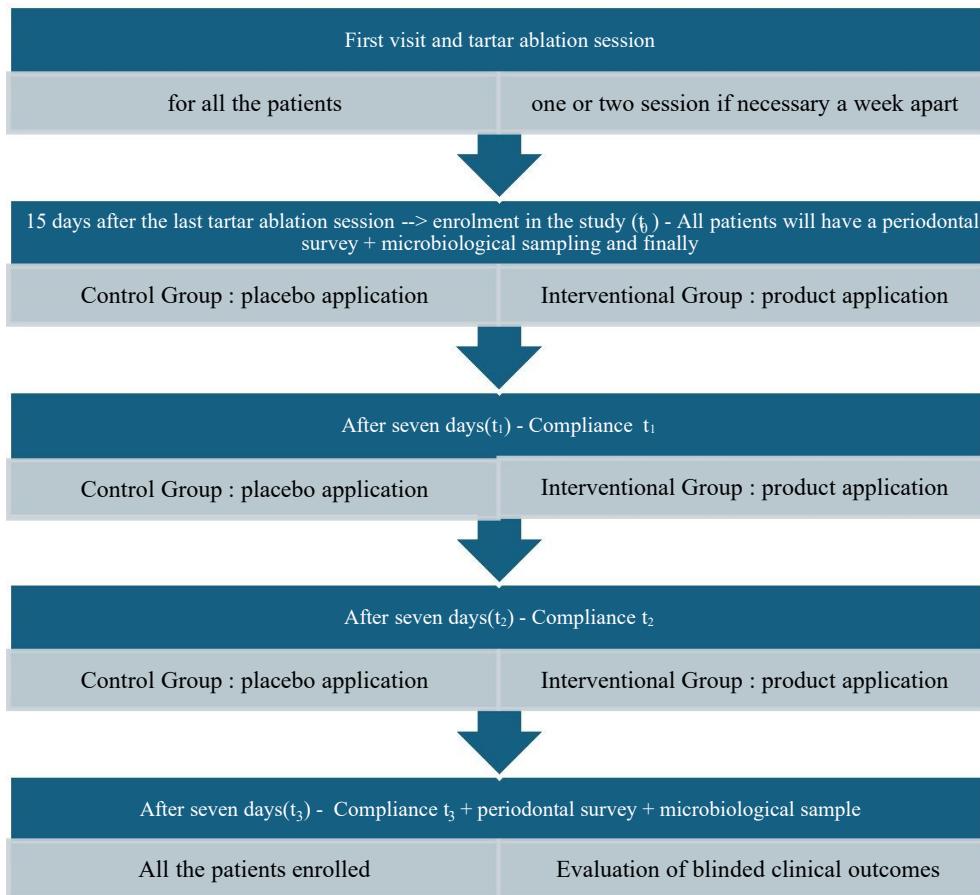


Clinical and microbiological study on local application of an Ozonated Olive Oil gel in the periodontal pockets: a randomized double-blind trial.

FLOW CHART: The study was conducted in full compliance with Good Clinical Practice and in accordance with the Declaration of Helsinki.



In this study have been enrolled 16 (sixteen) patients 8 male and 8 female who were randomized in two groups: Control group has randomized enrolled 5 males and 3 females and Interventional Group has randomized enrolled 5 females and 3 males. The average age of the patients enrolled in the study is 51 years old.

| Table 1 shows demographic data of patients enrolled in the study, divided into control group and intervention group | | | | | |
|--|------------|---------------------------------------|---------------------------|---|---------------|
| Group Control | | | Group Intervention | | |
| Pz A | Age | Gender | Pz B | Age | Gender |
| 1A | 29 years | F | 2B | 59 years | F |
| 3A | 61 years | M | 5B | 81 years | M |
| 4A | 30 years | M | 6B | 61 years | F |
| 8A | 68 years | F | 7B | 55 years | M |
| 12A | 63 years | M | 9B | 51 years | F |
| 14A | 44 years | M | 10B | 59 years | F |
| 15A | 28 years | M | 11B | 53 years | F |
| 16A | 28 years | F | 13B | 49 years | M |
| Control group average age | 43 years | Intervention group average age | 58 years | Average age patients enrolled (control + intervention) | 51 years |
| P value was calculated between the age of the two groups | | | | 0,0654 | |

All statistical tests were conducted at a significance level of .05 using Microsoft Excel Software. The significance value (p-value) was also calculated using data analysis with the t-test.

The International Standards Organisation Designation System (ISO) numbering drawn up by the WHO is used to indicate the tooth involved, while the following letters are used to indicate the site involved: the letter V to indicate the site located on the buccal side, the letter P for the site located on the palatal side, the letter L for the site located on the lingual side, the letter M for the mesial site and the letter D for the distal site. These letters can combine to indicate a specific site (e.g. MV17 - mesiovestibular site of 17). Table shows the PPD values, divided into the two groups Control and Intervention, found during the first and second survey in the three deepest sites surveyed. In addition, a further table showing the mm of PPD reduction from the beginning of the study to the end of the study, possibly expressing an improvement in the periodontal index, can be derived. Patients treated in group Control and those treated in group Intervention will be differentiated in such a way as to show significant differences between the two groups.

Table 2 shows PPD detections at the 3 selected sites measured with the periodontal survey at study enrolment (t_0) and at the end of the study (t_3) divided into groups: Control and Interventional

| Group Control | | | | Group Intervention | | | |
|---------------|------------------------------|------------------------------|------------------------------|--------------------|------------------------------|------------------------------|------------------------------|
| Pz A | Site 1 PPD Before - after | Site 2 PPD Before - after | Site 3 PPD Before - after | Pz B | Site 1 PPD Before - after | Site 2 PPD Before - after | Site 3 PPD Before - after |
| 1A | MV17 5mm – 5mm | DL46 5mm – 4mm | DV36 5mm – 4mm | 2B | MP14 8 mm – 6 mm | MP18 9 mm – 7 mm | DP28 7mm – 7mm |
| 3A | MV27 6mm – 5mm | DP17 7 mm – 7 mm | DV47 9 mm – 7 mm | 5B | V14 5 mm – 4 mm | DV45 4 mm - 3 mm | MV24 6mm – 4mm |
| 4A | DV47 10mm – 6mm | DL36 5 mm – 3 mm | DV16 7 mm – 3 mm | 6B | MV36 5 mm – 3 mm | DV16 5 mm – 3 mm | DV17 5mm – 4mm |
| 8A | DP27 4mm – 3mm | DV36 4 mm – 3 mm | DL37 4 mm – 3 mm | 7B | MP16 6 mm – 3mm | V27 4 mm – 3 mm | V44 4mm – 2mm |
| 12A | DV27 5mm – 6mm | DL37 6 mm - 6 mm | MP16 5 mm – 5 mm | 9B | DV34 7 mm – 2 mm | P14 8 mm – 2 mm | DP17 8mm – 3mm |
| 14A | MP13 7mm – 7mm | DP26 10 mm – 8 mm | MV26 12 mm – 10 mm | 10B | MV16 8 mm – 2 mm | DV26 5 mm – 3 mm | MV31 6mm – 5mm |
| 15A | ML47 4mm – 3mm | DL36 5 mm – 4 mm | MV27 5 mm – 5 mm | 11B | DP13 5 mm – 3mm | DV24 4 mm – 3mm | DV11 4mm – 2mm |
| 16A | ML45 5mm – 4mm | ML46 5 mm – 4 mm | MV16 6 mm – 5 mm | 13B | DP16 7 mm – 2 mm | DL44 5 mm – 2mm | DV33 5mm – 2mm |

Table 3 shows Δ PPD at the three selected sites measured with the periodontal survey at the time of study enrolment (t_0) and at the end of the study (t_1) divided into Control Group and Intervention Group.

| Group Control | | | | Group Intervention | | | |
|--------------------------------|----------------------------|------------------------|----------------------------|--------------------------------|---------------------------|---------------------------|------------------------|
| Pz A | Δ PPD Site 1 | Δ PPD Site 2 | Δ PPD Site 3 | Pz B | Δ PPD Site 1 | Δ PPD Site 2 | Δ PPD Site 3 |
| 1A | MV17 0 mm | DL46 -1 mm | DV36 -1 mm | 2B | MP14 -2 mm | MP18 -2 mm | DP28 0 mm |
| 3A | MV27 -1 mm | DP17 0 mm | DV47 -2 mm | 5B | V14 -1 mm | DV45 -1 mm | MV24 -2 mm |
| 4A | DV47 -4 mm | DL36 -2 mm | DV16 -4 mm | 6B | MV36 -2 mm | DV16 -2 mm | DV17 -1 mm |
| 8A | DP27 -1 mm | DV36 -1 mm | DL37 -1 mm | 7B | MP16 -3 mm | V27 -1 mm | V44 -2 mm |
| 12A | DV27 +1 mm | DL37 0 mm | MP16 0 mm | 9B | DV34 -5 mm | P14 -6 mm | DP17 -5 mm |
| 14A | MP13 0 mm | DP26 -2 mm | MV26 -2 mm | 10B | MV16 -6 mm | DV26 -2 mm | MV31 -1 mm |
| 15A | ML47 -1 mm | DL36 -1 mm | MV27 0 mm | 11B | DP13 -2 mm | DV24 -1 mm | DV11 -2 mm |
| 16A | ML45 -1 mm | ML46 -1 mm | MV16 -1 mm | 13B | DP16 -5 mm | DL44 -3 mm | DV33 -3 mm |
| Average Δ PPD | -0.875 mm | -1 mm | -1.375 mm | Average Δ PPD | -3.25 mm | -2.25 mm | -2 mm |

Table 3 shows the results of a difference made between the values in the previous Table, clearly showing whether there was a reduction or an increase or no change in PPD (Δ PPD). For each site identified in each group, the values of Δ PPD were averaged, resulting in:
- an average reduction of 0.875 mm for site 1 of the control group;
- an average reduction of 1 mm for site 2 of the control group;

Table 4 shows the summary values of the mean reduction of PPD at the three sites identified for each group and the p value was calculated on the mean reduction of PPD at the three sites identified for each group.

| Group Control | | | Group Intervention | | |
|---|-----------------------------|--|-----------------------------|-----------------------------|-----------------------------|
| Mean Δ PPD Site 1 | Mean Δ PPD Site 2 | Mean Δ PPD Site 3 | Mean Δ PPD Site 1 | Mean Δ PPD Site 2 | Mean Δ PPD Site 3 |
| -0.875 mm | -1 mm | -1.375 mm | -3.25 mm | -2.25 mm | -2 mm |
| Mean Δ PPD of all the sites of control group | -1.08 mm | Mean Δ PPD of all the sites of intervention group | -2.5 mm | p value | 0,0015 |

reduction of 2 mm for site 3 of the intervention group.

- an average reduction of 1.375 mm for site 3 of the control group;
- an average reduction of 3.25 mm for site 1 of the intervention group;
- an average reduction of 2.25 mm for site 2 of the intervention group;
- an average

Table 4 shows the summary values, listed above, of the mean reduction of PPD at the three sites identified for each group, then the mean reduction of PPD at all sites was calculated, which corresponds to -1.08 mm for the control group and -2.5 mm for the intervention group. For statistical purposes, the significance value (p value) on the mean reduction of PPD at the three sites identified for each group was calculated and it is 0.0015 therefore, it is less than 0.05 and this establishes that the mean reduction of PPD at the three sites identified of the intervention group is statistically significant compared to the mean reduction of PPD at the three sites of the control group.

Table 5 shows the data on the total microbial load found in the first and second microbiological sampling. The cell next to it shows the changes in percentage increase or decrease in the total microbial load detected by the two tests

| Group Control | | | | Group Intervention | | | |
|---------------|--|---|---------|--------------------|--|---|----------------|
| Pz A | Total microbial load in the first test | Total microbial load in the second test | % | Pz B | Total microbial load in the first test | Total microbial load in the second test | % |
| 1A | 315089 | 365101 | 15,87% | 2B | 9191825 | 883461 | -90,39% |
| 3A | 343899 | 324567 | -5,62% | 5B | 3960222 | 52360 | -98,68% |
| 4A | 442355290 | 7470638 | -83,11% | 6B | 112536 | 12093 | -89,25% |
| 8A | 645010 | 38298 | -94,96% | 7B | 4349829 | 177747 | -95,91% |
| 12A | 29928098 | 17246417 | -42,37% | 9B | 501307 | 394825 | -21,24% |
| 14A | 62634388 | 79553873 | 27,01% | 10B | 6649643 | 904892 | -86,39% |
| 15A | 14584057 | 14323174 | -1,79% | 11B | 8403042 | 12578 | -99,85% |
| 16A | 30809235 | 75890416 | 146,32% | 13B | 9010221 | 460648 | -94,89% |
| | Mean % Control group | -4,72% | | | Mean % Intervention gruppo | | -84,58% |

Table shows the data on the total microbial load found in the first and second microbiological sampling. The cell next to it shows the changes in percentage increase or decrease in the total microbial load detected by the two tests obtained by the following calculation:
[(total charge test 2 - total charge test 1) X 100]/ total charge test 1

It is clear from the percentage value obtained whether there is a decrease or increase in the total microbial load detected. The average value of percentage increase or decrease in group A results in an average reduction of 4.72%, while in group B an average reduction of 84.58% in the total microbial load was obtained.

As shown in the flow chart, compliance was collected at each follow-up meeting one week apart for 3 weeks. No side effects from the administration of the gel were recorded.

The evaluation of the study's limitations is important in order to identify any bias. The sample of 16 patients examined may be small in order to highlight any statistically significant differences sought by the investigator, which is why the study aims to be a pilot study and on the basis of the results that have been highlighted it may be a starting point for other investigators, also by virtue of the second limitation identified, namely monocentricity. In fact, the trial was carried out in a single centre.

Results show a significant reduction of PPD (Control Group -1,08mm versus Intervention Group - 2,5 mm; p value = 0,0015). Due to low number of subject statistical analysis of total microbial load was not performed (Control Group -4,8% versus Intervention Group -84,6%).

In conclusion, the gel based on ozonated EVO olive oil showed effectiveness as adjuvant treatment of periodontal pockets in non-diabetic young adults patients, anyway our study was underpowered because of slowness of inclusion.