

Original Article

The Effect of Systemic Administration of Zinc on The Gingival Crevicular Fluid Level of Total Oxidant Capacity in Type 2 Diabetic Patients with Stage II and III Periodontitis After Non-Surgical Periodontal Therapy: A Randomized Clinical Trial

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Abstract:

Aim: This study aimed to evaluate the effect of systemic administration of zinc supplementation on the total oxidant capacity in the gingival crevicular fluid level of controlled type 2 diabetic patients with stage-II and III periodontitis after non-surgical periodontal therapy.

Subjects and Methods: This clinical trial was registered at ClinicalTrials.gov Identifier: ID: NCT03923829 on April 23, 2019. Twenty-eight controlled type 2 diabetic patients with periodontitis stages II or III were randomly assigned into two groups, test and control where both received non-surgical periodontal therapy while the test group received 50 mg zinc one tablet/day for 12 weeks. Total oxidant capacity (TOC) level was measured in GCF of both groups at baseline and after 3 months. HbA1c level, GI, PPD and CAL were also reported at baseline and 3 months postoperatively.

Results: After 3 months a statistically significant decrease in mean TOC $\mu\text{mol/L}$ was reported in both groups being more significantly lower in test group. No significant difference in mean percentage HbA1c was reported after 3 months neither within each group nor between the two groups. In both groups there was a significant reduction in GI scores, PPD reduction and CAL gain after 3 months with no significant difference between them, while the test group showed significantly lower GI than the control group.

Conclusion: Systemic administration of zinc supplements as an adjunct to non-surgical periodontal therapy might improve the clinical parameters and oxidative stress in T2DM patients with stage II-III periodontitis.

Keywords: Periodontitis, Zinc, Type 2 Diabetes Mellitus, total oxidant capacity, GCF.

Introduction

Periodontitis is a common disease caused by complex interactions between pathogenic periodontal microbiota and the immune response of the host (Silva *et al.*, 2015). Occurrence and severity of periodontal diseases are influenced by a number of environmental and genetic factors in addition to the frequency of teeth brushing, age, carbohydrate intake and caffeine consumption (Abbass *et al.*, 2019).

The stages of periodontitis are determined by the severity of the disease which is decided according to the level of interproximal clinical attachment loss (CAL), radiographic bone loss and tooth loss in addition to the complexity, extent and distribution (Papapanou *et al.*, 2018). While the response to periodontal treatment differs since systemic diseases and risk factors could dramatically affect the prognosis of periodontal treatment. Hence, risk factors as smoking and systemic diseases as diabetes must be considered during detecting the rate of progression and upgrade the individual's grade to a higher level (Tonetti, Greenwell and Kornman, 2018).

Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia caused by impairments in insulin secretion, insulin action, or both (Burton-Freeman *et al.*, 2019). The severity of hyperglycemia over time is of paramount importance when it comes to the magnitude of its effect on the course of periodontal disease (Jepsen *et al.*, 2018).

It was speculated that periodontitis could be primarily caused by an overstated host response to pathogenic microorganisms and their products, resulting in an imbalance between the antioxidant and reactive oxygen species (ROS) in GCF (Savita *et al.*, 2015). DM was found to be a significant modifying factor for periodontitis where the degree of glycemic regulation in

diabetic patients could affect periodontitis grading (Jepsen *et al.*, 2018). Therefore, DM has been recognized as an important risk factor for periodontal diseases being related to a higher incidence and severity of periodontitis since chronic hyperglycemia was strongly associated to high prevalence of severe periodontitis (Albandar, Susin and Hughes, 2018). Periodontitis is aggravated by diabetes since it has several negative consequences over the periodontium as increased periodontal tissues destruction caused by decrease collagen turnover and impairment of neutrophil function. On the other hand, The oral cavity is a persistent source of infectious organisms that can exacerbate a patient's diabetes condition and function as a significant risk factor for the patient's glycemic control to deteriorate (Gurav and Jadhav, 2011).

Oxidative stress is an imbalance between the production of ROS or free radicals and the antioxidant defense. An imbalance between oxidants and antioxidants and lack of the antioxidant system in DM may induce tissue injury due to imbalance between production and scavenging of free radical (Bikkad *et al.*, 2014). Also, during inflammatory conditions as chronic periodontal disease, a great amount of prooxidant agents is produced. This overexposure to free radicals worsen the systemic inflammatory conditions leading to tissue damage (Marconcini *et al.*, 2017). Hence, the increased oxidative stress in chronic periodontitis patients with and without T2DM reveals the existence of a common factor involved in tissue damage and the excessive ROS generation is linked to more severe tissue destruction in periodontitis and is positively correlated to T2DM (Patil *et al.*, 2016).

Zinc (Zn) is one of the most common multipurpose trace minerals since it is involved in numerous biological activities and cellular functions including response to oxidative stress (Chasapis *et al.*, 2020). Patients with type 2 diabetes mellitus were found to have

hypo-zincemia and hyperzincuria, therefore zinc supplementation was suggested to help restore normal plasma zinc levels (Ranasinghe *et al.*, 2015). Thus, supplementing with 50 mg zinc gluconate daily for eight weeks was proved to improve superoxide dismutase (SOD) gene expression and enzyme activity (Nazem *et al.*, 2019). In addition, zinc could help improving the glycemic control in type 2 diabetic patients and decrease the negative effects of oxidative stress preventing diabetes complications (Khan *et al.*, 2013).

Therefore, the aim of this study was to evaluate the effect of systemic administration of zinc supplementation on the total oxidant capacity in the gingival crevicular fluid level of controlled type 2 diabetic patients with stage-II and III periodontitis after non-surgical periodontal therapy.

Subjects, materials, and methods:

Study settings:

The present double-blinded parallel grouped randomized controlled clinical trial included 28 patients (9 males and 19 females, aged 35 to 65 years) with controlled type 2 diabetic patients having stages II and III periodontitis. All patients completed the follow up with no dropouts. Patients were randomly assigned into two equal groups, the control group where non-surgical periodontal therapy was performed in addition to systemic administration of placebo tablets or the test group where non-surgical periodontal therapy in addition to systemic zinc supplementation was performed.

Subjects were selected from the outpatient clinic, Department of Oral Medicine and Periodontology, Faculty of Dentistry, Cairo University between October 2019 and November 2020. Screening of patients was continued until the target sample was achieved. Identifying and recruiting potential subjects was achieved

through patients' database. This clinical trial was registered in U.S. National Institutes of Health Clinical Trials Registry, ClinicalTrials.gov Identifier: ID: NCT03923829.

Eligibility criteria:

Inclusion criteria included: 1) Stages II and III periodontitis patients with 3 or 4 mm to ≥ 5 mm interdental CAL in at least two non-adjacent teeth and probing depth ranging from ≤ 5 mm to ≥ 6 mm with radiographic bone extending to middle third of the root or beyond with maximum number of teeth lost due to periodontitis ≤ 4 (Tonetti, Greenwell and Kornman, 2018). 2) Controlled T2DM with glycosylated hemoglobin HbA1c less than (7.5) (Sinclair *et al.*, 2011). 3) Patients with good compliance to oral hygiene and accepted the follow up appointments needed.

Exclusion criteria included: 1) Patients who received any periodontal therapy or antibiotics during the last six months (Hong *et al.*, 2019). 2) Patients received trace element supplements in the last three months. 3) Pregnant or lactating females (Laine, 2002). 3) Smoker patients (Shchipkova, Nagaraja and Kumar, 2010). 4) Patients receiving gastric or diuretic treatment (Farrell *et al.*, 2011; Braun and Rosenfeldt, 2013). 5) Patients with acute renal failure or any medical condition other than diabetes.

Power and Sample size calculation:

The aim of this study was to evaluate the effect of systemic administration of zinc supplements on the total oxidant capacity in the gingival crevicular fluid level (GCF) of controlled Type-2 diabetic patients with stages II and III periodontitis after non-surgical periodontal therapy. The minimum clinically important difference based upon an expert opinion was $80\% - 51.5387\% = 28.462$. Using a power of 80% and 5% significance level 14

patients in each group needed to be studied. This number was to be increased to a sample size of 17 per group to compensate for possible losses during the follow up. Sample size calculation was achieved using PS program (Power and sample size program: biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) (Muthuraj *et al.*, 2017).

Randomization:

Participants who fulfilled the inclusion criteria and provided an informed consent were randomly assigned to either control (SRP only) or test (SRP+zinc) groups using a randomization list with a 1:1 allocation ratio. The computer-generated randomization list (Research Randomizer computer software (Version 4.0). Retrieved on August 16, 2015, from <http://www.randomizer.org/>) was executed by a faculty member who was not involved in the patients' recruitment and the list was sent to the main supervisor for the purpose of concealment.

Allocation concealment mechanism:

Allocation concealment was achieved by sequentially numbered opaque sealed envelopes that contained the interventions to be performed to the enrolled subjects based on the randomized numbers in the randomization list. Consequently, the patients were allocated to either control group or test group after completing the step of supra and subgingival debridement for each patient. The sealed envelope containing treatment assignment was opened at that time and the number was picked by another person other than the operator.

Blinding:

The current investigation was a double-blinded clinical trial. Blinding included the patients, outcome assessor and the statistician. It was impossible for either the operator or the

patients to be blinded as the interventions were completely different.

Preoperative assessment:

Initial examination, including full mouth probing and radiographic examination was performed for the selected patients to confirm the diagnosis of stages II or III periodontitis. All participants meeting all inclusion and exclusion criteria were screened by comprehensive periodontal examination and full periodontal charts were obtained to determine the most periodontally affected tooth. Radiographic examination through periapical radiographs at the most periodontally affected tooth were taken using the long cone parallel technique at the time of the initial examination to confirm the diagnosis of stage II or III periodontitis. Glycated hemoglobin (HbA1c) was measured for all patients to confirm the diagnosis of controlled diabetes mellitus of being less than (7.5) at baseline and again 3 months following non-surgical periodontal therapy.

Both groups received non-surgical periodontal therapy including full mouth supra and subgingival debridement using ultrasonic scalers in addition to hand instruments under local anesthesia, which was completed over 2 visits. On the initial visit, supragingival scaling with ultrasonic device (Woodpecker ultrasonic dental scaler UDS-K, china) and hand instruments using sickle and Jacquette scalers were performed carefully without touching the marginal gingiva. On the next day (at beginning of second visit), the baseline value of GCF was collected. Then subgingival debridement was performed using Universal and Gracey curettes (Hu-Friedy universal and Gracey's curette; Hu-Friedy, Chicago, USA.). Local anesthesia was used for patient comfort when necessary. Patients were given careful instruction in self-performed plaque control measures: twice daily tooth brushing with a brush and tooth paste and once

daily interdental cleaning using dental floss or interdental brushes. Clinical photographs were taken at baseline, and 3 months postoperatively.

After non-surgical periodontal therapy, patients were randomly allocated to either test group receiving systemic administration of 1 tablet 50 mg elemental zinc as zinc sulphate (Solvazinc hard gelatin capsule, ALESRAA company, Egypt) to be used once a day for 12 weeks (Khan *et al.*, 2013) or control group receiving placebo tablets having the same shape and color like the zinc sulphate tablet.

Clinical Outcomes:

Clinical periodontal parameters included gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL). These parameters were recorded using Williams graduated periodontal probe (Martin™ graduated periodontal probe No. 43-357-00, KLS martin Group, Germany) at the most periodontally affected tooth and were rounded up to the nearest millimeters. Measurements were reported at baseline and 3 months postoperatively and were executed by an internal resident at the department of Oral medicine and Periodontology, Faculty of Dentistry, Cairo University.

PPD and CAL measurements were performed at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual). The readings from the six areas of the tooth were added and divided by six to give the mean CAL and PPD for the tooth.

Total Oxidant Capacity (TOC):

The level of total oxidant capacity (TOC) was measured in the GCF at the most affected tooth at baseline and 3 months after non-surgical periodontal therapy in both groups by using Enzyme linked immuno-sorbent assay (ELISA).

GCF collection:

All patients in both groups received supragingival scaling one day before taking GCF sample followed by subgingival debridement. In both groups, GCF samples were collected at baseline and 3 months after SRP was completed according to (Alwan, 2015) as follows:

To prevent plaque from contaminating the paper point, all clinically detectable supragingival plaque at the chosen location (the most periodontally affected site) was carefully removed without contacting the marginal gingiva. The target site was then carefully dried with an air syringe after the chosen tooth had been gently cleaned with water and separated with cotton rolls to avoid saliva contamination. For each collection site, one paper point (size 30) was utilized. It was carefully pushed into the gingival fissure until some resistance was felt, after which it was kept in situ for 30 seconds before being put in a plastic Eppendorf tube. Any paper point that was contaminated with blood, saliva, or plaque was thrown away. The sample from each site was kept at (- 40 C) for subsequent processing, after which GCF was extracted, and the TOC content was determined using the ELISA method.

Quantitation of TOC in GCF:

By adding 100 l of phosphate buffer saline to the eppendorf tube, mixing by vortex, and then centrifuging for 10 min. at 3000Xg, the TOC was recovered from the paper point. ToC was estimated using the supernatant. The PerOx (TOS/TOC) Immun-diagnostics Assay (ELISA) Kit (Catalog No. 5100 KO, Germany) was used to quantify the levels of human TOC in GCF. The purpose of the ELISA was to quantitatively assess the total oxidative status/capacity (TOS/TOC) of EDTA-plasma, serum, and cell culture supernatants.

Principle of the test: The interaction of a peroxidase with the peroxides in the sample was

followed by the transformation of TMB (Tetra-methylbenzidine) into a colored product to determine the presence of peroxides. The samples were measured at 450 nm in a microtiter plate reader after the addition of a stop solution. The calibrator that was given carried out the quantification.

Follow up visits:

The test group patients were recalled after one and three weeks for any possible complications after zinc administration. Then both groups were recalled after 3 months for collection of GCF sample and re-evaluation of the glycated hemoglobin (HbA1c). Clinical periodontal parameters including PPD, CAL and GI were reevaluated after 3 months using William's graduated probe.

Statistical analysis:

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed normal (parametric) distribution Gingival Index (GI) scores data which showed non-normal (non-parametric) distribution. Data were presented as mean, standard deviation (SD), median and range values. For parametric data, repeated measures ANOVA test was used to compare between the two groups as well as to study the changes within each group. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. For non-parametric data, Mann-Whitney U test was used to compare between the two groups. Wilcoxon signed-rank test was used to study the changes by time within each group. Pearson's and Spearman's correlation coefficients were used to determine the

correlation between TOC and different variables. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

Results:

Demographic data:

The study population in this randomized controlled clinical trial included a total of 28 controlled type 2 diabetic patients with periodontitis stages II or III (9 males and 19 females). The participants were randomly assigned into two equal groups; control group; received scaling and root planning (SRP) with placebo tablet and the intervention group; received SRP in addition to systemic administration of Zinc in the form of 50 mg elemental zinc as zinc sulphate (SRP+zinc), once a day for 12 weeks. The study was completed with 28 patients, with no dropouts or reported complications.

Patients treated with SRP alone in the control group had a mean (\pm SD) age of 53.4 (\pm 7.5) years while those treated with (SRP+zinc) in the test group had a mean (\pm SD) age of 55.1 (\pm 10.2). There was no statistically significant difference between mean age values in the two groups ($P = 0.603$). There were 3 males and 11 females in the test group represented by percentages as (21.4%, 78.6%) respectively. In control group there were 6 males and 8 females represented by percentages as (42.9%, 57.1%) respectively. There was no statistically significant difference between gender distributions in the two groups ($P = 0.420$). This study was reported according to CONSORT guidelines (Figure 1) (Moher *et al.*, 2012).

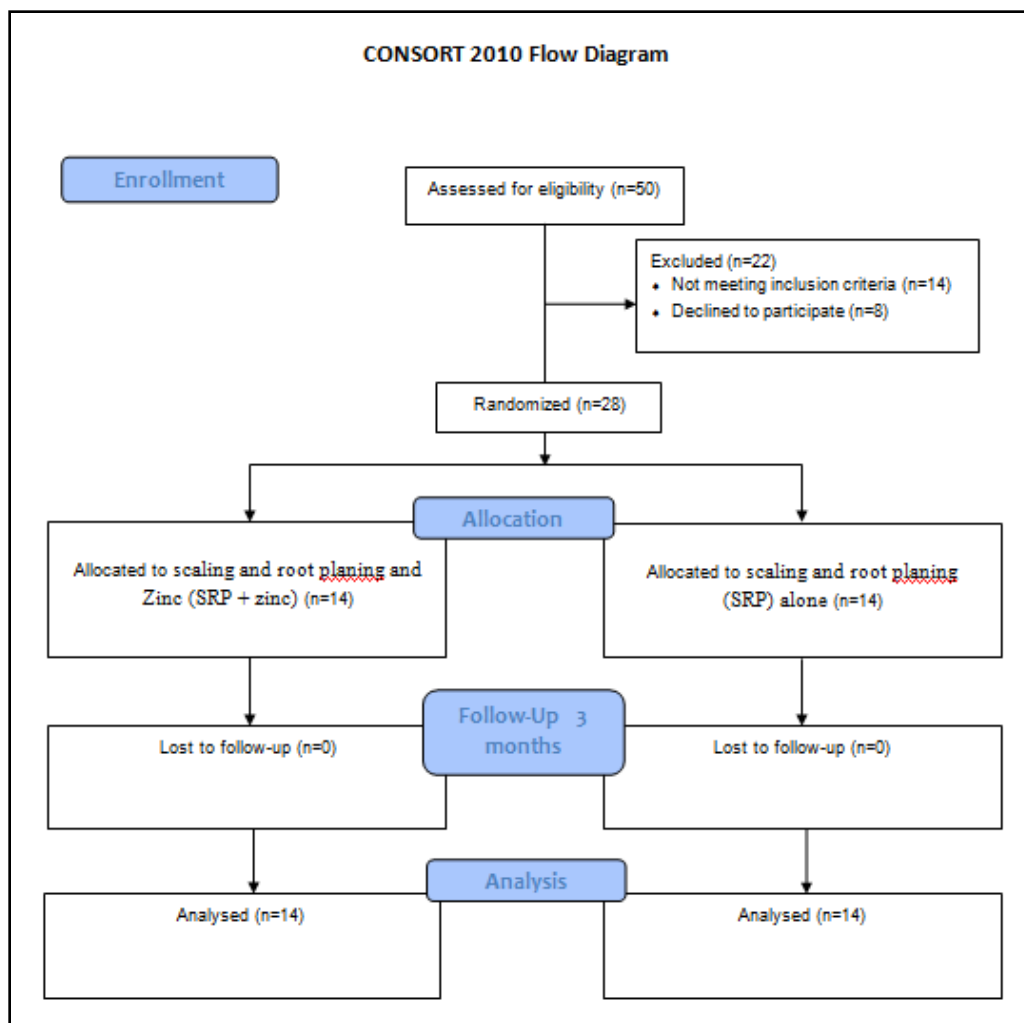


Figure (1): CONSORT Flow Diagram

I. Biochemical analysis:

1. Total oxidant capacity (TOC) ($\mu\text{mol/L}$):

The changes in mean ($\pm\text{SD}$) $\mu\text{mol/L}$ TOC and comparison at baseline as well as at 3 months between both test and control groups are presented in figure (2). The mean ($\pm\text{SD}$) $\mu\text{mol/L}$ TOC recorded for the test group at baseline was $358.9 (\pm 65.1)$ $\mu\text{mol/L}$, while at 3 months was $241.1 (\pm 31)$ $\mu\text{mol/L}$. Statistical analysis revealed a statistically significant reduction (P -value < 0.001 , Effect size = 0.685) in the mean $\mu\text{mol/L}$ TOC at 3 months compared to baseline values.

The mean ($\pm\text{SD}$) $\mu\text{mol/L}$ TOC observed for the control group at baseline was $347 (\pm 85.6)$

$\mu\text{mol/L}$, while at 3 months was $277.4 (\pm 52.6)$ $\mu\text{mol/L}$ showing a statistically significant decrease (P -value < 0.001 , Effect size = 0.441) in the mean $\mu\text{mol/L}$ TOC at 3 months compared to baseline values.

At baseline, there was no statistically significant difference between mean $\mu\text{mol/L}$ TOC in the two groups (P -value = 0.734, Effect size = 0.005). However, after 3 months, the test group showed statistically significantly lower mean $\mu\text{mol/L}$ TOC than the control group (P -value = 0.035, Effect size = 0.159).

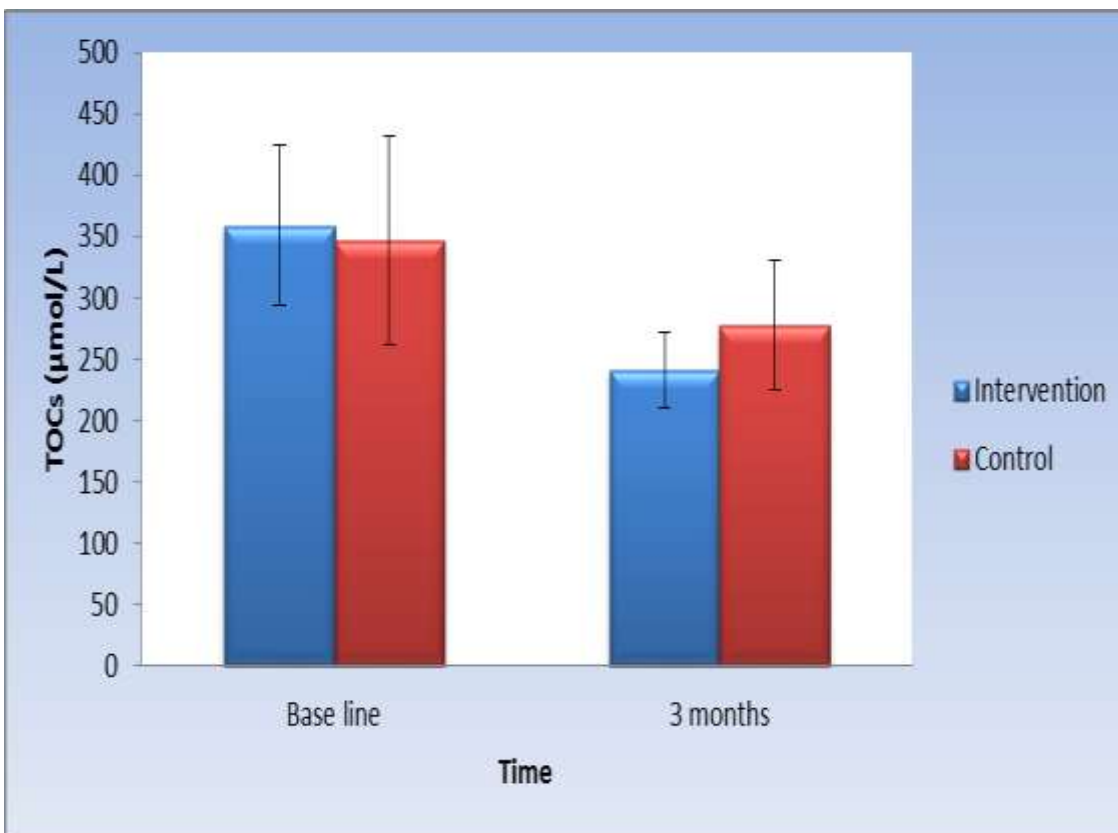


Figure (2): Bar chart representing mean and standard deviation values for TOC in the two groups

2. Changes in glycated hemoglobin (HbA1c) (%):

The changes in mean (\pm SD) μ mol/L TOC and comparison at baseline as well as at 3 months between both test and control groups are presented in figure (3). In the test group, the mean (\pm SD) percentage (HbA1c) recorded at baseline was 7.36 (\pm 0.26) %, while at 3 months was 7.46 (\pm 0.89) % with no statistically significant changes in mean percentage HbA1c after three months (P -value = 0.709, Effect size = 0.005).

In the control group, the mean (\pm SD) percentage (HbA1c) observed at baseline was 7 (\pm 0.4) %, while at 3 months was 7.21 (\pm 1.42) %. There was also no statistically significant change in mean percentage HbA1c after 3 months (P -value = 0.472, Effect size = 0.02). As for the test group, baseline values showed statistically significantly higher mean percentage HbA1c than control group (P-value = 0.010, Effect size = 0.23). However, after 3 months, there was no statistically significant difference between mean percentage HbA1c in the two groups (P -value = 0.571, Effect size = 0.012).

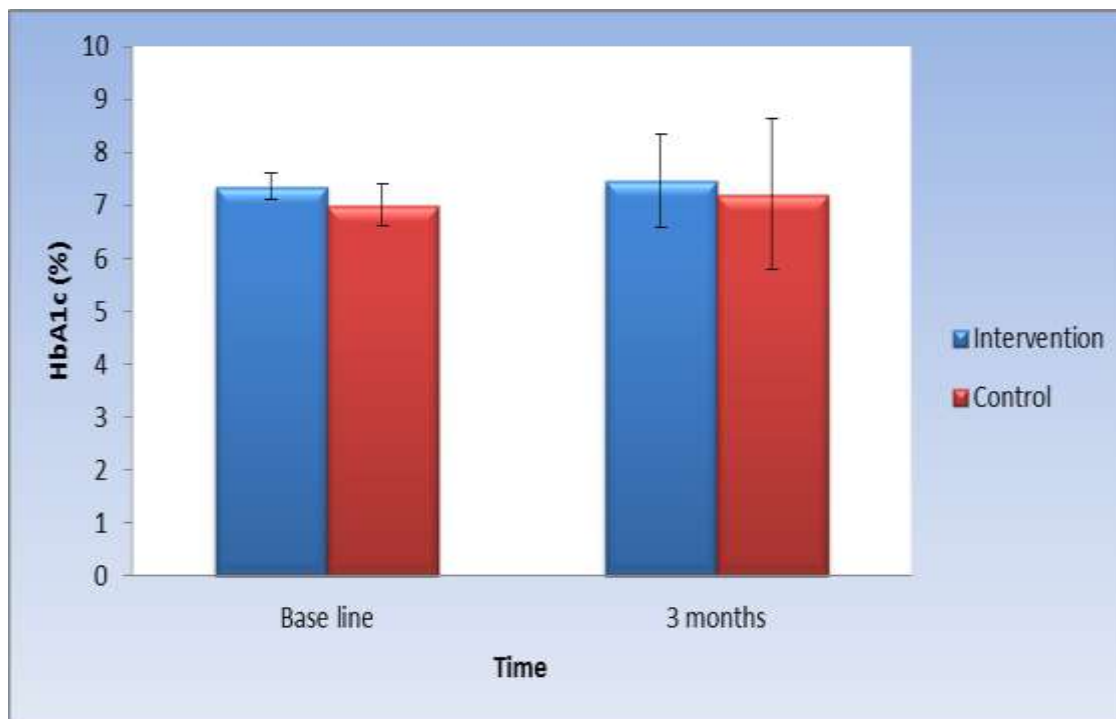


Figure (3): Bar chart representing mean and standard deviation values for HbA1c in the two groups.

II. Clinical parameters:

The changes in the mean (\pm SD) mm of PPD and CAL and median (range) of GI score and comparison at baseline as well as at 3 months between both test and control groups are shown in table (1).

1. Probing Pocket Depth (PPD) (mm):

In both groups, there was a statistically significant reduction in mean mm PPD after 3 months (P -value <0.001). Also, at baseline as well as after 3 months, there was no statistically significant difference between mean mm PPD in the two groups (P -value = 0.542 and 0.227 respectively).

2. Clinical Attachment Level (CAL) (mm):

In both groups, there was a statistically significant gain in mean mm CAL after 3 months (P -value <0.001). At baseline as well as after 3 months, there was no statistically significant difference between mean mm CAL in the two groups (P -value = 0.248, 0.852 respectively).

3. Gingival Index (GI) Scores:

In both groups, there was a statistically significant decrease (P -value = 0.001) in GI scores from baseline to 3 months. At baseline, there was no statistically significant difference between median GI in the two groups (P -value = 0.289, Effect size = 0.245). However, after 3 months, the test group showed statistically significantly lower median GI than the control group (P -value = 0.016, Effect size = 0.863).

Table (1): Changes and comparison in clinical parameters between both groups at baseline and after 3 months

Parameter	Period	Test group	Control group	P-value
		Mean (\pm SD)	Mean (\pm SD)	
Probing pocket depth (PPD)	Baseline	2.97 (\pm 0.92)	2.81 (\pm 0.38)	0.542
	3 months	1.58 (\pm 0.73)	1.87 (\pm 0.49)	0.227
	P-value	P <0.001*	P <0.001*	
Clinical attachment level (CAL)	Baseline	4.97 (\pm 1.28)	4.5 (\pm 0.75)	0.248
	3 months	3.6 (\pm 1.19)	3.53 (\pm 0.77)	0.852
	P-value	P <0.001*	P <0.001*	
Gingival Index Score in median (range)	Baseline	2 (2-3)	2 (2-3)	0.289
	3 months	0 (0-1)	1 (0-2)	0.016*
	P-value	0.001*	0.002*	

Discussion:

One of the major systemic risk factors for periodontal disease is diabetes mellitus (DM) which plays a significant role in the disease's initiation and progression (Casanova, Hughes and Preshaw, 2014). While periodontal disease was proved to adversely affect the glycemic control worsening the diabetes complications since diabetic patients with periodontitis had higher levels of inflammatory mediators in their saliva and GCF than non-diabetic patients with periodontitis (Chapple and Genco, 2013; Genco and Borgnakke, 2020).

Hyperglycemia cause an increased extracellular and intracellular free radical concentrations which cause oxidative stress in addition to the decreased levels and activities of antioxidant protein (glutathione), enzymes (catalase, glutathione peroxidase, superoxide dismutase), micronutrients (selenium and zinc) and vitamins such as C, E and A. This stimulates production of ROS resulting in oxidative stress which stimulates the production of inflammatory

cytokines (Tatsch *et al.*, 2012). Zinc plays an important role as an antioxidant protection in people with T2DM. Zinc mineral provide defense through being an important cofactor for more than 300 enzymes, including superoxide dismutase and by its ability to reduce and neutralize free radicals (Carocho and Ferreira, 2013). It was further observed that the plasma zinc concentration is reduced in T2DM patients which is linked to a large amount of mineral loss in the urine (Saharia and Goswami, 2013).

This study was conducted specifically on T2DM patients due to the high percentage and prevalence of this disease among Egyptian population (Hegazi *et al.*, 2015). In addition, high prevalence of periodontitis was observed to be accompanied with T2DM (Al Shafey and Khalil, 2021). Thus, the present clinical study aimed to evaluate the effect of systemic administration of zinc on the clinical parameters and GCF level of TOC in controlled T2DM patients with stages II or III periodontitis after non-surgical periodontal therapy .

The proved antioxidant capacity of zinc administration with diabetic patients had made this mineral an interesting topic to investigate (Carocho and Ferreira, 2013; Toma, Makonnen and Yimer, 2013). Therefore, zinc was chosen in this study as a supplement based on several studies that confirmed the deficiency of this mineral in T2DM patients (Anderson *et al.*, 2001; Al-Marouf and Al-Sharbatti, 2006; Saharia and Goswami, 2013; GOWDA and RANGAREDDY, 2021). Also, it was observed that serum zinc level in Egyptian T2DM patients was significantly low with a negative relationship among serum zinc, fasting blood sugar and HbA1c levels (Soliman, 2019).

Zinc supplement was prescribed for 3 months only according to (Khan *et al.*, 2013) since excessive zinc supplementation could lead to copper deficiency via interacting with it in a competitive manner leading to hypocupremia (Hoffman II, Phylky and Fleming, 1988). Patients receiving diuretic treatment were excluded from this study since diuretics were shown to induce zincuria that could lead to decrease effectiveness of the zinc (Braun and Rosenfeldt, 2013). Patients receiving gastric treatments were excluded since many gastric medications as proton pump inhibitors could impair zinc absorption during supplementation (Farrell *et al.*, 2011).

For minimizing any confounders, patients previously treated with SRP or antibiotics during the last 6 months or those who received any trace element supplements in the previous 3 months were excluded from this study (Hong *et al.*, 2019). Similarly, smokers were excluded because smoking could alter the composition of the subgingival biofilm through raising the prevalence of periodontal pathogens (Shchipkova, Nagaraja and Kumar, 2010).

All patients in both groups received supragingival scaling one day before taking GCF

sample followed by subgingival debridement on the next day to minimize contamination of the paper point by the plaque and to enable easier periodontal examination and GCF sampling (Sinclair *et al.*, 2011). GCF samples were obtained using size 30 paper point as recommended by (Alwan, 2015) from the most periodontally affected site at the most periodontally affected tooth. In this study, GCF was used to determine the level of TOC since GCF is closely related to the periodontal tissues where periodontal disease starts in addition to its ability to provide a close reflection to the periodontal condition more efficiently than the salivary markers (Zia *et al.*, 2011).

The TOC in GCF samples was determined using a commercially available human-specific enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (TOC- ELISA kit) which are relatively accurate tests (Lutfioğlu *et al.*, 2017). Assays of TOC have the advantage of analyzing the combined effectiveness of contributing species, which may be greater than the sum of the effects of the individual ROS. Also, these assays are more efficient, cheaper, and less time-consuming than performing large numbers of individual assays (Maxwell, Dietrich and Chapple, 2006).

In this current clinical trial, there was a statistically significant decrease ($P < 0.001$) in TOC level within the control group. This came in accordance with (Muthuraj *et al.*, 2017) who reported a marked decrease in 8-hydroxydeoxyguanosine (8-OHdG) level which is a biomarker for oxidative stress at 3 months after SRP in GCF of T2DM patients with chronic periodontitis. These results were also in line with (Wei *et al.*, 2010) who observed a significant decrease in the level of total oxidant status (TOS) after SRP in serum, saliva and GCF of chronic periodontitis patients after 4 months compared to baseline levels ($p < 0.05$). However,

their study did not include diabetic patients which could stress on the effect of SRP in reducing oxidative stress in periodontitis patients. Also, the results of the present study agreed with those of (Vincent *et al.*, 2020) who examined the effect of non-surgical periodontal treatment on GCF TOS in chronic periodontitis patients with T2DM showing a statistically significant reduction ($P < 0.001$) at 6 weeks compared to baseline values.

However, the results of TOC level in control group in this clinical trial were superior to those obtained by (Koromantzos *et al.*, 2012) who did not have a statistically significant effect on the serum d-8-iso prostaglandin F2a (d-8-iso) level which is a marker of oxidative stress at 6 months after SRP. This discrepancy in the results could be due to the difference in the population who were uncontrolled T2DM with moderate to severe periodontitis plus the oxidative stress marker in their study was evaluated in the serum not in GCF.

In the test group who took zinc supplements, there was also a statistically significant decrease ($P < 0.001$) in the mean $\mu\text{mol/L}$ TOC at 3 months compared to baseline values. These results are consistent with (Anderson *et al.*, 2001) who prescribed 30 mg/d Zn gluconate for 6 months in T2DM patients. Their study showed statistically significant reduction in plasma thiobarbituric acid reactive substances (TBARS) which is a biomarker for oxidative stress at 6 months compared to baseline values. Despite the difference between their study and the current trial where oxidative stress marker was evaluated in the plasma not in GCF and their follow up was 6 months, yet both studies confirm the ability of zinc to reduce oxidative stress.

An indirect comparison could also be performed with a study conducted by (Nazem *et al.*, 2019) who reported a statistically significant increase in enzyme activity of superoxide

dismutase (SOD) among T2DM patients in the group receiving 50 mg zinc gluconate for 8 weeks. Despite the difference of biomarker evaluation in serum not in GCF and the different follow up period, yet their results support the results of the current trial proving that Zinc is an important cofactor for more than 300 enzymes, including superoxide dismutase. This mineral was proved to help reducing and neutralizing the free radicals and hence decreasing the oxidative stress (Carocho and Ferreira, 2013). However, the results of (Seet *et al.*, 2011) were not in agreement with this current study, since they showed no statistically significant difference in oxidative stress related biomarkers, F2-IsoPs, F4-NPs, COPs and allantoin, as well as HETEs, at 3 months after administration of 240 mg/day of zinc gluconate in T2DM patients. The variation in the follow up period, sample size, dose of zinc administration and method of evaluation could be behind this variability of results .

When comparing the mean TOC level between both groups in this study at baseline, no statistically significant difference was found ($P = 0.734$). However, after 3 months, the test group showed statistically significantly lower mean $\mu\text{mol/L}$ TOC than the control group ($P = 0.035$). The tenable explanation of this finding may be related to the antioxidant effect of zinc on GCF total oxidant capacity. However, no direct comparison could be made due to lack of clinical trials in the literature that combined the effects of zinc and SRP on the GCF total oxidant capacity in controlled T2DM with periodontitis. Nevertheless, the results of this study could be supported by the most recent systematic review and meta-analysis specifically on the effects of Zn supplementation on oxidative stress and inflammatory markers conducted by (Hosseini *et al.*, 2021) which proved that serum malondialdehyde (MDA) level (biomarker for oxidative stress) is significantly reduced in groups receiving Zn compared to placebo groups.

Despite the differences in type of patients, sampling methods, and type of tests used for evaluation of oxidative stress, yet their results are consistent with this clinical trial confirming the antioxidant effect of zinc .

When comparing the mean percentage HbA1c 3 months after SRP in the control and test groups of this study, no statistically significant change was found ($P= 0.472, 0.709$) respectively. This agrees with (Engebretson *et al.*, 2013) and (Santos *et al.*, 2009) who reported that SRP did not significantly improve HbA1c levels at 3 and 6 months in T2DM patients with chronic periodontitis.

However, (Goel, Pradhan and Bhattarai, 2017) managed to reach a statistically significant reduction in HbA1c level after SRP in moderately controlled T2DM patients with HbA1c values between 6-8% with chronic periodontitis. In addition to a systematic review by (Baeza *et al.*, 2020) which reported a significant reduction in % HbA1c ($p<0.00001$) from the beginning to the end of the follow-up after non-surgical periodontal therapy claiming that this reduction in the levels of glycosylated hemoglobin was attributed to the effect of the periodontal therapy.

When comparing the zinc group to other trials, no studies were found correlating the combined effects of zinc and SRP on the mean percentage HbA1c in controlled type 2 diabetes with periodontitis, hence no direct comparison could be performed. Nevertheless, a study conducted by (Anderson *et al.*, 2001) could support the results of this study since they reported no statistically significant changes in mean percentage HbA1c at six months after administration of 30 mg/d of zinc gluconate for 6 months in T2DM patients. However, their population were not suffering from chronic periodontitis and their diabetic status was uncontrolled ($HbA1C \geq 7.5\%$) in addition to the different dose and duration of zinc

administration. While the results of (Jayawardena *et al.*, 2012) and (Nazem *et al.*, 2019) were not in agreement with this study, since they could prove that zinc supplementation in T2DM patients was associated with a statistically significant reduction in HbA1c levels.

At baseline, the test group showed statistically significant higher mean percentage HbA1c than control group ($P=0.010$). However, after 3 months, there was no statistically significant difference between mean percentage HbA1c in the two groups ($P=0.571$). The tenable explanation of this finding may be related to the baseline controlled HbA1c diabetic patients involved in this study. This is confirmed by (Chen *et al.*, 2021) in their systematic review and meta-analysis reporting that periodontal therapy improved glycemic control in T2DM patients, especially in those with a higher baseline HbA1c level. They further added that periodontal therapy significantly improved glycemic control in studies with a higher baseline HbA1c levels than in studies with lower baseline values.

Several studies were consistent with the results of this clinical trial concerning the enhanced clinical parameters after SRP in diabetic patients as (Agarwal *et al.*, 2016) reported a statistically significant decrease in mean PPD and CAL at 6 months ($P < 0.005$) after SRP for moderately controlled T2DM patients and chronic periodontitis. Similarly, (Vincent *et al.*, 2020) and (Soi *et al.*, 2021) confirmed a statistically significant decrease in GI, PPD and CAL values after SRP in T2DM patients with chronic periodontitis after 6 weeks and 6 months respectively. On the contrary, (Rodrigues *et al.*, 2003) found no statistically significant changes in mean mm CAL at 3 months after non-surgical periodontal therapy for T2DM patients with periodontitis.

When observing the results of the control group in this clinical trial, a statistically

significant direct (positive) correlation between TOC and PPD ($r = 0.536$, $P = 0.048$) was found at baseline. An increase in PPD was associated with an increase in TOC and vice versa. However, there was no statistically significant correlation between TOC and the other variables (HbA1c, CAL and GI) ($P = 0.397$, 0.077 , 0.109) respectively. While on observing the results after three months, no statistically significant correlation was found between TOC and the four different variables ($P = 0.451$, 0.238 , 0.230 , 0.535) respectively. In the test group (SRP+zinc) of this study, at baseline there was no statistically significant correlation between TOC and all the four different variables ($P = 0.291$, 0.332 , 0.210 , 0.259) respectively. Also, at 3 months there was no statistically significant correlation between TOC and all the four different variables ($P = 0.614$, 0.820 , 0.349 , 0.103) respectively.

The results of (Vincent *et al.*, 2020) supported the results of this study concerning the direct positive correlation between TOC and PPD, however it was not in agreement with this study regarding the correlation between GI and total antioxidant capacity (TAOC), since they found that baseline values of GI were negatively correlated with TAOC in chronic periodontitis patients whereas PPD and oxidative stress were positively correlated ($P < 0.05$).

Conclusions:

- Both groups showed a statistically significant reduction in TOC proving that SRP was efficient at reducing the oxidative stress within the GCF.
- The antioxidant effect of zinc was highlighted in this study through its ability to reduce the TOC within the test group with statistically significant difference over the control group after 3 months.
- Regarding the glycemic control, no statistically significant difference was found

in the mean percentage reduction of HbA1c within each group and between the two groups after 3 months compared to baseline values.

- Concerning the clinical periodontal parameters PPD, CAL and GI, both groups showed a statistically significant decrease in mean mm PPD, mm CAL gain and median (range) GI after 3 months.
- After 3 months; there was no statistically significant difference between mean mm PPD and mean mm CAL in the two groups however, the test group showed statistically significantly lower median GI than the control group.

Recommendations:

- Further longitudinal and multicenter clinical studies with longer follow up period and larger sample size are still needed to explore the potential effect of different doses and duration of zinc administration on the TOC within serum, GCF and saliva, in addition to its potential effect on uncontrolled diabetic patients.
- Further investigations are mandatory to assess the combining effect of SRP with Zinc on the controlled and uncontrolled diabetic patients with periodontitis with enough follow up period to give us more reliable results for the synergistic effects of both interventions.

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