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To evaluate the effect of oral zinc supplementation on salivary MMP-8 levels in periodontitis: A randomized, double-blind, placebo-controlled study

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ABSTRACT

Background and objective: Zinc is an essential micronutrient that plays a crucial role in maintaining oral health. Periodontitis is a widespread oral disease characterized by inflammation and destruction of the gums and surrounding tissues. The objective of this study was to evaluate the effect of oral zinc supplementation as an adjunct to scaling and root planing in the treatment of periodontitis by evaluating its effects on clinical parameters and salivary MMP-8 level.

Methods: 42 patients were enrolled in the study from the periodontology OPD of a tertiary care hospital in India. The subjects were divided into two groups: group 1 and group 2 consisting of 21 patients each of moderate to severe periodontitis. Group 1 was given oral zinc supplementation along with scaling and root planing (SRP) and group 2 was given placebo tablet along with SRP for 1 month. Several periodontal parameters were assessed, including Papillary bleeding Index (BI), Gingival index (GI), Probing pocket depth (PPD) and Clinical attachment level (CAL) at baseline as well as at 1 month post treatment. Around 3 ml of whole unstimulated saliva was collected for MMP-8 estimation by ELISA method at baseline as well as at 1 month.

Results: The data was analyzed using SPSS version 26. All clinical parameters and MMP-8 level in saliva were comparable at baseline. 1 month after respective treatment modalities were performed in each group, it was found that all clinical parameters and salivary MMP-8 level showed significant differences with group 1 (zinc + SRP) showing highly significant decrease in GI, PPD and CAL ($p < 0.01$) and significant decrease in BI and salivary MMP-8 level when compared to group 2 (placebo + SRP) ($p < 0.05$).

Conclusion: When compared with scaling and root planing alone, the administration of oral zinc supplementation along with scaling and root planing showed greater reduction in clinical parameters and salivary MMP-8 levels in patients with moderate to severe periodontitis. Zinc has a positive effect in management of periodontitis and can serve as an easy, cost effective, harmless and beneficial adjunct in treatment of periodontitis.

1. Introduction

Periodontitis is a multifactorial disease caused by multiple microorganisms leading to progressive destruction of the attachment apparatus with the formation of the periodontal pocket, gingival recession, or both.[1] It is an inflammatory condition of the tooth supporting structure and slow progressing in nature, which persuades an immune response and results in loss of supporting tissues of the teeth. This disease is mainly caused by the release of toxic substances by microorganisms present in the dental plaque and calculus.

Mechanical therapy i.e., scaling and root planing (SRP) is considered the gold standard procedure in the treatment of periodontitis. However,

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Table 1
Mean ± SD values of the different clinical parameters tested in groups 1 and 2 at follow-up.

	GI	BI	PPD	CAL
GROUP 1 (n = 21) SRP + Zn (at follow up)	0.89 ± 0.4	0.67 ± 0.27	3.63 ± 1.01	4.32 ± 1.2
GROUP 2 (n = 21) SRP + Placebo (at follow up)	1.23 ± 0.47	0.9 ± 0.41	4.6 ± 0.94	5.68 ± 1.58
p value (Group1 vs Group 2)	0.009**	0.028*	0.003**	0.003**

**p < 0.01 is statistically highly significant, *p < 0.05 is statistically significant, Wilcoxon rank sum test of significance and Independent *t*-test (unpaired *t*-test) of significance applied.

due to limited access to the root surface, various adjuncts are used in the treatment of periodontal disease. There is now extensive evidence supporting the effects of dietary supplements on various inflammatory diseases including periodontal disease. Hence, growing interest has been seen in functions of micronutrients such as zinc in relation to periodontal disease.[2]

Zinc is essential for healthy periodontal tissue as it has an immunological effect on oral soft tissues.[3] Zinc shows anti-inflammatory, antioxidant and pro-apoptotic activities. It is also present in many DNA repair proteins. Zinc causes activation of an antioxidant enzyme, superoxide dismutase (SOD). SOD inhibits production of reactive oxygen species, thereby protecting damage of DNA.

The human body does not have any dedicated storage system for zinc, so it needs to be incorporated in one's diet daily.[4] Zinc deficiency or insufficient level of zinc in body has been found to adversely impact the periodontal health and disease.[5] Supplementation of zinc has been said to be useful in periodontal and other oral diseases by many researchers.[6–8]

Various inflammatory molecules play a direct role in the degradation of periodontium.[9] One of the inflammatory molecules is the matrix metalloprotienase (MMP) which represent a calcium-dependent zinc--containing super family of proteases that are released from different cell types and act as a major regulator to maintain physiologic extracellular collagen matrix homeostasis as well as pro-inflammatory proteins to cause progression and increased severity of periodontitis.[10]

Among MMPs, Matrix metalloproteinase–8 (MMP-8) is a major collagenolytic MMP which is involved in extracellular matrix destruction and remodelling event and has a direct as well as an indirect positive correlation with the severity of inflammation in periodontitis patients.[11] Various studies show that MMP-8 acts an important biomarker in periodontitis. MMP-8 has been correlated with bleeding on probing, clinical attachment loss, and probing pocket depth.[12,13] MMP-8 shows a unique ability to degrade types I and III collagen.[14,15]

Zinc has a key role in MMP-8 reaction.[16]

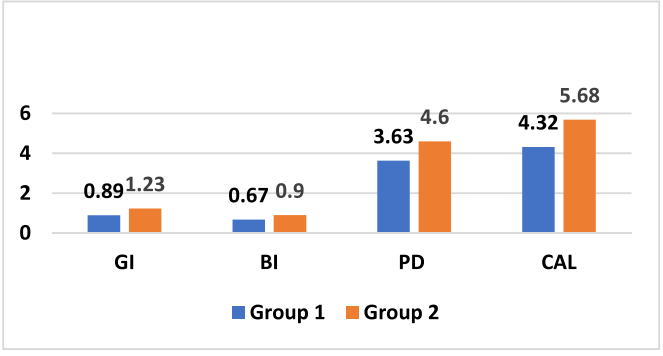


Fig. 1. Mean values of Clinical parameters of group 1 & 2 at follow up.

Table 2
Mean ± SD values of salivary MMP-8 levels tested in groups 1 and 2 at follow-up.

	GROUP 1	GROUP 2
Value of salivary MMP-8 (ng/dl)	307.37 ± 1.62	309.05 ± 2.38
p value (Group1 vs Group 2)	0.03*	

**p < 0.01 is statistically highly significant, *p < 0.05 is statistically significant, Independent *t*-test (unpaired *t*-test) of significance applied.

2. Objectives and rationale

Our objectives were firstly to assess and compare the clinical effects of oral supplementation of zinc as an adjunct to scaling and root planing (SRP) in the treatment of periodontitis. Secondly, it was to evaluate and compare the expression of salivary MMP-8 after oral zinc supplementation as an adjunct to scaling and root planing (SRP) in patients with periodontitis.

Reason for using MMP-8 is that it's co-factor, zinc, affects the enzyme reaction. Zinc is also found to act as an inhibitor when there is an increase in the production of this MMP-8. This advantage makes zinc ideal as an adjunctive treatment that inhibits MMP-8.[17,18] This property of MMP-8 is exclusive to it and hence it was measured in this study instead of other MMPs such as the gelatinases MMP-2 and MMP-9 which are usually used in such studies.

3. Study design and methods

The present placebo controlled randomized clinical study assessed 42 patients with moderate to severe periodontitis. All patients came for the follow up without any no dropouts. Patients were randomly assigned into two equal groups, the first group (Group 1) where non-surgical periodontal therapy was performed and oral hygiene instructions were given in addition to systemic administration of zinc tablets or the second group (Group 2) where non-surgical periodontal therapy and oral hygiene instructions were given in addition to administration of placebo tablets was performed.

4. Study design

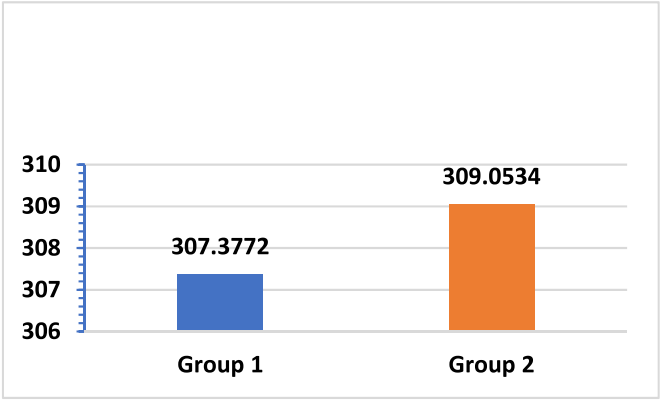
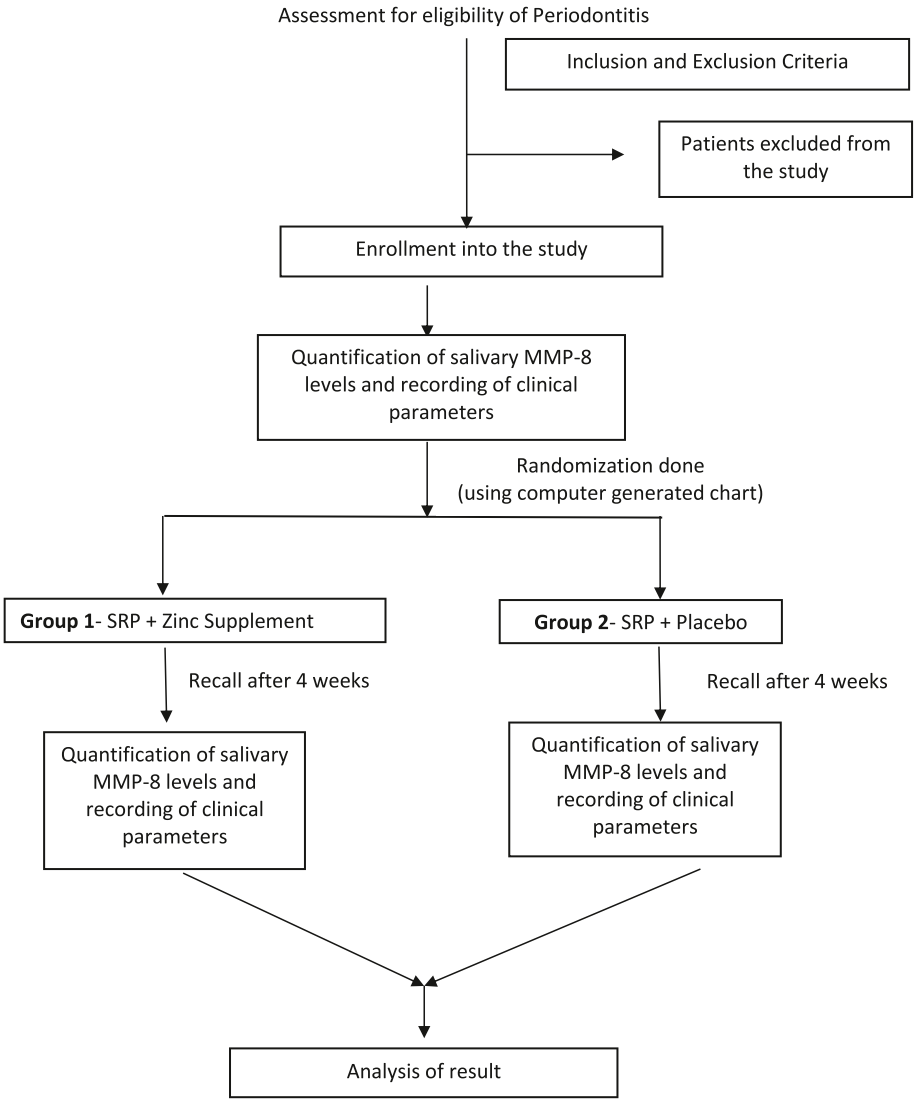


Fig. 2. Mean values of MMP of group 1 & 2 at follow up.



Study Design Flow Chart.

4.1. Site and study participants

Subjects were selected from the outpatient clinic of Department of Periodontology, of a tertiary care Hospital. Clinical parameters were evaluated at baseline (pre-treatment), followed by re-evaluation of clinical parameters at 1 month after scaling and root planing and biochemical parameter (Salivary MMP-8 level) at baseline and 1 month after treatment.

4.2. Inclusion criteria

Criteria for inclusion in the study was healthy patients, aged 18–60 years, of untreated moderate - severe (≥ two tooth sites with clinical attachment loss ≥3 mm), generalised (>30 % of teeth affected) periodontitis, with a slow-moderate progression, fulfilling Stage II/III Grade A/B periodontitis based on Papapanou et al. (2018).[19] Patients included were systemically healthy and had no history of periodontal therapy in the last 6 months.

4.3. Exclusion criteria

Excluded were pregnant or lactating women, patients who are heavy smokers or had a history of alcohol abuse, patients who had taken zinc supplements in last 6 months, Patients who had undergone periodontal therapy in last 6 months, Patients who had received antibiotic therapy in last 6 months, Patients who were regularly using anti-inflammatory drugs, Patients with any systemic disorder affecting periodontal health, Patients who were regular users of mouthwash or subgingival irrigation with known antimicrobial activity within the past one month and patients having teeth with endo-perio complications.

4.4. Sample size determination

- The determination of sample size was done on the basis of a previous study by **Gupta et al., 2015.**[20]
- Difference in the mean MMP-8 level (μ1−μ2) was 158.65 in periodontitis (348.89) and control (190.91) and the average population variance (σ^2) was 172.99.
- The sample size (n) = 2 ($Z\alpha/_2 + Z_{[1-\beta]}$)2 × σ2/(μ1−μ2)^2, based on formula by **Charan and Biswas (2013)**,[21] assuming 0.05 level significance ($Z\alpha/_2 = 1.96$) and 90 % power ($Z_{[1-\beta]} = 1.28$) was **18.6** in each group:

$$n = \frac{2\left(Z_{\alpha/2} + Z_{[1-\beta]}\right)^2 \times \sigma^2}{(\mu 1 - \mu 2)^2}$$

$$n = \frac{2\,(1.96 + 0.84)^2 \times 172.89^2}{(348.89 - 190.91)^2}$$

$$n = 18.6$$

Considering any dropouts, we enrolled **21 patients in each group**.

4.5. Randomization, allocation concealment and consent

The required quantity of Zinc acetate tablets and placebo tablets of same size in a precoded (CODE I and CODE II) identical appearing, opaque sealed envelopes were made. Envelope labelled as CODE I contained 30 tablets of Zinc acetate in a zip lock pouch bag. Similarly, envelope labelled as CODE II contained placebo for zinc in equal quantities in zip lock pouch bags. The placebo used was carboxymethyl cellulose tablet. Patients were randomized at a ratio of 1:1 using an online, web based (http://www.jerrydallal.com/random/random_block_size_r.htm) sequence generator system which is a website for generating chart for randomizing subjects to a single treatment. Computer generated chart is used so that unequal balance between the two groups could get avoided.

The study was double-blinded. The randomization chart was held by the supervisor and was not broken until the completion of data analysis. Only the supervisor provided the precoded envelopes to the patients (according to the randomization chart) at later treatment visit. Patients were instructed to take one tablet once a day for 30 days. The investigator had no access to the randomization chart. Hence, both the patient and the investigator were blinded. The participants were explained in detail about the purpose of the study, the methodology involved and the related risks and benefits, in a language well understood by them. A written consent in local language and a thorough medical and dental history were taken from all the participants. Before the commencement of the study, ethical clearance was obtained from the Ethical Committee of the university (Ref. Code: *III PGTSC-IIA/35*).

4.6. Data collection

Baseline demographic, biochemical and clinical parameters were assessed and collected by a masked investigator of the study team. Assessment of the clinical parameters i.e., Gingival index (GI), Papillary bleeding Index (BI), Probing pocket depth (PPD) and Clinical attachment level (CAL), were measured at baseline before scaling and root planing (first time the patient reports) and at 1 month after respective treatment modalities were performed, in both groups. Probing pocket and clinical attachment level were measured with a University of North Carolina-15 (UNC-15) probe. All the clinical parameters were assessed by the same investigator at different recall visits. After completion of all measurements, to avoid bias, the intra-examiner repeatability was assessed, showing acceptable reproducibility and reliability.

4.7. MMP-8 measurements

Whole unstimulated saliva was collected on day 1 and after 1 month by draining method by Navazesh[22] and centrifuged to obtain the supernatant. To measure salivary MMP-8 level, enzyme linked immune-sorbent assay (ELISA) was performed using Elabscience™ human Matrix Metalloprotienase 8 (MMP-8) ELISA Kit. ELISA was chosen due to its cost effectiveness and easy accessibility as compared to the point of care diagnostic methods available for measuring MMP-8.

4.8. Statistical methods

The results were analyzed using descriptive statistics and making comparisons among various groups. Discrete (categorical) data were summarized as proportions and percentages (%) and quantitative data were summarized as mean ± SD.

Intragroup comparison was done for gingival index (GI) and bleeding index (BI), within group 1 and 2 at baseline and follow-up using Wilcoxon signed rank test of significance. Paired student t-test of significance was used for comparison of. probing pocket depth (PPD), clinical attachment level (CAL) and MMP-8.

Wilcoxon rank sum test of significance was used for intergroup comparison between the groups at baseline and follow-up for gingival index (GI) and bleeding index (BI). Independent *t*-test of significance (also known as unpaired *t*-test of significance) was used for intergroup comparison of probing pocket depth (PPD), clinical attachment level (CAL) and MMP-8.

5. Results

The mean values, standard deviation, standard error of mean along with the respective p value of the comparison was tabulated. All relevant graphs were made. The data was collected and entered in MS Excel spreadsheet. It was analyzed using SPSS version 26. Data analysis was carried out by using descriptive statistics and other relevant tests of significance. The p value was set at 0.05 to be significant and p value less than 0.01 was considered as highly significant. Confidence level was set at 95 % and power of the study was fixed at 0.08.

After statistical analysis it was found that there was a highly significant difference ($p < 0.01$) in the Gingival index (GI), Probing pocket depth (PPD) and Clinical attachment level (CAL)of the study participants between the two groups at follow-up. The mean value table shows that the score of GI and the values of PPD and CAL of group 1 was significantly lower than group 2. (Table 1 and Fig. 1).

Also, there was a significant difference ($p < 0.05$) in the bleeding index of the study participants between the two groups at follow-up. The mean value table shows that the score of bleeding index of group 1 was significantly lower than group 2 (Table 1 and Fig. 1).

For MMP-8 it was found that there was a significant difference in the values of MMP-8 of the study participants between the two groups at follow-up. The value of MMP-8 at follow-up was significantly more in group 2 (Table 2 and Fig. 2).

6. Discussion

Periodontitis is a chronic inflammatory disease which is multifactorial in origin that leads to loss of attachment and bone destruction in tooth supporting tissues such as the periodontal ligament. It is plaque-associated infection caused by the accumulation and maturation of pathogenic biofilms on the surface of the hard tissues of oral cavity. Hence, this disease begins as gingivitis in response to the bacterial load. Unlike other diseases, single microorganism does not cause it. Rather, it is caused by multiple pools of bacteria existing in the sub-gingival sulcus as dental plaque which even till now, are not all identifiable.

Gold standard treatment of periodontitis involves subgingival and supragingival mechanical debridement concurrent with supervised oral hygiene maintenance, which results in substantial decrease in the subgingival microbial load and clinical parameters.[23–25]

Systemic antibiotics and topical mouth washes are usually employed as adjuncts to mechanical therapy to deter recolonization of pathogens but are found to have limited long-term effectiveness. Also, concerns regarding antibiotic use and abuse leading to bacterial resistance to antibiotics have led to demand for newer and nobler treatment approaches for periodontitis.[26,27]

There is a wide range of studies and evidence supporting the positive effects of dietary supplements and micronutrients like zinc as adjuncts in the treatment of inflammatory conditions[1] and the positive effect of zinc supplementation on reduction in periodontal disease state as compared to patients without zinc supplementation.[28–30]

In this randomized clinical study, a total of 42 subjects (21 in each group) with moderate to severe periodontitis were included for clinical and biochemical evaluation. Mean age of patients in group 1 was 41.8

and in group 2 was 36.9. Total of 24 Males and 18 females were present in the study. All subjects in our study were systemically healthy, received regular treatments and were well motivated for maintaining good oral health.

The dosage of zinc given to the patients was Zinc Acetate 50 mg tablet (containing 15 mg of elemental zinc) once a day for 30 days. No complications or side effects were reported by the patients, (which was expected as zinc has a high therefore, the concentration and the dosage given can be considered safe to prescribe.

In our study, the intergroup comparison at baseline showed that the differences in mean of clinical parameters (GI, BI, PPD and CAL) at baseline between both the groups were statistically insignificant ($p > 0.05$) which shows that both the groups were comparable at baseline.

The intergroup comparison data inferred that, zinc + SRP Group exhibits greater reduction than Placebo + SRP in gingival index (GI) at follow up visit which was highly significant ($p < 0.01$) (Fig. 1 and Table 1). This improvement in Group 1 can be attributed to the anti-inflammatory and antimicrobial properties of zinc. Almost similar reduction in GI ($p < 0.001$) was seen in the study conducted by Alqawi AA. et al. (2022),[29] Uçkardes et al., 2009)[27], and Rösing CK et al. (2017).[30]

This is also in agreement with the animal studies by Orbak R et al. (2006)[31] and Seyedmajidi SA et al. (2014).[32]

In Bleeding index (BI) at follow-up in Group 1 it was observed that highly statistically significant decrease in bleeding from baseline to follow up ($p < 0.01$). This decrease might have been due to SRP and zinc supplementation and maintenance of oral hygiene by the patient as we have thoroughly counseled the patient for maintaining good oral hygiene throughout the study. Similar results were also obtained in Group 2 where statistically significant decrease is seen in bleeding scores were observed from baseline to follow up ($p < 0.01$). This decrease might be due to SRP and maintenance of oral hygiene by the patient throughout the study.

On Intergroup comparison of BI, significant difference was observed in mean of BI between the two groups at follow up ($p < 0.05$) (Table 1 and Fig. 1). This implies that zinc has a positive effect on bleeding on probing as is evident from the improvement in bleeding index (BI) score. Similar results were found in an animal study done by Tofrizal T et al. (2022)[33] in which Papillary Bleeding index at 11th day was reduced more in rats fed with zinc supplements as compared to rats with low zinc diet.

Statistically significant mean reduction in probing pocket depth (PPD) was observed in both Group 1 and Group 2 at 1 month of follow up. On Intragroup comparison of Group 1, the PPD highly significantly decreases from baseline to follow up ($p < 0.01$). Group 2 also demonstrated a highly significant decrease in PPD from baseline to follow up ($p < 0.01$). On comparing the PPD at follow-up between Group 1 and Group 2 (intergroup comparison), it was found that the reduction in PPD in Group 1 was more at follow up ($p < 0.01$) (Table 1 and Fig. 1). Similar results were obtained by Salih SM et al. (2014)[28] and contrary results to this were obtained by Alqawi AA et al. (2022).[29] Similar contrasting result was seen in an animal study by Seyedmajidi SA et al. (2014)[31] where there was no significant difference regarding probing pocket depth between zinc fed rats and non zinc fed rats. ($P = 0.07$).

In our study, highly statistically significant differences in gain in Clinical attachment level (CAL) ($p < 0.01$) were seen in Group 1 and Group 2 at follow up visit. The CAL gain in Group 1 is highly statistically significant from baseline to follow up with ($p < 0.01$). Similar results were seen in Group 2 ($p < 0.01$) but the gain was greater in Group 1. This gain in CAL was similar to study done by Salih SM. et al. (2014).[27] They have suggested that several potential mechanisms might be at play on the cellular level. Zinc might protect cells against oxidative damage by inhibiting the formation of reactive oxygen species (ROS). This contrasts with a study by Alqawi AA. et al. (2022).[29]

All the clinical parameters in our study indicated betterment in periodontal health for patients who took oral zinc supplementation. Furthermore, because in all of our patients SRP was performed, our data are consistent with the notion that taking zinc supplements may have beneficial effects above and beyond of SRP alone.

MMPs are proteolytic enzymes, belonging to the zinc protease super family, are involved in the physiological degradation of basement membranes and extracellular matrix proteins. They are divided into several groups. An important source of MMP-8 in humans is degranulation triggered by neutrophils (neutrophil-type MMP-8), but MMP-8 (mesenchymal cell-type MMP-8) is de-novo secreted and expressed in minute amounts by non-PMN-lineage cells like, fibroblasts, smooth muscle cells, macrophages, epithelial cells and endothelial cells.[34] MMP-8 is one of the essential members of the MMPs family and belongs to the collagenase group possessing a novel ability to decompose collagen type III and I which are present within the periodontal ligament.

Salivary concentrations of MMP-8 are increased at sites affected by gingivitis and periodontitis, and the levels of MMP-8 also correlate with clinical periodontal disease severity.[35–37] Evidence suggests that the level of MMP-8 decreases after periodontal treatment i.e. scaling and root planing, along with reduction in clinical signs of inflammation.[20,35,38] So, the saliva of patients was analyzed in this study for MMP-8.

In the present study, the differences in mean MMP-8 level at baseline between both the groups were not statistically significant ($p > 0.05$) which shows that both the groups were comparable at baseline.

On intragroup comparison, MMP-8 level in Group 1 and Group 2 significantly decreased after treatment from baseline to foll ow up (311.68 ± 1.96 to 307.377 ± 1.99) for Group 1 and (311.31 ± 1.15 to 309.05 ± 2.38) for Group 2 both of which were statistically highly significant differences ($p < 0.01$). (Table 2 and Fig. 2).

This reduction of salivary MMP-8 in SRP group can be correlated to studies by Rangbulla et al. (2017)[38] and Gupta N et al. (2014)[20] and Sexton et al. (2011).[35]

The intergroup comparison data inferred that; Group 1 exhibited greater reduction than Group 2 in MMP-8 at follow up visit which was significant ($p < 0.05$). This better improvement in Group 1 can be attributed to the inhibitory properties of zinc in regard to MMP-8 and also its anti-inflammatory and antimicrobial properties. Our finding was similar to the findings that were observed in the study conducted by Kasuma N et al. (2016).[17] They determined a significance level of ($p = 0.00$) between MMP-8 and zinc consumption. They also mentioned that the reason for this relationship could be due to zinc's important role in oxidative stress and also as an anti-inflammatory agent.

The results of our study are also in agreement to a follow up animal study conducted by Kasuma N et al. (2021),[6] which concluded that zinc supplementation is effective in reducing MMP-8 level in periodontitis thereby lowering the disease severity. Various reasons for this suppression of MMP-8 due to zinc supplementation can be found in literature. Zinc acts as MMP-8's cofactor that affects enzyme reaction and as an inhibitor[17,18] when there is an excess in this enzyme production which is shown to happen in periodontitis. Zinc is found to enhance the production of TIMP (Tissue inhibitor of Matrix metalloprotienases) which inhibits MMP-8 production and slow down the collagenase activity.[39] Zinc supplementation has shown to suppress osteoclast differentiation and induce osteoblast mineralization On periodontitis followed with bone loss condition.[40,41]

The possible limitation of our study includes a small sample size. This raises the requirement of a longitudinal study having a larger sample size for evaluating the role of zinc. Differences in zinc dosage, treatment protocols, disease severity, observation intervals, and measurements, as well as the flow rate of saliva, has to also be taken into consideration and thus further studies need to be conducted in order to understand if the above-mentioned factors play a role and affect the results in any possible way.

7. Conclusion

Oral zinc supplementation has a positive effect when used as an adjunct to scaling and root planing as it shows significant improvement in all the clinical parameters of the periodontal disease and also shows statistically significant reduction in salivary MMP-8 level. Additionally, it also helped in preventing the progression of the periodontal lesions. and it has the advantage of being easy-to-administer, having no harmful side effects and being a cost-effective dietary supplement with additional systemic benefits. Reduction in MMP-8 levels post conservative therapy, strengthens the fact that it can be used as biomarker as a diagnostic tool in periodontitis. The MMP-8 can also be used for monitoring the progress of management therapy in periodontitis cases and for determining their prognosis. The results of this study provide valuable information for healthcare providers and highlight the need for further investigation into the potential therapeutic benefits of zinc supplementation in the management of periodontitis. The long-term effects of zinc supplementation have to be evaluated through more studies done over a longer duration of time and having a larger sample size.

Contribution of each author

Author 1: Contributed to conception, design, data acquisition and statistical interpretation. Drafted and critically revised the manuscript.

Author 2: Contributed to conception, design, data acquisition and statistical interpretation. Drafted and critically revised the manuscript and did overall supervision of the study.

Author 3: Contributed to biochemical analysis, data acquisition and interpretation.

Author 4: Contributed to study designing and data acquisition.

Author 5: Contributed to data acquisition and revision of manuscript.

Author 6: Contributed to data acquisition and interpretation.

Author 7: Contributed to clinical assistance.

Author 8: Contributed to biochemical analysis, data acquisition and interpretation.

Author 9: Contributed to critical revision of the manuscript.

All authors gave their final approval and agreed to be accountable for all aspects of the work.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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