Preliminary considerations.

The present clinical trial is being conducted at the University of Pisa. Sixty patients were considered eligible for the experimental part of the study. Inclusion criteria were: diagnosis of diabetes mellitus (both type 1 and 2), diagnosis of periodontitis, age between 18 and 70 years old. Exclusion criteria were: pregnancy, conditions required antibiotic prophylaxis, psychiatric disorders, severe physical handicap, carcinoma, immunosuppressive therapy, hospital in-patient, vitamins supplements or antibiotic taken within 3 months or periodontal therapy received within 6 months. At each time point of the follow-up, the following clinical parameters were recorded:

- PPD. Standard periodontal probe at 6 sites (mesio-buccal, med-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) per tooth (excluding the third molars). This parameter is measured as the distance from the free gingival margin to the apical extent of the pocket/sulcus.
- Marginal BOP or full mouth bleeding score: assessed following the PPD measurements at 6 sites per tooth, 30 second after applying the periodontal probe with a constant force. BOP is recorded as 1 (present) or (if absent). A percentage is going to be derived.
- Overall plaque amount or full mouth plaque index measured dichotomously and then a percentage value is going to be derived.

At each time-point, samples of saliva and digital blood were taken in order to dose the following parameters:

- Whole saliva sample (not stimulated)
  - Total antioxidant capacity (SAT test)
- Digital blood sample or venous blood sample
  - Total antioxidant capacity (PAT test)
  - dROMs test (total amount of reactive oxygen species)
  - Glycated Haemoglobin HBA1C

At baseline, patients were randomly allocated to one of the following treatment groups:

1. Professional non-surgical periodontal treatment (ultrasonic and mechanical debridement) + chlorhexidine mouthwash
2. Professional non-surgical periodontal treatment (ultrasonic and mechanical debridement) + anti-oxidant mouthwash
3. Professional non-surgical periodontal treatment (ultrasonic and mechanical debridement) + domiciliary ozone delivery system.

The mean age of the cohort resulted 60.9 years old.

The mean body mass index 27.8.

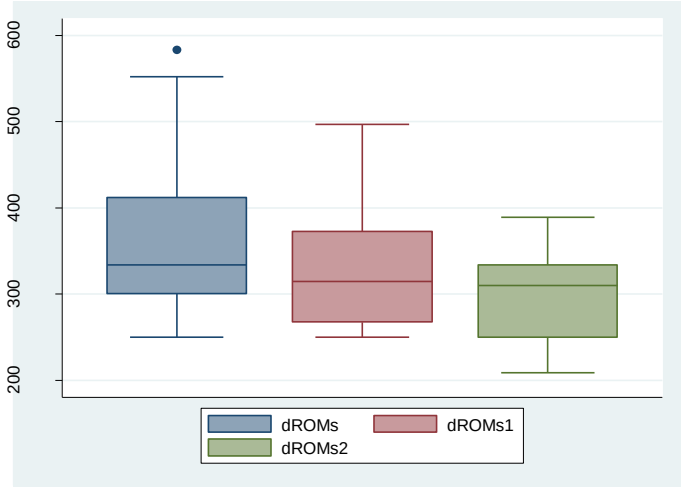
The tables below report mean and standard deviation of each of the variable taken into account.

dROMs

Time point	*Variable*	*Mean*	*Std. Dev.*	*Range*
Baseline	dROMs T_0	357	87.8	Middle level of OS
1 month	dROMs T_1	327	71.6	Low level of OS
3 months	dROMs T_2	296	51.8	Normal range

Time point	*Variable*	*Mean*	*Std. Dev.*	*p-value*
T_0-T_1	ΔdROMs$_1$	-26.8	55.9	**0.0296**
T_0-T_2	Δ dROMs$_2$	-62.0	160	**0.0198**

The tables above report mean values of the amount of plasmatic free-radicals at each time-point for the entire cohort. At baseline, the mean **dROMs** was 357, a value that belongs to a pathological range. Three months after non-surgical periodontal treatment, the mean value recorded was 296 which is normal. Using the Wilcoxon-signed rank test for matched data, the difference between dROMs at baseline and dROMs after three months appeared statistically significant (p-value=0.0198). It is remarkable that, stratifying data for anamnesis of major complications of DM, dROMs was higher in patients that had a previous history of DM complications.

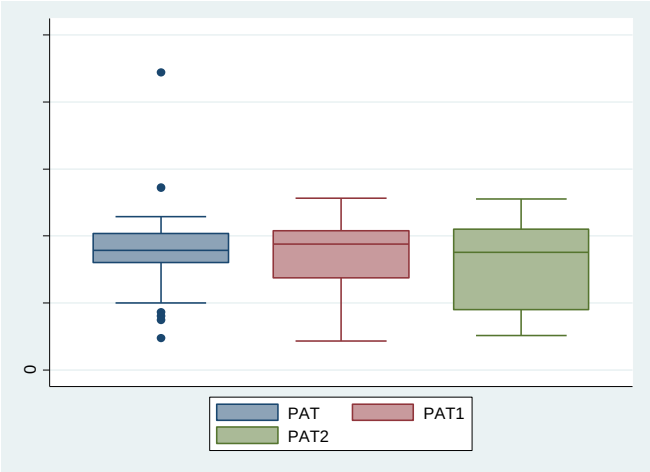


PAT

Time point	*Variable*	*Mean*	*Std. Dev.*	*Range*
Baseline	PAT	1789	739	Deficiency
1 month	PAT T_1	1680	588	Deficiency
3 months	PAT T_2	1528	674	Deficiency

Time point	*Variable*	*Mean*	*Std. Dev.*	*p-value*
T_0-T_1	ΔPAT$_1$	-112	859	0.3313
T_0-T_2	ΔPAT$_2$	-303	1004	0.2736

The tables above report mean values and longitudinal matched differences for the plasmatic antioxidant capacity (**PAT**). At baseline patients presented a deficiency status that would not change after three-months.

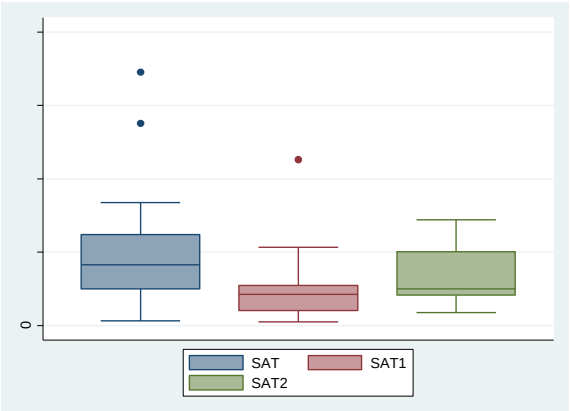


SAT

Time point	*Variable*	*Mean*	*Std. Dev.*	*Range*
Baseline	SAT	2006	1495	Border-line
1 month	SAT1	974	906	Shortage
3 months	SAT2	1372	760	Optimal

Time point	*Variable*	*Mean*	*Std. Dev.*	*p-value*
T_0-T_1	ΔSAT_1	-1188	1823	**0.0020**
T_0-T_2	ΔSAT_2	-656	1450	0.0605

The tables above report mean values and longitudinal matched differences for the salivary anti-oxidant capacity. At baseline the SAT test resulted in a mean value of 2006 which is to be considered “high”. High values are related to a possible inflammatory status. After three months, the **SAT** came back to an optimal status.

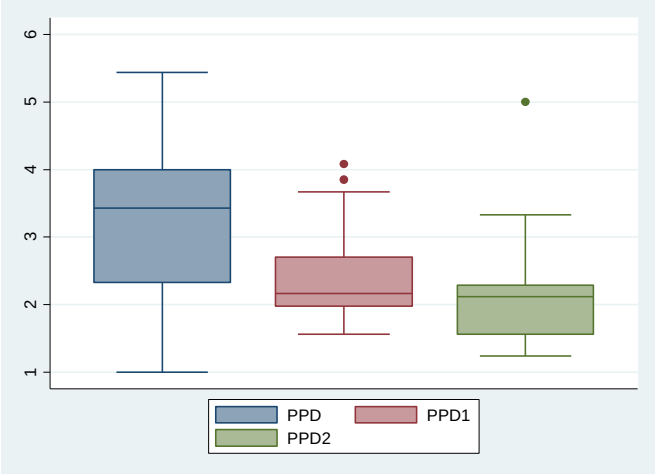


PPD

Time point	*Variable*	*Mean*	*Std. Dev.*	*Range*
Baseline	PPD	3.23	1.05	Pathological
1 month	PPD1	2.39	.706	Normal
3 months	PPD2	2.13	.85	Normal

Time point	*Variable*	*Mean*	*Std. Dev.*	*p-value*
T_0-T_1	ΔPPD_1	-.79	1.17	**0.0051**
T_0-T_2	ΔPPD_2	-.95	1.00	**0.0009**

Periodontal probing depth was pathological at baseline (mean value= 3.23) and it resulted significantly reduced after three months (mean value=2.13).

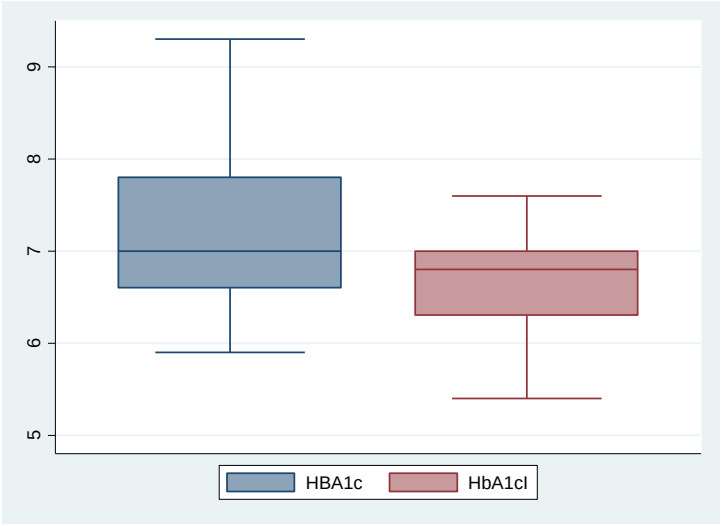


HbA1c

Time point	*Variable*	*Mean*	*Std. Dev.*
Baseline	HbA1c (I)	7.16	0.89
3 months	HbA1c (II)	6.64	0.40

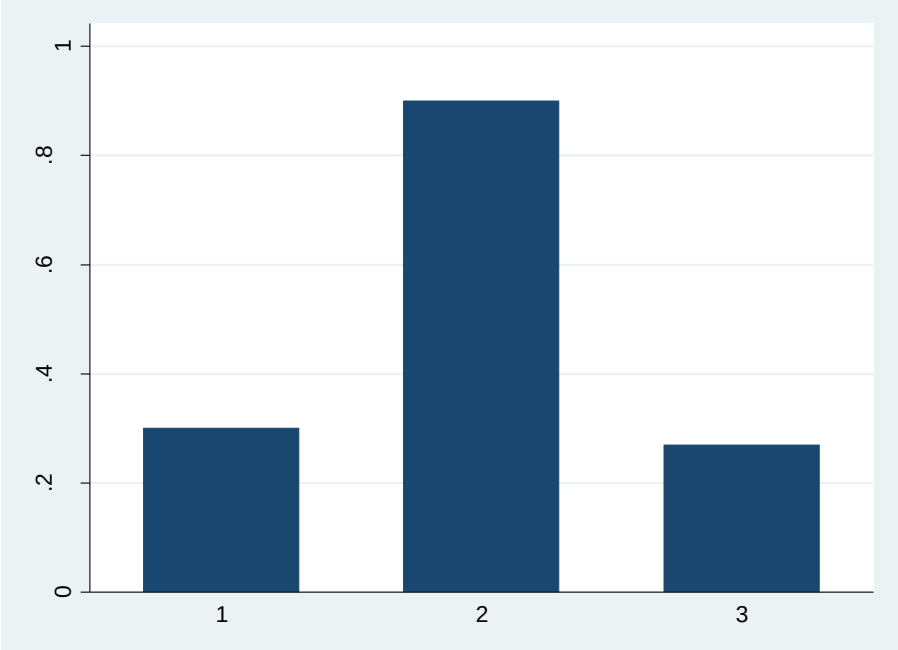
Time point	*Variable*	*Mean*	*Std. Dev.*
T_0-T_2	ΔHbA1c	.56	.70

Each patient has been diagnosed with diabetes and they had their glycated hemoglobin being monitored by their physicians. Mean **HbA1c** at baseline was 7.16 and it went down to 6.64 after three-months. The reduction is almost significant (p-value=0.08).

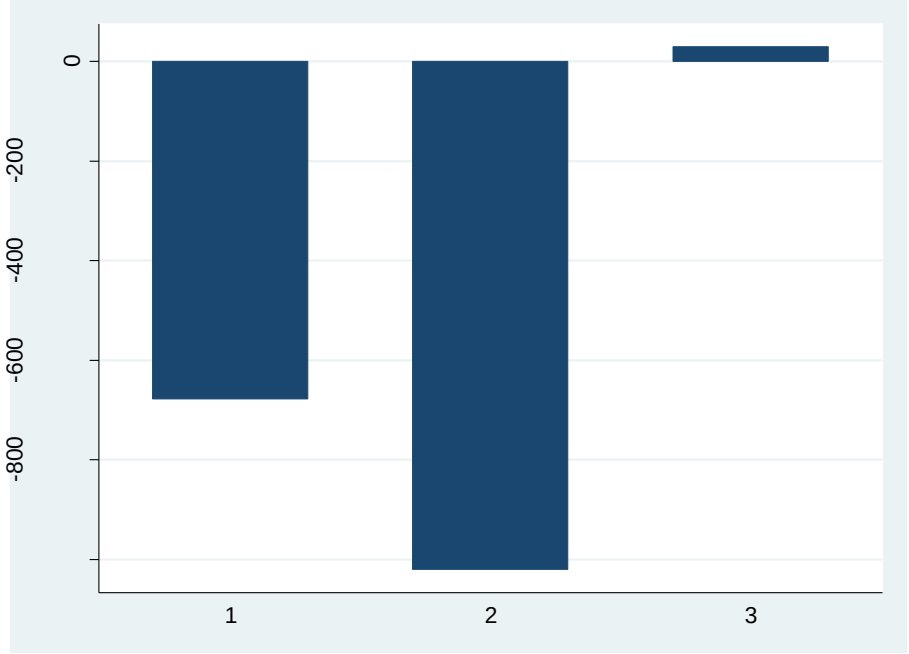


The *Kruskal-Wallis equality-of-populations rank test* showed that **Ozone test group** had better outcomes in terms of SAT and HbA1c reduction over time. Those results are not significant but they give a hint for further investigations.

GRAPH for mean variation of glycated hemoglobin by group of treatment.

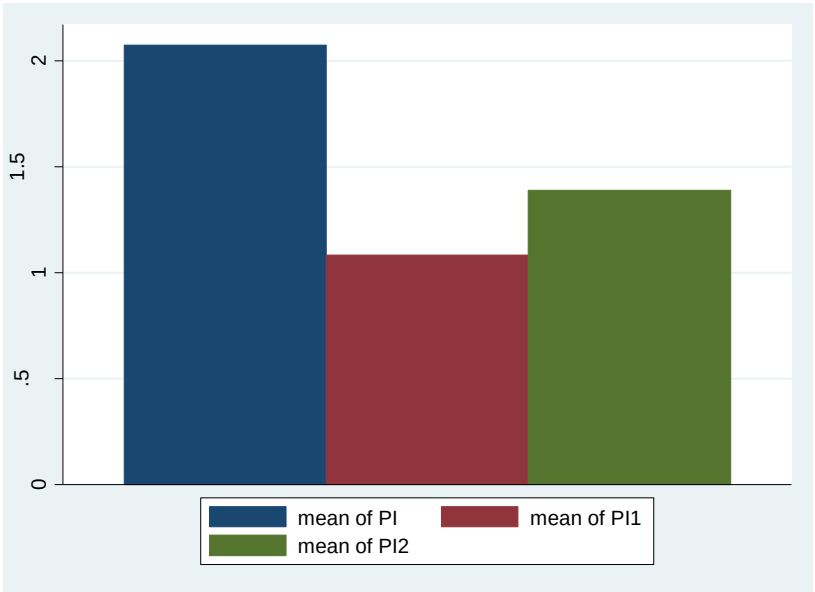


GRAPH for mean variation of salivary antioxidant capacity by group of treatment.



PLAQUE INDEX

Time point	*Variable*	*Mean*	*Std. Dev.*	*Range*
Baseline	PI	2.07	0.87	Pathological
1 month	PI1	1.08	0.92	Normal
3 months	PI2	1.38	0.97	Normal



DISCUSSION

The design of the present study is the one of a prospective randomized clinical trial.

Preliminary outcomes suggested that there could be a relation between oxidative stress and periodontitis in diabetic patients. Those results are in line with previous literature.

Glycated hemoglobin represents the most used parameter to watch the glycemic status of a diabetic patient over time. Recent literature, anyway, highlights how this parameter could not be considered the only marker to prevent diabetes complications.

Oxidative stress takes part in the pathogenesis of each one of the major complications of diabetes, periodontal disease being included.

The partial results of the present study suggested that the SAT test could be useful in assessing the risk of a diabetic patient of developing secondary morbidities related to oxidative stress. The salivary antioxidant capacity can increase secondarily to the presence of a chronic inflammatory status. Chronic inflammation causes free-radicals release which in turn can elicit the production of an excess of reactive antioxidants.

Pisa, June 2016