BMC Oral Health

RESEARCH

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"The effects of non-surgical periodontal treatment plus zinc and magnesium supplementation on oxidative stress and antioxidants enzymes in type 2 diabetes patients: a quasi-experimental study"

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Abstract

Background Periodontal Disease (PD) associated with Type 2 Diabetes Mellitus (T2DM) is a chronic condition that affects the oral cavity of people living with T2DM. The mechanisms of the interaction between type 2 Diabetes Mellitus and Periodontal diseases are complex and involve multiple pathophysiological pathways related to the systemic inflammatory process and oxidative stress. Non-surgical periodontal treatment (NSTP) is considered the standard for the management of this disease; however, patients with systemic conditions such as type 2 Diabetes Mellitus do not seem to respond adequately. For this reason, the use of complementary treatments has been suggested to support non-surgical periodontal treatment to reduce the clinical consequences of the disease and improve the systemic conditions of the patient. The use of zinc gluconate and magnesium oxide as an adjunct to non-surgical periodontal treatment and its effects on periodontal clinical features and oxidative stress in patients with Periodontal diseases -type 2 Diabetes Mellitus is poorly understood.

Methods A quasi-experimental study was performed in patients with periodontal diseases associated with T2DM. Initially, 45 subjects who met the selection criteria were included. 19 were assigned to a control group [non-surgical periodontal treatment] and 20 to the experimental group (non-surgical periodontal treatment + 500 mg of magnesium oxide and 50 mg of zinc gluconate for oral supplementation for 30 days) and the data of 6 patients were eliminated. Sociodemographic characteristics, physiological factors, biochemical parameters, and clinical features of periodontal diseases were assessed.

Results In this research a change in periodontal clinical characteristics was observed, which has been associated with disease remission. Additionally, a shift in MDA levels was presented for both groups. Furthermore, the

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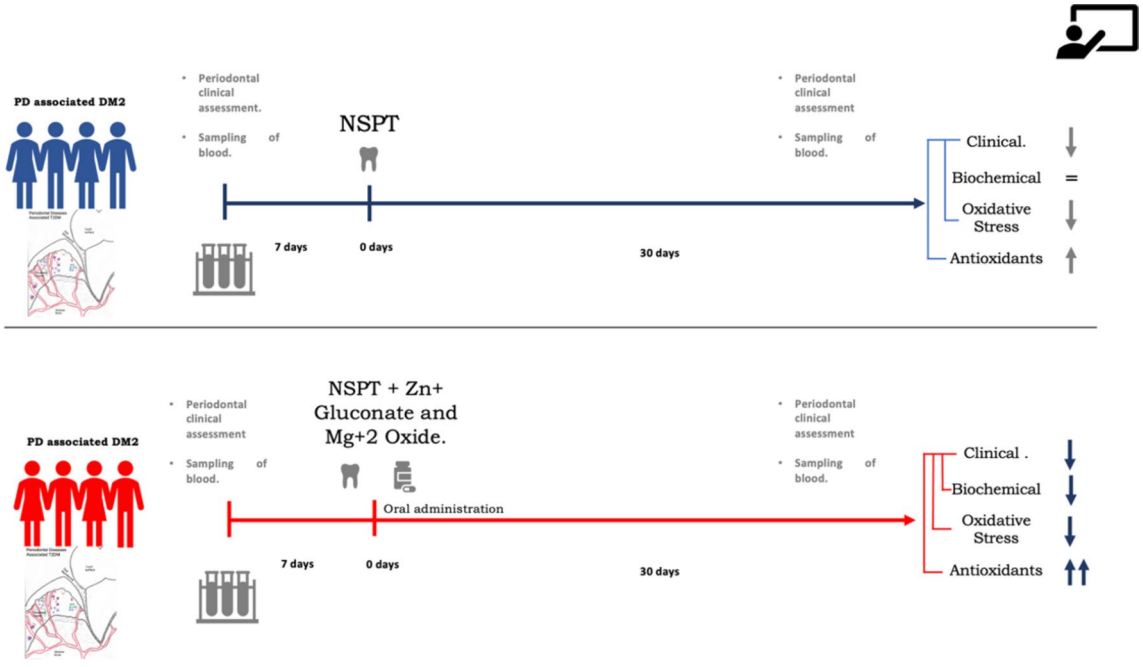
supplementation group showed an increase in antioxidant enzymes when compared to the group that only received NSPT.

Conclusion The use of Zinc gluconate and magnesium oxide can serve as a complementary treatment to non-surgical periodontal treatment, that supports the remission of PD as a result of regulation-reduction of oxidative biomarkers and increase in antioxidant enzymes activity.

Trial Registration https://www.isrctn.com ISRCTN 14,092,381. September 13º 2023. Retrospective Registration.

Graphical Abstract



Keywords Type 2 diabetes mellitus, Periodontal diseases, Non-surgical periodontal treatment, Zinc and magnesium, Antioxidant, Oxidative stress

Introduction

Periodontal Diseases (PD) are characterized by a chronic inflammatory process that affects the anatomical structures supporting the tooth. On the other hand, Type 2 Diabetes Mellitus (T2DM) is a non-transmissible chronic disease characterized by high glucose levels [1, 2]. Research studies on the relationship between PD and Diabetes Mellitus 2 (DM2) have reported an increase in oxidative stress (OS) and the presence of free radical-reactive oxygen species (ROS) as a response to damage in periodontal tissues and the severity of PD [3–8] The formation of ROS promotes the process of lipid peroxidation resulting in increased levels of aldehydes such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) locally or systemically [9] which has been associated with oral problems in patients with DM2. The antioxidant defense system is activated to counteract the damage caused by oxidative stress. Its primary function is to neutralize and stop the oxidizing actions of free radicals to prevent damage to cells and tissues [10] This system has various enzymes, from which Superoxide dismutase (SOD) and Catalase (CAT) stand out [11, 13]. However, in patients with PD associated with T2DM, the number of antioxidants enzymes is deficient. This contributes to the uncontrolled increase of free radicals and consequently the progression of both pathologies [14].

On the other hand, its deficiency of Zinc (Zn^{+}) and Magnesium (Mg^{+2}) in patients with PD-DM2 has been reported [15, 16] These chemical elements are abundant in the body and are basic components of our diet [19]. The importance of studying these micronutrients lies in their involvement in multiple biologic functions, such as metabolic processes related to glucose homeostasis, where Zn^{+} participates in secretion, storage and transport processes [20, 21]. Similarly, Mg^{+2} is involved in insulin sensitivity processes through its participation in the activation of ISR-1 and oxidative phosphorylation. In muscle contraction, they are involved in ions channels [18]. Additionally, they regulate the system immune by participating in signaling pathways in cellular

subpopulations. Finally, it been recognized that these elements contribute to the activation of intracellular pathways that promote the production and activity of antioxidant enzymes, where they are essential co-factors. The suggested recommendations for magnesium intake in adults are at least 100 mg per day, while for zinc, a consumption of 15 mg per day [15–21]. The use of Zn^{+} and Mg^{+2} as a dietary supplement has been shown to be effective in addressing cardiovascular problems and dyslipidemia in patients with DM2 [22].

Regarding the therapy for PD associated with T2DM, clinical practice guidelines suggest non-surgical periodontal treatment (NSPT) as the standard treatment [23]. Its goal is focused on the remission of PD and the stability of tissues through the regulation of oxidative stress [24] and the inflammatory process [25] thus reducing the clinical parameters of the disease, improving the oral and systemic health of patients who receive it.

Nonetheless, in patients with chronic conditions such as DM2 combined with poor glycemic control, the response to this treatment of periodontal tissues is not as expected [26]. Due to this, the use of treatments that complement NSPT has been proposed. In this regard, supplementation with Zn^{+} and Mg^{+2} has been reported to have benefits in addressing deficiencies of these elements described in patients with DM2 [27, 28]. Meanwhile, Yu et al., 2021 described the supplement's influence on the reduction of OS and increasing antioxidant enzymes [29, 30].

Therefore, the aim of this study was to evaluate the effects of NSPT plus Zn^{+} gluconate and Mg^{+2} oxide in patients with PD associated with DM2. It has been proposed as a hypothesis that supplementation with trace elements as complements to NSPT supports the improvement of periodontal clinical conditions through the regulation of OS and the increase of antioxidant enzymes SOD and CAT.

Materials and methods

Study design

A quasi-experimental, prospective, and longitudinal study was performed, patients with T2DM and PD under different medical treatments for diabetes control (oral hypoglycemic agents, insulin and combinations) who met the inclusion criteria were selected by convenient sampling method were included (males and females, aged 18–60 who attended the periodontal department for the first time and had at least 10 teeth in the oral cavity). Following written informed consent; a periodontal examination and clinical probes (biochemical analyses) was conducted.

Initially, 45 patients were included and assigned into to two groups by consecutive cases sampling: The control group received Non-Surgical Periodontal treatment (NSPT) and oral hygiene instruction for 30 days; the experimental group, in addition to NSPT and oral hygiene instruction, received 500 mg/day of magnesium oxide and 50 mg of zinc gluconate in oral administration for 30 days. During the research process, 6 patients were eliminated for not continuing their periodontal treatment, supplementation period and for missing their analytical test, leaving ($n=19$) in the control group and ($n=20$) in the experimental group. We excluded anyone reporting previous NSPT, pregnancy, any condition requiring antibiotics prior to dental treatment, and hypersensitivity to Zn^{+} and Mg^{+2} supplementation. This study was approved by the research ethics committee of the Institute of Health Science from Universidad Veracruzana and the ethical committee of Dr. Rafael Lucio Hospital (F.10/2022.) and received code of ISRCTN 14,092,381 13/09/23.

Periodontal examination

Participants underwent periodontal examination conducted by the principal investigator who trained as a dental surgeon in conjunction with the periodontics specialist to evaluate periodontal clinical parameters such as Periodontal Probing Depth (PPD) using a standard manual periodontal probe (North Caroline, Hu-Friedy). This test was estimated by considering the distance from the gingival margin to the bottom of gingival sulcus and the periodontal pocket at six sites per tooth (expressed in mm.).

Bleeding on probing (BoP) was recorded, the periodontal probe was inserted to the bottom of the gingival sulcus or periodontal pocket, applying light force and gently moving along the tooth surface. If bleeding occurred upon removing the probe and waiting a few seconds, it was considered a positive site. Three buccal sites (mesial, middle, distal) and three palatal o lingual sites (mesial, middle, distal) were evaluated. The number of site positives with bleeding was identified, and the result was divided by the total number of sites evaluated per tooth. Periodontal Inflamed surface area (PISA) was measured using the algorithm suggested by Nesse et al. 2008 [31], and bacterial dental plaque index (DBPI) by Turesky-Glickman modified to Quigley Hei (1970) [32].

All patients were instructed in oral hygiene using the Stillman-modified brushing technique, dental flossing and monitoring of bacterial dental plaque before the periodontal intervention. Non-surgical periodontal treatment was conducted by quadrants, with weekly sessions over course of one month using manual instruments (Gracey curettes -Hu-Friedy) and ultrasonic instruments (DTE ultrasonic system) with local anesthesia.

Biochemical analyses

Venous blood samples were obtained from all patients at baseline and four weeks following NSPT. Patients were scheduled at 7:00 am (they were asked to attend with a 12-hour fast,

following the recommendation to eat a light dinner and avoid alcohol consumption). Serum glucose levels, lipid profile, serum magnesium, and serum zinc were quantified. Clotted blood samples were centrifuged (1500 rpm 10 min RT) for serum separation. Aliquots were frozen at -80 ºC for storage prior to analysis. MDA levels were analyzed using a commercial kit for lipid peroxidation (Abcam, USA, Catalogue Nº AB233471, lot. GR3359468-1). The activity of the antioxidant enzymes SOD and CAT was determined using commercial kits (SOD Abcam, USA, Catalogue AB65354, lot. GR3362240-1; CAT Abcam, Catalogue AB83464, lot GR3301240-1). During each session of the NSPT, patients were monitored before the intervention with tests for: fasting blood glucose, blood pressure, and oxygen saturation.

Table 1 Sociodemographic, physiological, biochemical and clinical characteristic of patients with PD associated T2DM before NSPT

	Mean (± SD)		
	Control Group (NSPT)	Experimental Group (NSPT + Zn and Mg)	p value
Age in years	51.7 ± 12	54.35 ± 8.6	0.4620
Diabetes Duration years	8.4 ± 7.6	5.9 ± 5	0.3890
Sex, Female	63.2%	80%	0.2427
BMI	29.8 ± 5.4	30.6 ± 6	0.8810
Weight	75.6 ± 19	73.4 ± 13	0.9125
% Body fat	40.0%	40.4%	0.8653
Glucose (mg/dL).	194.3 ± 89	191 ± 78	0.9034
Mg (mg/dL).	1.85 ± 0.16	1.87 ± 0.1	0.6090
Zn (mM/L).	7.2 ± 0.3	6.575 ± 0.3	0.2108
HDL-Cholesterol (mg/dL).	40.3 ± 9	39.2 ± 8	0.9723
LDL-Cholesterol (mg/dL).	112.26 ± 36	112.5 ± 47	0.9391
Total Cholesterol (mg/dL).	220.1 ± 57	196.3 ± 50	0.2495
Triglycerides (mg/dL).	350.85 ± 87	233.9 ± 31.2	0.6465
Fasting blood glucose (mg/dL).	252 ± 24	269.8 ± 33	0.8513
Systolic blood pressure (mmHg)	126.2 ± 17	125.1 ± 13	0.8186
Diastolic blood pressure (mmHg)	80.3 ± 9	79.57 ± 7	0.9500
PPD (mm)	2.8 ± 0.6	3.12 ± 0.7	0.1213
BoP (%)	0.44 ± 0.1	0.5 ± 0.1	0.0765
PISA ($mm^{3)}$	707.6 ± 394	674.5 ± 359	0.9609
DBPI (%)	2.01 ± 0.1	2.2 ± 0.3	**0.0396***

The variables study represents the mean and standard deviation

PD = Periodontal Diseases

T2DM = Type 2 Diabetes Mellitus

NSPT = Non-surgical periodontal treatment

Mg = Magnesium

Zn = Zinc

BMI = Body Mass Index

HDL-C. High Density Lipoprotein

LDL-C = Low Density Lipoprotein

PPD = Periodontal probing depth

BoP = Bleeding on probing

PISA = Periodontal inflammated surface areas

DBPI = Dentobacterial plaque index

*: Statistical significance $p < 0.05$

**: Statistical significance $p < 0.001$

Statical analyze: Mann-Whitney U Test / Chi-square Test

Statistical analyses

Data were analyzed using GraphPad Prisma 8 statical analysis program. Data are presented as mean ± standard deviation, percentage and numbers.

The normal distribution of continuous variables was analyzed using the Shapiro-Wilks test. A Poisson test was performed. The paired t-test was also used to compare the results within groups before-and-after periodontal intervention. The Chi-square test was used to compare the qualitative variables. The independent samples t- test was used to compare the results within control-and-experimental groups post-periodontal intervention; otherwise, the Mann-Whitney U test, was employed. The statistical significance level was set at $p < 0.05$.

Results

45 patients with PDassociated DM2 were included initially in the study. They were assigned into two groups [control group ($n = 19$), experimental group ($n = 20$)]. 6 patients were eliminated (3 for not completing their NSPT and supplementation dosing scheme and 3 for not attending their final laboratory tests). A comparison of sociodemographic, biochemical, and clinical periodontal characteristics of study participants at baseline not revealed significant differences (Table 1).

Non-surgical periodontal treatment reduces periodontal clinical characteristics in patients with PD associated with DM2

To evaluate the effects of NSPT in the periodontal tissue of patients with PD, clinical characteristics of the disease were measured.

Before treatment, differences between groups in DBPI ($p = 0.031$) were identified. In the control group, a mean of PPD 2.8±0.6, BoP of 0.44±0.1, PISA of 707.6±394 y DBPI de 2.01±0.1was observed, values that decrease after NSPT (PPD 2.03±0.3, BoP 0.0008±0.02, PISA 298.9±68.5, DBPI 1.55±0.4) Fig. 1A.

In the experimental group, a mean of DoP 3.1±0.7, BoP of 0.5±0.07, PISA of 674.5±359 y DBPI de 2.2±0.3

A

Clinical periodontal parameters in PD associated to T2DM patients before-and-after periodontal intervention.

	Control Group. (NSPT)		p value	Experimental Group. (NSPT + Zn^{+} y Mg^{+2})		p value
	Before	After		Before	After	
PPD	2.8±0.6	2.03±0.3	**0.001*** **	3.1±0.7	2.1±0.3	**0.001*** **
BoP	0.44±0.1	0.008±0.02	**0.001*** **	0.5±0.16	0.05±0.07	**0.001*** **
PISA	707.6±394	298.9±68.5	**0.02****	674.5±359	267.75±146.0	**0.001*** **
DBPI	2.01±0.1	1.55±0.4	**0.001*** **	2.2±0.3	1.8±0.3	**0.001*** **

The variables study represent the mean and standard deviation.
PD= Periodontal Diseases
T2DM= Type 2 Diabetes Mellitus
NSPT= Non-surgical periodontal treatment.
Mg^{+2}= Magnesium
Zn^{+}= Zinc
PPD= Periodontal probing depth in mm
BoP= Bleeding on probing.
PISA= Periodontal inflammated surface area in mm2
DBPI=Dentobacterial plaque Index.
*= Statistical significance p<0.05
**=Statistical significance p<0.01
***=Statistical significance p<0.001
Statical analyzes= Paired t-Test.

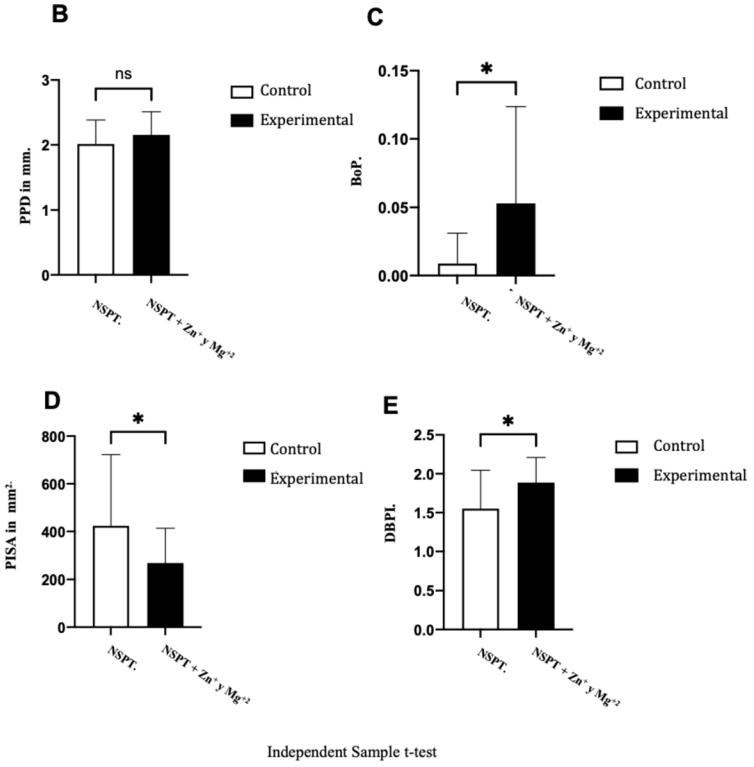


Fig. 1 Effect of non-surgical treatment on periodontal clinical parameters **A**) changes in the periodontal clinical characteristics of patients with PD association with T2DM before and after periodontal interventions **B**) Comparision of levels PPD after periodontal intervention **C**) Decrease of BoP after NSPT **D**) Decrease in PISA after NSPT puls Zn^{+} and Mg^{+2} supplementation **E**) Comparative levels in DBPI after NSPT

was identified as baseline, which decreased their values after periodontal intervention plus supplementation (Dop 2.1±0.3, CAL 3.07±1.2, BoP 0.05±0.07, PISA 267.7±144, DBPI 1.8±0.3) Fig. 1A.

The comparison of periodontal clinical characteristics after periodontal interventions between groups showed clinical differences in clinical variables such as BoP ($p=0.013$) PISA ($p=0.044$), and DBPI ($p=0.016$) Fig. 1B, C,D, E.

Non-surgical periodontal treatment regulates oxidative stress decreasing lipidic peroxidation in patients with PD associated with DM2

To evaluate the effects of non-surgical periodontal treatment (NSPT) plus Zn^{+} gluconate and Mg^{+2} oxide supplements in lipidic peroxidation related to oxidative stress, malondialdehyde levels in serum samples from patients with PD and DM2 were measured.

At baseline, mean of level of 4.21±4 nM de MDA for the group control was found, which after NSPT decreased to 2.2±0.9 ($p<0.0487$). In the experimental group, we observed levels of 3.57±nM MDA were observed after the NSPT plus Zn^{+} and Mg^{+2} supplementation decreased levels to 2.04±0.7 nM de MDA ($p<0.0001$).

There was no significant difference in the mean serum levels of MDA between the two groups ($p<0.4606$) after intervention Fig. 2.

Zn^{+} gluconate and Mg^{+2} oxide supplementation plus non-surgical periodontal treatment increases the activity of antioxidant enzymes after 30 days

To evaluate the antioxidant effects of Zn^{+} gluconate and Mg^{+2} oxide supplementation activity of superoxide dismutase (SOD) and catalase (CAT) enzymes were measured.

Both groups showed an increase in the serum levels of SOD and catalase after treatments (control group: SOD 1.02±0.2 vs. 2.11±0.1 U/ µProt., $p<0.0001$, CAT 57.52±5.2 vs. 88.22±2.5 U/µProt; $p<0.0001$; experimental: SOD 1.07±0.9 vs. 3.49±0.4; $p<0.0001$, CAT: 55.05±4 vs. 109.24±5 U/µProt; $p<0.0001$) (Fig. 3A).

However, comparison of the activity levels of SOD and CAT after treatment between groups showed that supplementation of Zn^{+} and Mg^{+2} had a significant higher increase of these enzymes' activity (Fig. 3B **and C).**

Discussion

Periodontal diseases cause alteration in the oral cavity and are related to systemic diseases such as type 2 diabetes mellitus. The late diagnosis of T2DM and poor

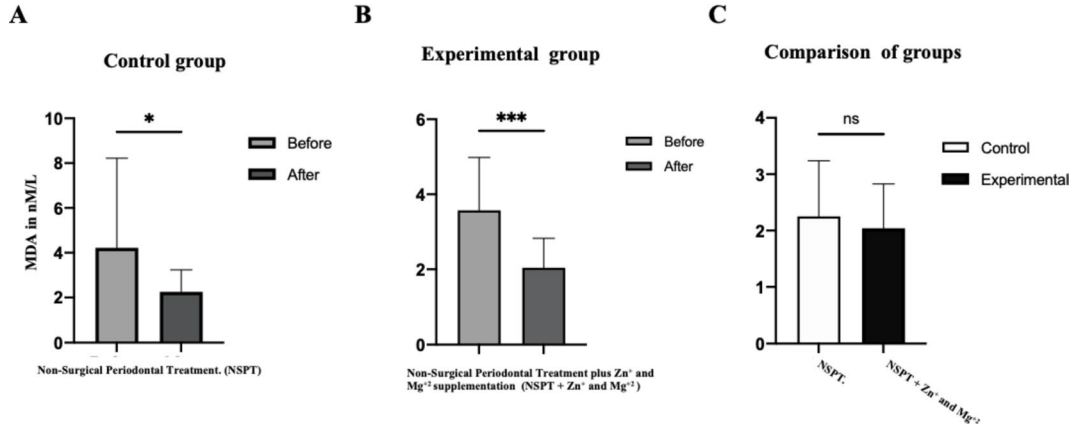


Fig. 2 Effect of non surgical periodontal treatment plus Zn^{+} and Mg^{+2} supplementation. **A**) Decrease MDA levels after NSPT. **B**) Decrease MDA levels after NSPT Plus ZN^{+} and Mg^{+2} supplementation. **C**) Comparitive MDA levels after periodontal interventions. The value of the bars represents the mean and standard deviation of MDA levels in the groups

A

Activity of antioxidant enzymes in serum of patients with PD associated to T2DM before and after treatment.

	Control				Experimental		
	before	after	p		before	after	p
SOD	1.0	2.1 ****	0.0001	SOD	1.0	3.4 ****	0.0001
CAT	57.5	88.2 ****	0.0001	CAT	57.0	109.2 ****	0.0001

PD: Peridontal disease
T2DM: Type 2 Diabetes Mellitus
SOD: Superoxide dismutase in U/mg prot.
CAT: Catalase U/mg prot.
*: Statistical significance p<0.05
**: Statistical significance p<0.001
***: Statistical significance p<0.005
***: Statistical significance p<0.0001

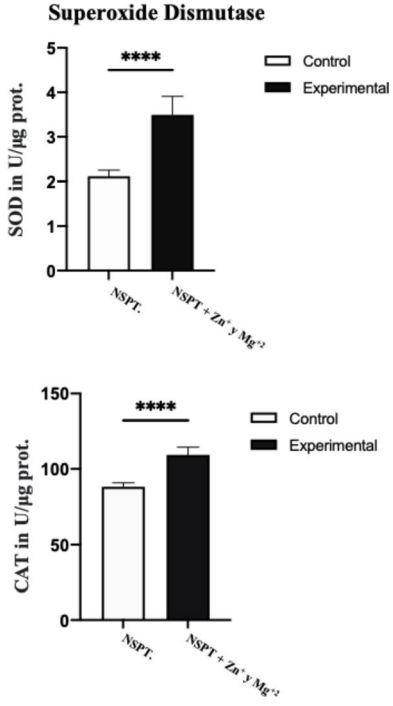


Fig. 3 Effect of non surgical periodontal treatment plus Zn^{+} and Mg_{+2} supplementation in antioxidant enzymes. **A**) Increase of activity SOD and CAT after NSPT in patients with PD associated T2DM. **B**) Comparison of SOD activity after NSPT plus Zn^{+} and Mg^{+2} supplementation. **C**) Comparison of CAT activity after NSPT plus Zn^{+} and Mg^{+2} supplementation

metabolic control could be influence the progression of PD. The severity of PD has been linked to an increase in OS and the alteration of its regulation system.

For the management of PD associated to DM2, non-surgical periodontal treatment has been suggested. However, some patients with chronic diseases do not seem to respond favorably to this treatment. In the case of patients with T2DM, it has been considered that the duration of diabetes influence in wound healing.

The objective of this research was to evaluate the effects of NSPT plus Zn^{+} gluconate and Mg^{+2} oxide for 30 days in patients with PD associated with DM2.

To our knowledge, this is the first research to report the use of Zn^{+} gluconate and Mg^{+2} oxide plus NSPT in populations affected by PD-DM2.

The results showed that the group of patients with PD and DM2 have deficiencies in levels of Zn^{+} and Mg^{+2} at baseline. This information adds to what has been reported in other studies, where DM2 has been associated with low levels of Zn^{+} and Mg^{+2} and their influence

on the development of complications such as nephropathies and PD [15, 16].

The alterations in the levels of these elements refer to their contribution in the pathophysiology of DM2 and its complications because of the dysregulation of cellular pathways where Zn^{+} and Mg^{+2} are co-factors [33–35].

Nevertheless, the pathways related to the alteration in the levels of Zn^{+} and Mg^{+2} in patients with DM2 and its co-morbidities are still unclear. In patients with chronic kidney diseases associated with DM2, deficiencies of Zn^{+} have been described to be related to the decrease in intestinal zinc absorption, uremic toxicity, as well as its bioavailability related to tubular dysfunction and glomerular filtration[36]. Further research is required to evaluate how PD influences deficiencies of these elements when associated with DM2.

The results prove the reduction of DPP, BoP, PISA, and DBPI after periodontal intervention in both groups. Mailao et al.,2015 mention that NSPT, reduces dental biofilm, dental calculus, and endotoxins followed by a change in the periodontal clinical characteristics [37], conditioning the root surfaces to be biologically acceptable and stopping the progression of PD [38]. This could suggest a positive change in the clinical response to treatment, resulting in the reduction of OS, antioxidant activity, and inflammation. More research is needed on the host response mechanisms to NSPT related to the observed clinical changes.

In this research, it was also identified that patients with DM2 and PD presented elevated levels of MDA. Research reports have linked deficiencies of Zn^{+} and Mg^{+2} to increased OS in periodontal tissues [39, 40]. Lipid peroxidation is the main mechanism of damage caused by ROS, which generates an increase in MDA levels [9]. This is an oxidative state biomarker that is related to micro and macrovascular damage during PD. Its increase in patients with DM2 and PD could indicate greater severity and inflammatory state [39, 41].

After 30 days of NSPT, a decrease in MDA levels was detected. This result contributes to the findings on the effects of NSPT on oxidative stress biomarkers. Several studies have reported that a reduction in these markers occurs 3 months after periodontal intervention [38, 42]. In this research, a reduction at 30 days after treatment is shown and clinical improvement of periodontal tissues that could be related to the decrease in MDA is identified, too. The correct execution of NSPT, proper adherence to patients' instructions, and the response of periodontal tissue to the therapy are factors that support the reduction in treatment time reported in this study [44].

On the other hand, research has shown that OS damage in periodontal disease is regulated and controlled by an endogenous system of antioxidant enzymes [5]. However, in patients with PD and DM2 with deficiencies in Zn^{+} and Mg^{+2}, the expression of antioxidant enzymes is affected, reducing their concentration and activity [45].

The results of this research demonstrate that patients presented low levels of enzymatic activity units for SOD and CAT, increasing their levels after 30 days of NSPT. Sukhtankar et al., 2013 suggest that during the inflammatory process in PD, SOD enzyme levels rise as a result of increased superoxide anion on periodontal tissues and decreases after 2 months of treatment [46]. This suggests that the antioxidants enzymes, in addition to acting on oxidative stress, could take part in recovery and wound healing processes on periodontal tissues.

Continuing with studies of NSPT and its effects in DM2 patients, Hass-Nogueira et al., 2021, proposed the use of supplements that support the remission of PD and promote periodontal stability [25, 26]. The use of essential oils, ginger, melatonin, probiotics, vitamin C, among other has been highlighted [45–49].

In this regard, this work proposes the use of zinc gluconate and magnesium oxide as a co-adjuvant of NSPT in patients with PD associated with DM2. Other studies reported the use of co-supplementation with Zn^{+} and Mg^{+2} in women with gestational DM, as they show a reduction in biomarkers of OS and an increase in total antioxidant capacity (TAC) after 6 months of use [42]. With this information, the benefits of Zn^{+} and Mg^{+2} supplementation for the management of other disease conditions are recognized.

As a result of the effects of NSPT plus the supplementation of Zn^{+} gluconate and Mg^{+2} oxide proposed in this research, it was possible to observe that, 30 days after the periodontal treatment, levels of enzymatic activity for SOD and CAT increased to a greater extent compared to the group that only received NSPT. These results add valuable information to all the research describing the participation of Zn^{+} and Mg^{+2} as co-factors in the synthesis of antioxidant enzymes and how supplementation increases their activity [53, 54]. This research suggests that the increase in the levels of antioxidant enzymatic activity by SOD and CAT is one of the response mechanisms to protect and recovery of periodontal tissue affected by PD associated with DM2. The role of these enzymes in periodontal healing processes should be further evaluated.

Further research is necessary to describe the cellular biological pathways involved in the regulation of oxidative stress, the inflammatory process, and the subsequent antioxidant response following supplementation with Zn^{+} and Mg^{+2}. It could be claimed that these elements reduce the expression of cellular nuclear factors such as NF-kB and Nrf2 in periodontal and immune cells.

In this research, it was expected that the epidemiological behavior of periodontal disease and diabetes mellitus would be similar in our groups with respect

to gender. However, we were able to observe that more female patients were present. This is consistent with previous research which reports that women are more susceptible to both conditions and exhibit a higher degree of severity [55]. It has been considered that variation on hormonal levels, inflammatory processes and altered bone metabolism are factors contributing to the suffering periodontal diseases. Further research is needed on the supplementation of Zn^{+} and Mg^{+2} in women and its potential influence on metabolism and hormonal regulation.

Conclusion

The results of this research show that NSPT plus supplementation with zinc gluconate and magnesium oxide promote an increases SOD and CAT enzymatic activity. This systemic effect could be related to the changes in periodontal clinical parameters and the stability/remission of periodontal diseases. In addition, it is reaffirmed that NSPT decrease MDA levels. Nevertheless, further research is needed to understand the potential mechanisms by which patients with PD and DM2 present deficiencies of Zn^{+} and Mg^{+2} and how these elements participate in the regulation, expression, activity of antioxidant enzymes and its possible participation in periodontal wound healing.

Research agenda

The response of periodontal tissues following periodontal treatment depends on factors such as the modalities of periodontal treatment, clinical characteristics, the use of complementary treatments, and the systematic health status of patients receiving it.

Therefore, it's necessary to:

1. Evaluated the use of these supplements in different modalities of periodontal treatment (surgical periodontal treatment, tissue regeneration, and osteointegration processes).
2. Verify if the benefits observed using zinc gluconate and magnesium oxide after non-surgical periodontal treatments are maintained over time.
3. Compare the effects of non-surgical periodontal treatment plus supplementation in patients without associated systemic conditions and with a longer exposure time (3–6 months).
4. Finally, we suggest evaluating the cellular pathways by which Zn^{+} and Mg^{+2} is taking part in the decrease of oxidative stress and regulation of antioxidant enzymes in periodontal tissues.

Abbreviations

T2DM	Type 2 Diabetes Mellitus
PD	Periodontal Diseases
NSPT	Non-surgical periodontal treatment
OS	Oxidative Stress
ROS	Reactive oxygen species
MDA	Malondialdehyde
4-HNE	4-hydroxy-2-nonenal
SOD	Superoxide dismutase
CAT	Catalase
Zinc	Zn^{+}
Magnesium	Mg^{+2}
DM2	Diabetes Mellitus 2
PPD	Periodontal Probing Depth
BoP	Bleeding on probing
PISA	Periodontal inflamed surface area
DBPI	Dento bacterial plaque index
PD-DM2	Periodontal Diseases associated to Diabetes Mellitus 2

Acknowledgements

This research acknowledges the Dr. Rafael Lucio High Specialty Center and the clinical analysis laboratory of the hospital, where patients' recruitments and laboratory analyses were conducted. We also thank the Translational Medicine Laboratory and the Flow Cytometry Units of the, SHI which allowed us access to their facilities for the development of laboratory assays. Likewise, we thank the Immunology Department of CINVESTAV.

Author contributions

All authors have conjointly given final approval and agreed to be accountable for all aspects of the work. JCAM: was the principal investigator and has contributed to conceptualization, methodology, data collection, formal analysis, writing – original draft, visualization, investigation, review, and editing. MFM: Methodology, supervision, visualization, review, and editing. MSLM: Methodology, supervision, visualization, review, and editing. MEGR: Methodology, supervision, visualization, review, and editing. JCRA: Methodology, supervision, visualization, review, and editing. CRCL: Methodology, supervision, visualization, editing, statistical analysis. FJNG: Methodology, supervision, visualization, review, and editing. VHMM: Methodology, supervision, visualization, review, and editing. MGNG: Conceptualization, methodology, data collection, formal analysis, writing – original draft, visualization, investigation, review, and editing, project administration.

Funding

This research was partially supported by a grant from the of Sciences Health Institute of the Universidad Veracruzana. José Carlos Alarcón-Moreno was a recipient of a CONACYT Scholarship with the folio number 785006, enrolled in the national postgraduate program in health sciences.

Data availability

The data that support the findings of this study are available from institutional repository by of Sciences Health Institute, Universidad Veracruzana, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of corresponding author: Nachón-García María Gabriela.

Declarations

Ethics approval and consent to participate

This study was approved by the research ethics committee of the Institute of Health Science from Universidad Veracruzana and the ethical committee of Dr. Rafael Lucio Hospital Xalapa, Veracruz. México.(F-011/2018-F.10/2022.) The registration date for the clinical trial on https://www.isrctn.com. received code of (ISRCTN 14092381. September 13º 2023. Retrospectively Registered**)** All participants in this research were given an informed consent form. The patients who agreed to participate signed the requirement sheet, a procedure approved by the Ethics and Investigation Committee. Committee Names: "Comité de Ética en Investigacion del Instituto de Ciencias de la Salud de la Universidad Veracruzana". COBIOETICA-30-CEI-001-20180131. References number (Nº 011/2018 - Nº010/2022). "Centro de Alta Especialidad "Dr. Rafael Lucio" Subdirección de Enseñanza y Capacitación. Departamento de Investigación". COFEPRIS 16CI 30087028. Reference Number (Nº48/18).

Informed consent
The participants who made up the samples signed the informed consent voluntarily, accepting their participation, publication of the results and, where appropriate, intraoral photographs that do not allow their identification and the authors were committed to safe confidentiality.

Consent for publication
"Not applicable".

Competing interests
The authors declare no competing interests.

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Received: 1 May 2024 / Accepted: 30 July 2024
Published online: 04 August 2024

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