The efficacy of systemic atorvastatin as an adjunctive therapy for treatment of chronic periodontitis and its correlation with salivary osteoprotegrin level: a randomized placebo-controlled double-blinded clinical trial

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Abstract

Objective: To examine the efficacy of systemic atorvastatin as an adjunctive therapy for chronic periodontitis in conjunction with conventional periodontal therapy, as compared to a placebo and its correlation with changes in salivary OPG levels.

Intervention: All patients underwent full-mouth scaling and root planning, then were randomly allocated to one of the two study groups: group A (Intervention), which received atorvastatin 20 mg/d, or group B (Control), which received a placebo capsule.

Outcome measures: The primary outcome was improvement in periodontal parameters, including clinical attachment loss, pocket depth, and bleeding on probing from baseline to six months of treatment, while the secondary outcomes were reduction in radiographic intra-bony defect depth as well as variations in salivary **osteoprotegrin** levels from the baseline to six months.

Results: There was a statistically significant difference in clinical attachment loss, pocket depth, and depth of intra-bony defect between pre- and post-treatment values in the atorvastatin group. At 6 months follow up, the atorvastatin group showed statistically significant lowering in clinical attachment loss, pocket depth, and depth of intra-bony defect, as well as statistically significant elevation of the salivary osteoprotegrin level as compared to the placebo group. However, no change was observed in bleeding on probing values between the two groups. Also, there was a statistically significant correlation between PD and salivary OPG level (r = -0.255, P = 0.174). However, there was no statistically significant correlation between CAL and salivary OPG level (r = -0.294 and P = 0.15). Furthermore, there was no statistically significant correlation between IBD and salivary OPG level (r = -0.09 and P = 0.636).

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Conclusion: Systemic atorvastatin could be used effectively as an adjunctive therapy for the treatment of periodontitis, leading to enhanced treatment outcomes. Systemic atorvastatin elevates salivary OPG levels, which promote bone regeneration and reduce alveolar bone loss.

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INTRODUCTION

Statins could inhibit competitively the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in the mevalonate pathway that produces cholesterol in the liver.Aside from cholesterol lowering effect of statins that have been routinely utilized in cardiovascular disease (CVD), they displayed extra-pleiotropic benefits such as; enhanced bone formation as well as anti-inflammatory, immunosuppressive, anti-oxidant, anti-thrombotic, and angiogenesis effects, have drawn increasing attention **(1-5).**

Lovastatin, a substance that occurs naturally, was the first statin to be found. Since then, six more statins have been released on the market. While fluvastatin, atorvastatin (ATV), rovastatin, and pitavastatin are synthetic statins, Simvastatin and pravastatin are semi-synthetic statins **(6, 7).**

The impact of statins on bone mineral density (BMD) has been the subject of conflicting prior research, however, current data points to both their bone-forming and bone-sparing effects. According to earlier studies, statins are strong inducers of the bone-anabolic proteins such as; vascular endothelial growth factors (VEGFs) and bone morphogenetic protein-2 (BMP-2), which support osteoblast development and bone mineralization. In addition, it was discovered that they promoted the activity of alkaline phosphatase in human periodontal ligament (PDL) cells **(1, 8-13).**

Additionally, statins promote the synthesis of osteoprotegrin (OPG), which may be the cause of their ability to preserve bone. Blocking the intermediates generated from mevalonate that activate osteoclasts may intensify this impact. Statins may thereby lessen bone loss by inhibiting the HMG-CoA reductase pathway.It is noteworthy that hydrophobic statins like pravastatin could not promote BMP-2 expression on osteoblasts, but lipophilic statins like simvastatin and ATV did **(14-17).**

Chronic periodontitis is a chronic inflammatory disease characterized by the loss of attachment of connective tissue and alveolar bone. The process of bone resorption associated with periodontitis requires the interaction of cytokines produced by immune cells in the inflamed tissues, such as B- and T-lymphocytes, and resident cells, such as human gingival fibroblasts (HGF) and PDL cells. The activation of osteoclasts is controlled by the interaction between RANKL (receptor activator of nuclear factor kappa beta ligand), which is found on periosteal osteoblasts, fibroblasts, and B- and T-lymphocytes, and its receptor RANK, which is found on mononucleated osteoclasts that will eventually become bone-resorbing osteoclasts **(18, 19).**

The soluble decoy receptor, OPG, binds to RANKL when its concentration is high enough to prevent RANK-RANKL interaction. This inhibits osteoclast development and activation, which in turn prevents bone resorption. According to some research, a high dosage of ATV may boost the formation of OPG and hence reduce bone resorption, **(20-22).**

In the context of periodontitis, the many physiologic effects of statins provide an appealing therapeutic prospect. There is evidence that statins have antibacterial qualities, making them an effective weapon against infections that impede healing, particularly in situations when the condition is resistant to conventional systemic antibiotic therapy **(6, 23).**

Likewise, statins demonstrated an anti-inflammatory action through inhibiting pro-inflammatory cytokines such as IL-1B and IL-6, reducing the number of inflammatory cells. Furthermore, statins induce the generation of nitric oxide (NO), which explains how they influence the interaction between leukocytes and endothelium. The C-reactive protein (CRP) level, a clinical indicator generated by the liver in response to pro-inflammatory cytokines, is

decreased as a result of the statins' reduction of inflammation **(2, 24-27).**

In a number of animal studies investigating the effects of statins on tooth attachment and alveolar bone loss, protective effects and the capacity for new bone formation have been demonstrated. However, only a small number of studies examined how individuals with chronic periodontitis responded to statins, particularly simvastatin and ATV. Therefore, it is unclear whether statins could help treat periodontal disease and encourage the growth of new bone

In light of the multiple biologic effects of statins, including their protective effect on bone, we decided to examine the effects of ATV, a lipophilic statin, as a potential adjunctive treatment for chronic periodontitis as compared to placebo combined with conventional scaling and root planning (SRP), as well as its correlation with changes in salivary OPG levels.

METHODS

This study is a randomized placebo-controlled double-blinded clinical trial with a 1:1 allocation ratio in parallel groups.

The Faculty of Dentistry at Fayoum University's outpatient clinic served as the recruitment site for eligible subjects. After being notified of the whole process, recruited individuals who matched the inclusion criteria were requested to sign consent forms to participate in the research.

Eligibility criteria: In order for patients to be included in the study, they must meet the following criteria: 1) they should have severe chronic periodontitis with pocket depth (PD)≥5 mm or clinical attachment loss(CAL) ≥ 4 mm as well as vertical bone loss ≥3 mm on periapical radiographs**(28)**; 2) should be aged over 20; 3) should not have had periodontal therapy within the last 6 months; and 4) should have at least 15 remaining teeth, while **Criteria for exclusion were**; 1) Patients with any systemic condition that may affect periodontal health and bone formation, such as; those who are pregnant, nursing, or postmenopausal women; DM, CVD, metabolic syndrome, osteoporosis, AIDS and chronic alcoholism **(29, 30)**; 2) having local factors that may aggravate periodontal diseases such as; orthodontic and prosthetic appliances and parafunctional habits **(31-34)**; 3)smoking (patients wereconsidered smokers if theysmoked at least 10 cigarettes per day on a regular basis for at least 5 years)**(35);**4) Patients who are taking any medication that may have an impact on the study's results, such as; glucocorticoid treatment, bisphosphonate treatment, hormone replacement therapy, chronic treatment with NSAIDS, antibiotic treatment, use of statins for six months prior to the study, or periodontal therapy for the previous 6 months.

Participants and Intervention: All patients completed thorough full-mouth SRP. Under local anaesthesia, subgingival debridement was achieved using periodontal Gracey curettes and an ultrasonic scaler (NSK Disease Markers 3 non-optical ultrasonic scaler, Kanuma-shi, Japan; Lustra Gracey periodontal curettes, Dentsply, Surrey, UK). Patients were then randomly allocated (see randomization below) to one of the two study groups: group A (Intervention), which received atorvastatin 20 mg/d (ATOR, EIPICO), or group B (Control), which received a placebo (Capsules were prepared to be identical to ATV capsules in shape, colour and odour).

Outcome measures: The primary outcome wasimprovement in periodontal parameters including PD (in mm), CAL (in mm), and bleeding on probing (BOP in %) from baseline to 6-months of treatment**,** while **the Secondary outcomes**werereduction in radiographic intra-bony defect depth (IBD in mm) from the baseline to six monthsand variations in salivary OPG levels (pg/ml) from the beginning to six months.

Data gathering: All data were recorded at baseline and after 6 months of treatment

Periodontal examination: Evaluation of periodontal parameters such as; PD, CAL, and BOP was performed manually using Williams' graduated periodontal probe periodontal (PCP-12; Hu-Friedy, Chicago, IL, USA), CAL and PD measurements were collected and recorded on six surfaces per tooth (mesio-buccal, mid-buccal, disto-buccal, and mesio-lingual, mid-lingual, disto-lingual, or palatal surface)**(36, 37)**. The CAL measurement represents the

distance between the cement-enamel junction of the tooth and the deepest part of the pocket, while PD measures the distance between the gingival margin and the deepest part of the pocket. The PD score of an individual tooth was calculated by summing the PD values measured on each tooth surface and dividing by the number of dental surfaces examined. The CAL score for each tooth was then calculated using the same method(38).Total mean of CAL and PD was computed for each patient and was recorded to the nearest millimeter**(39).** Sulcus depths between 0 and 2 mm were regarded as normal **(40).**

Four surfaces per tooth were examined for BOP readings (mesial, distal, buccal, and lingual or palatal surface). BOP was examined directly after the PD measurement (30 seconds after applying the periodontal probe) and was reported as absent (0) or present (1). The proportion of teeth displaying BOP was recorded.

Evaluation of intra-bony defects using radiography (IBD)(41, 42): Using the parallel technique, a 1.5mm EzSensor HD sensor (Vatech, Korea) was utilised for radiographic evaluation. The bite was registered using acrylic resin (Duralay, Reliance, IL, USA). To assure the same occlusion in the subsequent radiography, it was initially recorded prior to the radiography. To evaluate the depth of IBD, radiographs were obtained at baseline and six months later using the same voltage, amperage, exposure duration, and occlusion record. Images were captured in DICOM format and processed using EzDent-i 3.1.6 (Vatech, Korea). On the radiograph, the distance between the cemento-enamel junction (CEJ) and the base of the defect (BD) was measured to identify the IBD. The tooth with the most significant defect was evaluated. Image calibration was performed using software prior to measurement. as shown in **figure (1)**.

Collection of salivary OPG samples(43): A standard method was used to collect complete un-stimulated saliva**.**Before samples collection, patients were refrained from eating, drinking and smoking. Then, patients were instructed to swallow first, tilt their heads forward, and expectorate all of their saliva into a tube for five minutes at about 30 seconds interval without swallowing. All saliva samples were centrifuged at 2000 g after collection, and the separated supernatants were kept at 80 °C for further analysis.

Measurement of salivary OPG (using ELIZA): Using a commercially available human enzyme-linked immunosorbent assay (ELISA) kit, salivary samples were examined for OPG (BioVendor Research & Diagnostic Product European Union). Analyses were conducted in accordance with the manufacturer's procedure. All ELISA analyses were carried out twice. A sandwich-type ELISA in which an anti-human monoclonal antibody has been adsorbed onto microwells reacted with the sample's OPG. The assay kit's standard curves were utilized to compute the results. At 450 nm, the color's intensity was assessed. The amount of OPG present was expressed in picograms per millilitre (pg/mL).Saliva samples were taken at the beginning of the study and after 6 months of treatment.

Calculating the sample size: Using the sample t-test and a prior study by Fajardo et al. **(4)**, the sample size was computed for the likelihood of type I error (α) = 0.05, the power (1-β) of 0.9, and the effect size of 0.764 using the mean and SD of CAL in the intervention group before and after treatment. It was determined to be a total of 21 participants. To compensate for dropout loss, the sample size was raised to 30 (15 in each group). The G*Power 3.1.9.7 software was used to calculate sample size

Randomization and blinding: Following the patients' enrollment agreement, the allocation sequence was established using computer-generated randomization (www.rand.org) at a 1:1 ratio. The Faculty's statistical unit was in charge of creating the sequence. Randomization table was sent to faculty of pharmacy, whereidentical looking containers marked asA (Intervention) and B (Placebo) were prepared. The primary investigator (S.R) was responsible for allocation, enrollment, recording of baseline characteristics and treatment delivery without knowing the

intervention versusplacebo containers.The outcome assessor (E.M.) was blinded since she was not engaged in patient assignment or therapy administration. Only radiologic evaluation pre- and post-treatment was done by the same investigator (S.M) who was unaware of clinical data and the delivered treatment.

Statistical methods: Variables were statistically described in terms of mean standard deviation (SD) or percentages. Continuous data were examined for normality using the Shapiro-Wilk test, and homogeneity of variances was determined using the Levene test. Student t test was used to compare two independent samples for normally distributed (Parametric) continuous data, while Mann-Whitney test was used to compare two independent samples for non-normally distributed (non-parametric) continuous data. When the continuous variables within a group were normally distributed, the paired t test was used to compare them, whereas the Wilcoxon rank test was used for non-normally distributed (non-parametric) data. P-values below 0.05 were regarded as statistically significant. To determine the correlation between distinct parameters, the Pearson correlation test was utilized.

RESULTS

The 30 participants consisted of 15 in Group A (ATV), 15 in Group B (Placebo).The 15 of group A were 9 females (60%) and 6 males (40%) with mean ages of 44.4 ± 12.2, while the 15 of group B were 7 females (46.7%) and 8 males (53.3%) with mean ages of 40± 10.1. All means and SD of the studied variables (PD, CAL, BOP, IBD and salivary OPG) were calculated and recorded as presented in **table (1).**

Table (1): The studied variables at baseline and after 6 months

Parameters	At Baseline		P-value	At 6 months		P-value
	Atorvastatin	Placebo		Atorvastatin	Placebo	
PD (mm)	6.13±0.99	5.6±1.056	0.165	3.8±0.775	5.21±0.01	≤0.001
CAL (mm)	7.07±1.75	6.27±1.75	0.24	4.47±1.552	6.27±1.75	0.018
BOP (%)	26.11±21.15	25.86±16.65	0.97	19.83± 19.45	20.93±13.9	0.708
IBD (mm)	3.99±0.75	3.86±0.68	0.687	3.03±0.66	3.61±0.734	0.032
Salivary OPG (ng/ml)	64.47±15.9	63.59±14.9	0.878	79.23±9.81	64.46±14.071	0.002

As shown in **table (2),** there was no statistically significant difference between ATV and placebo groups in any of the studied parameters at baseline. However, at 6 months follow up the ATV group showed statistically significant difference than placebo in which there was significant lowering in PD, CAL and IBD, ,whereas there was significant elevation of salivary OPG level. On the other hand, no change was observed in BOP values between the two groups neither at baseline nor at 6 months of treatment **(Figure 1).**

Table (2): comparison between Atorvastatin and placebo regarding the studied variables

Parameters	Visit	Test	Statistics	Mean difference	95% CI		Effect size	P-value
					lower	upper		
PD (mm)	At Baseline	Student's t	-1.427	-0.533	-1.29	0.232	-0.5211	0.165
	At 6 months	Mann-Whitney U	33	1	1	2	0.706	≤0.01
CAL (mm)	At Baseline	Student's t	-1.25	-0.8	-2.11	0.51	-0.456	0.221
	At 6 months	Mann-Whitney U	56	2	1.5	3	0.5022	0.018
BOP (%)	At Baseline	Student's t	-0.0364	-0.25	-14.49	13.9	-0.0133	0.971
	At 6 months	Mann-Whitney U	103	4.5	-10.57	12.9	0.084	0.709
IBD (mm)	At Baseline	Student's t	-4.067	-1.107	-0.64	0.43	-0.148	0.687
	At 6 months	Student's t	2.26	0.58	0.054	1.12	0.84	0.032
Salivary OP	At Baseline	Student's t	-0.155	-0.87	-12.42	10.67	-0.0566	0.874
(ng/ml)	At 6 months	Student's t	-3.34	-14.77	-23.84	-5.7	-1.218	0.002

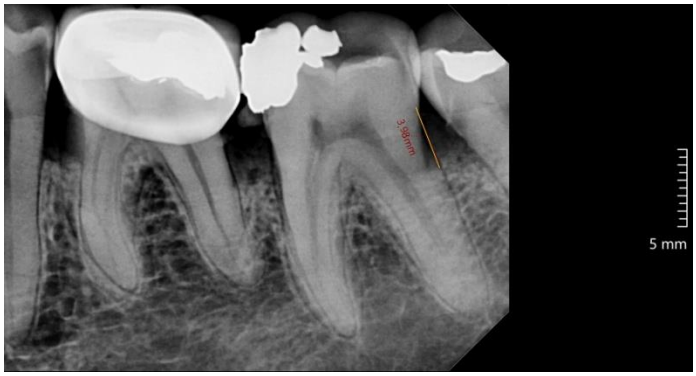

Figure (1): Radiographic picture showing evaluation of intra-bony defect using software

When we performed paired analysis within each group (ATV and placebo) comparing pre- and post-treatment values, results revealed statistically significant difference between pre- and post-treatment values in ATV group as shown in **Figure (3).** Regarding PD, CAL and IBD there was statistically significant reduction (PD reduction is shown in **figure 4**)

Concerning salivary OPG level there was statistically significant elevation after 6 months of treatment. However, only BOP values were significantly different in placebo group at baseline compared to 6 months of treatment as shown in **table (3) and figure (3).**

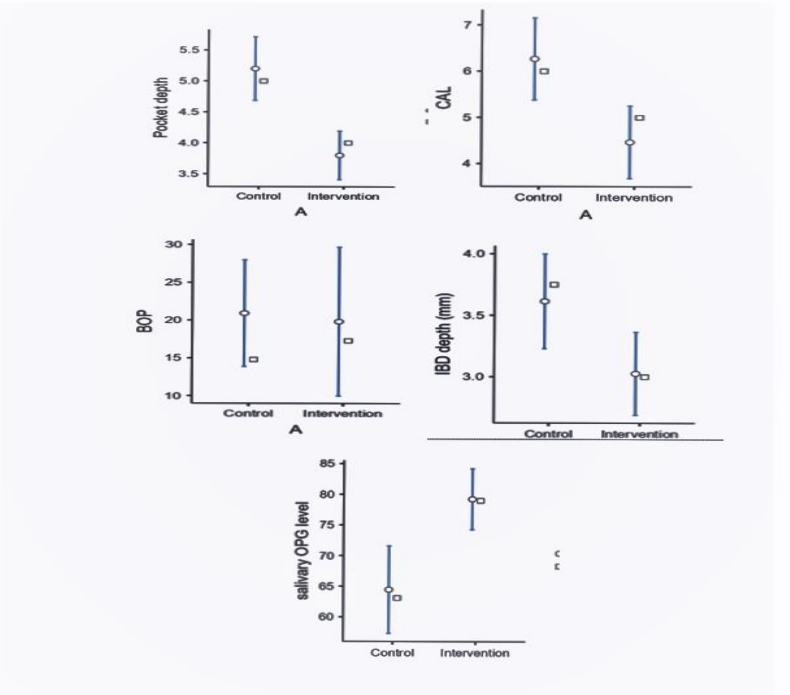

Figure (2): At 6 months, comparison of the studied variables in the atorvastatin group versus the placebo group

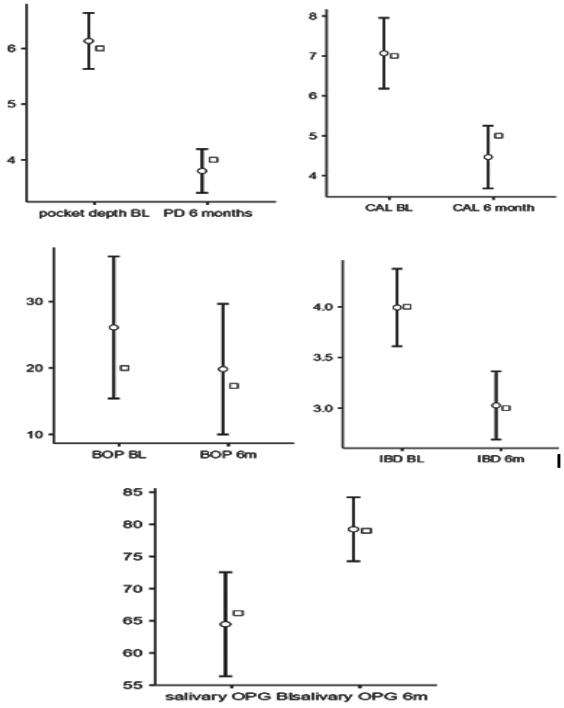

Figure (3): Comparison of the studied variables between baseline and 6 months of treatment values in the atorvastatin group

Pearson correlation analysis revealed no statistically significant correlation between PD and salivary OPG level (r=-0.255, P=0.174). Also, there was no statistically significant correlation between CAL and salivary OPG level (r=-0.294 and P=0.115). Furthermore, there was no statistically significant correlation between IBD and salivary OPG level (r=-0.09 and P=0.636).

Table (3): Comparison of the studied variables between baselines to 6 months in the 2 study groups

Baseline -6 months	Group	Test	Statistics	P-value
PD (mm)	Atorvastatin	Wilcoxon W	120	≤0.001
	Placebo	Wilcoxon W	6	0.149
CAL (mm)	Atorvastatin	Wilcoxon W	105	≤0.001
	Placebo	Wilcoxon W	6	0.149
BOP (%)	Atorvastatin	Wilcoxon W	37	0.097
	Placebo	Wilcoxon W	36	0.014
IBD (mm)	Atorvastatin	Paired Student's t	4.812	≤0.001
	Placebo	Wilcoxon W	6	0.174
Salivary OPG (ng/m	Atorvastatin	Paired Student's t	-5.74	≤0.001
	Placebo	Wilcoxon W	11	0.363

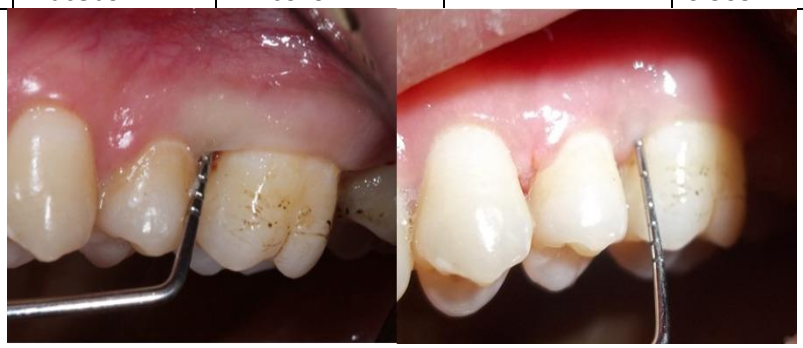

Figure (4): Clinical photograph showing reduction of pocket depth measurements before and after treatment in atorvastatin group

DISCUSSION

Periodontal diseases are considered infectious diseases. Accordingly, the use of antimicrobial agent has been the primary pharmacological approach in its treatment as an adjunct to SRP. However, bacterial resistance and immunosuppression constitute a major concern in the use of antibiotics**(44).**

Statins have been one of the suggested adjunctive therapies that have shown promising results in the treatment of periodontal diseases. The pleiotropic effects of statins have been clearly demonstrated by in vitro studies. These effects include regulation of immune response, inflammation and bone regeneration.Statins could exert their effects on modulation of oseoclastic and osteoblastic differentiation by acting on the OPG-RANK-RANKL signaling system, which has been investigated as possible biomarkers in periodontal regeneration **(45, 46).**

Furthermore, lower levels of alkaline phosphatase and osteocalcin that occur with the use of statins have been linked to regulation of osteoclastic differentiation, whereas there were decreases in MMP-1 that induce collagen degradation **(12, 46-50).**In addition, the anti-inflammatory effect of statins was revealed through their ability to reduce cytokines including IL-6 and IL-8 **(51, 52).** Aside from their bone modulatory and anti-inflammatory actions, statins could affect periodontal condition through controlling dyslipidemia **(53).** Although not yet conclusive, current evidence suggests association of dyslipidemia and worsening of periodontitis **(54)**

The efficacy of statins as prophylactic treatment in periodontal disease has been well documented in animal studies **(50, 55-63).** Also, clinical studies were conducted to investigate the topical and systemic use of statins to control periodontitis with promising results **(4, 41, 42, 64-72).**However, to date, there is no consensus on their efficacy in the treatment of periodontal diseases**.**

Owing to the multiple beneficial effects of statins and their possible use as an alternative therapy to overcome the adverse effects of antimicrobials and to augment periodontal treatment outcome, they have been a topic of interest in our research.

Of note, most of the researches performed on statins in periodontitis focused on their prophylactic effect. However, in the clinical setting, management of established periodontitis is the main point of concern as it is the state at which patients usually seek dental management.

Another point of interest is the adjustment of dose and mode of administration which varies between animals and human studies. Accordingly, we conducted the present study to evaluate the efficacy of 20 mg/d ATV as an adjunct to SRP compared to placebo and SRP in terms of PD, CAL, BOP, IBD. In addition, we analyzed the variation in salivary OPG levels, which are believed to play a crucial role in bone regeneration.

Our results have shown greater improvement in periodontal parameters such as; PD, CAL and IBD in the ATV group than in the placebo group after 6 months of treatment. In ATV group, values of these parameters were significantly lowered after 6 months of treatment than baseline values. Although no change has been observed in BOP values, but considering CAL, PD and IBD is more reliable as they are the main periodontal parameters that determines severity and prognosis of periodontitis **(28).**

On the other hand, there was evident elevation in salivary OPG level which indicates bone regeneration. Although we could not find significant correlation between PD and CAL in relation to salivary OPG level, however, the inverse relation has been revealed with significant lowering in periodontal measurements and elevation of salivary OPG. Given the obvious improvement of IBD level, overall data suggests greater periodontal and bony regeneration with ATV than placebo.

In accordance with our results, several studies revealed the efficacy of statins in the treatment of periodontitis. However, topical use of statins has been recommended than systemic use due to fewer adverse effects and adequate concentrations at the site of inflammation which allows obtaining the possible antimicrobial and osteogenic effects of statins**(53, 73, 74)**

Nevertheless, the choice of proper local delivery system that provides adequate release and biocompatibility could be challenging and the most suitable carrier is not yet conclusive **(75).**For this reason, we preferred to use systemic administration with better patient compliance and less variation in application technique. This rationalizes the need for re-evaluation of these medications as one of the available therapeutic options in treatment of periodontitis

Despite the evident benefits of statins in treatment of periodontitis, recent systematic reviews of clinical trials pointed to considerable heterogeneity of periodontal parameters examined and indicated a weak recommendation of statins as an adjunct to SRP **(53, 73).**

With thecurrent available evidence on the efficacy of topical application of ATV as an adjunct to SRP in improvement of periodontal parameters as well as IBD, to our knowledge, only two clinical trials investigated the systemic use of statins **(4, 41, 67, 69, 70).**Results of **Fajarado et al. (2010)** were in line with the present study in which significant alveolar bone gain as well as PD and CAL reduction was evident in the ATV group relative to the placebo group. Similarly, **Fentoğlu et al. (2012)** found an improvement in all clinical parameters with ATV treatment **(4, 67)**

Moreover, numerous studies showed the positive association bone regeneration and increase in OPG levels **(12, 47-50)**These findings are consistent with our results as there was reduction in IBD as well as elevation of OPG within atorvastatin group post-treatment than pre- treatment.

To sum up, our research added to the current evidence that statins could be used successfully as an adjunctive therapy in the management of periodontitis with improvement in treatment outcome. In particular, systemic administration of ATV demonstrated promising results regarding most clinical parameters of periodontal disease. Taking advantage of the multiple benefits of statins, including their anti-inflammatory, immune-modulatory, and anti-microbial properties, ATV seems to be an excellent alternative to antibiotics in the treatment of periodontitis. Furthermore, the present study confirms the assumption that statins contribute to the elevation of salivary OPG levels, induce bone regeneration, and reduce alveolar bone loss.

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Conflict of interest:

No conflicts of interest are disclosed by the authors.

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