td>Academic year*Order of intervention</td>
<td>0.184</td>
</tr>
</tbody>
</table>
Table 3.2: Plaque (RMNPI) change score (Post – Pre) between two Brushing Flossing and Flossing Brushing

<table>
<thead>
<tr>
<th></th>
<th>Mean (SE)</th>
<th>Mean difference (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brushing Flossing</td>
<td>0.042 (1.043)</td>
<td>-0.058 (-3.335, 3.219)</td>
<td>0.971</td>
</tr>
<tr>
<td>Flossing Brushing</td>
<td>0.101 (1.427)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no significant three-way interaction between intervention, academic year and order of intervention. Therefore, we assessed if there was two-way interaction. There were no significant two-way interaction between intervention and order of intervention, academic year and order of intervention, and intervention and academic year.

There was no significant difference of plaque score (change from baseline) between brushing flossing and flossing brushing (P=0.971).