Cone-beam computed tomography analysis of the effects of topical application of 1% sodium alendronate gel in the surgical treatment of periodontal intrabony defects: a 6-month human randomized controlled clinical trial

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Abstract

Aim: The primary aim of this randomized split-mouth triple-blind placebo-controlled clinical trial was to assess, by means of cone-beam computed tomography (CBCT), the effect of surgical topical use of 1%- Sodium alendronate (ALN) on the reduction of periodontal intrabony defects.

Material and methods: Sixty-four intrabony defects from 32 patients with periodontitis were randomly treated with either 1%-ALN gel or placebo gel during periodontal surgeries. Periodontal clinical parameters were recorded at baseline and at 90 (T1) and 180 days (T2) after surgical treatment. Bone defects were evaluated by digital subtraction radiography (DSR) and CBCT at baseline and T2.

Results: At T2, intergroup analysis showed significantly better periodontal clinical parameters, higher positive effects on periodontal bone repair ($p<0.001$) detected through DSR and greater bone filling detected through CBCT ($p<0.001$) for the ALN group. Moreover, the sensitivity of DSR in relation to CBCT increases with higher bone filling gain, i.e., small filling gains (≤0.5-to-<1.0 mm) are not detected by DSR.

Conclusions: Topical application of 1%-ALN gel in intrabony defects showed better clinical results and significantly greater bone filling detected through CBCT compared to a placebo gel after 6 months.

Keywords: Alendronate; Periodontitis. Cone-beam computed tomography. Bone regeneration. Substraction technique.

Introduction

Periodontitis is considered as an inflammatory disease caused by bacteria in the dental biofilm leading to loss of teeth supporting tissues, namely the periodontal ligament, cementum, and alveolar bone. Since teeth support comprises 3 diverse tissues, the treatment of the disease is also equally challenging like its anatomy. Attempts to treat periodontal disease range from nonsurgical to surgical periodontal therapies, with or without systemic or local anti-microbial agents, as well as regenerative therapies with different types of biomaterials. Overall, periodontal therapy aims to arrest the disease process and prevent its recurrence, as well as regenerate the lost supporting tissues (Kao *et al*., 2015).

Over the years, several techniques aimed at regenerating periodontal attachment tissues have been proposed, including the use of bone grafts (Matarasso *et al*., 2015) , guided tissue regeneration (Chen *et al*., 2010; Kao *et al*., 2015) and biomolecules such as growth factors (Matarasso *et al*., 2015) and bisphosphonates (BPs) (Reddy *et al*.,

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2005; Dutra *et al*., 2017). Of particular interest in this study, although promising results have emerged on the use of local BPs in infra-bone defects, these findings need to be confirmed for future studies (Donos *et al*., 2019).

BPs are drugs that act to decrease the amount and activity of osteoclasts, thus reducing bone loss (Reddy *et al*., 2005; Dutra *et al*., 2017). Sodium alendronate (ALN) is a powerful inhibitor of bone resorption and it has been advocated that ALN is a favorable carrier of biomolecules for periodontal bone repair (Ishizaki *et al*., 2009; Killeen *et al*., 2012; Toker *et al*., 2012; Storrer *et al*., 2016). Due to its ability to inhibit the differentiation of osteoclast cell precursors and the performance of fully differentiated osteoclasts, studies have been carried out to evaluate the effectiveness of ALN in therapies for bone and periodontal disorders (Killeen *et al*., 2012; Toker *et al*., 2012; Storrer *et al*., 2016; Sheokand *et al*., 2019).

Human studies have investigated the effectiveness of topical application of 1%-ALN in non-surgical (Sharma and Pradeep 2012; Pradeep e al., 2015; Dutra *et al*., 2017; Sharma *et al*., 2017; Sheokand *et al*., 2019) and surgical (Reddy *et al*., 2005; Veena *et al*., 2010) periodontal procedures. In general, authors reported additional benefits in probing depth (PD) and clinical attachment level (CAL) improvements with the adjunctive topical use of ALN in comparison to scaling and root planing alone. One of the major limitations of most of these studies is the evaluation of bone filling in periodontal defects using conventional or digital radiographies (Reddy *et al*., 2005; Veena *et al*., 2010; Pradeep *et al*., 2015; Dutra *et al*., 2017; Sharma *et al*., 2017; Sheokand *et al*., 2019). Furthermore, no study with surgical procedures and ALN reporting results with the use of CBCT has yet been released.

Conventional or digital radiographies are techniques most commonly used in the assessment of the periodontium. Unfortunately, these techniques provide two-dimensional (2D) images lacking any information about the third dimension which hampers a true differentiation between buccal and lingual cortical plates and obfuscates the evaluation of the periodontal defects (Mol 2004; Brägger 2005; Anter *et al*., 2016).

Due to this drawback, there was a need for a more accurate imaging technique to be used in the assessment of periodontal conditions, with special regards to the imaging of the three-dimensional (3D) structures such as infra-bony defects, buccal and lingual cortical plates and furcation involvement (Zimmermann *et al*., 2004; Kumar *et al*. 2017). The use of cone-beam computed tomography (CBCT) in diagnosing periodontal problems yielded good results, but unfortunately presented other important factors such as cost, accessibility, and radiation dose, thus preventing its routine use in dental clinics (Anter *et al*., 2016). Additionally, Anter *et al*. (2016) stated that CBCT primarily remains as a research tool for clinical trials as it effectively maximizes the validity of scientific evidence.

In this scenario, the aim of this randomized controlled clinical trial was to assess, by means of CBCT, the effect of surgical topical use of 1% ALN on the reduction of periodontal intrabony defects after the interval of 6 months. Additionally, to verify the diagnostic validity of digital subtraction radiography (DSR) in relation to CBCT at the gain in bone filling.

Materials and Methods

The Institutional Ethics Committee approved the present randomized split-mouth triple-blind placebo-controlled clinical trial study (protocol #3701316.2.0000.5149). Participants provided an informed written consent and all study procedures were conducted according to the Helsinki Declaration of 1975, as revised in 2000. The study was registered at Clinicaltrials.gov (NTC0247611). Clinical and radiographic findings from this clinical trial were previously reported by Carvalho Dutra *et al*. (2019).

Study population

The study population comprised patients registered in the periodontal screening program of the School of Dentistry from the Federal University of Minas Gerais, from March 2018 to December 2018.

Participants of both genders, aged between 35 and 60 years old, good general health, and diagnosed with periodontitis (stage II or III) (Tonetti *et al*., 2018) were recruited by convenience. Specifically, patients had to present at least two contralateral teeth with proximal sites showing PD ≥5mm, CAL >3mm, vertical bone defects, and contact with the adjacent teeth (sites that previously did not respond to non-surgical periodontal therapy). Moreover, they also should have the same number of roots and be located at the same dental arch with <2mm difference in their PD baseline values (Carvalho Dutra *et al*., 2019). Furcation lesions, premature occlusal contact, or prostheses were excluded.

Reduction of intrabony defect in mm3 after 6 months, as the primary study outcome, was used for sample size calculation. It was based on intrabony filling gain from previous studies using CBTC (Zimmermann *et al*., 2004; Kumar *et al*., 2017) and considered a 0.05 significance level, 0.80 study power of 80% and a 0.50 size effect. Based on and a 20% minimum difference between study groups (mean values in intrabony defect changes), 25 sample units per group were determined to be necessary as a minimum sample size. A sample loss of approximately 20% was considered and 32 sites per group were initially defined as appropriate. Hence, after applying the inclusion and exclusion criteria, 32 individuals were enrolled in the study (split-mouth design). The final sample comprised 64 sample units: 32 contralateral periodontal sites allocated to either gel A or gel B.

All clinical trial procedures and analysis were performed as gel A and B. They were provided in identical syringes identified as gel A or gel B, without any further information except the expiration date so participants and researchers were kept blinded. Treatments were only identified a posteriori: gel A = 1% sodium aleandronate (ALN) and gel B = placebo. The 1% AL gel (approved by Health Surveillance Agency - ANVISA, Brazil #1677300740028) was prepared according to Reddy *et al.* (2005) and the placebo gel had the same chemical composition without the ALN. In order to keep patients and researchers blinded, the ALN and placebo gels were dispensed in identical syringes labeled as gel A or gel B.

Periodontal clinical examinations were performed at 3 times: T0 (baseline), T1 (after 90 days), and T2 (after 180 days). Radiographic examinations were performed at T0 and T2 by means of standardized periapical digital radiographies and analyzed using the DSR technique (Mol 2004; Silva *et al.*, 2010; Nibali *et al.*, 2011). Thus, each periodontal intrabony defects was classified as having gain of mineralized tissue when the resulting image was light gray (highest mineral density = positive effects), loss of mineralized tissue when dark gray (negative effects) and without changes (no effects) (Silva *et al.*, 2010; Carvalho Dutra *et al.*, 2019).

Bone filling was assessed using tomographic images acquired by cone-beam computed tomography (CBCT) (Anter *et al.*, 2016), using 3D Kodak 9000 (Eastman Kodak Company, Rochester, NY, USA) performed at T0 and T2. The study flowchart and exclusion criteria is shown in Figure 1.

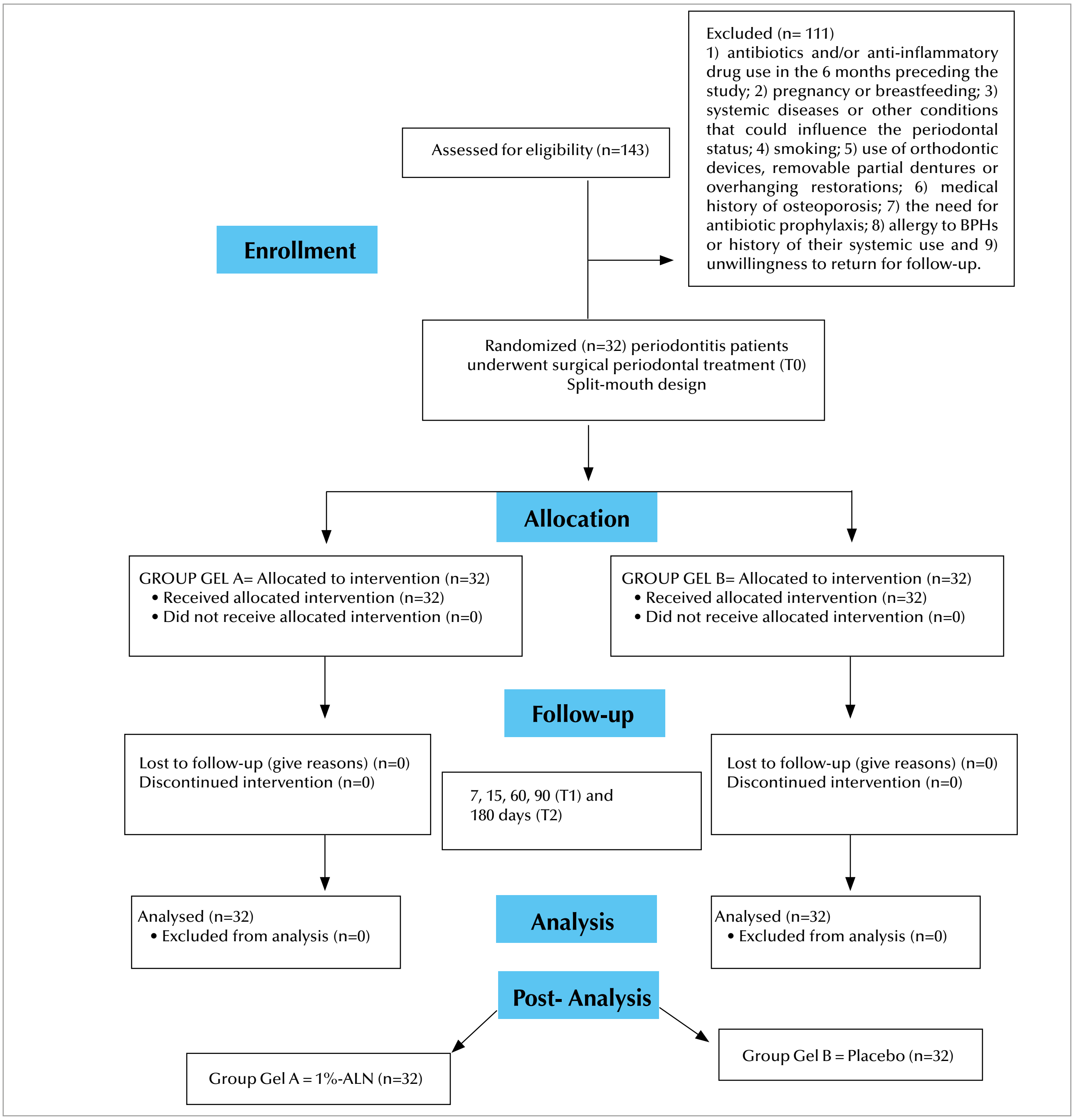

Figure 1. Flow Diagram (Consort).

The images obtained by digital radiographs and CBCT were taken following good dental practices and the ALARA (As Low As Reasonably Achievable) principles (Hayashi *et al.*, 2018).

Periodontal parameters and digital subtraction radiographies

The following clinical parameters were assessed during the screening phase to determine periodontal diagnosis, as well as at baseline and 3 and 6 months after periodontal treatment: PD, CAL, and bleeding on probing (BOP). The methodology for radiographic evaluation, clinical and DSR results were previously reported by Carvalho Dutra *et al.* (2019).

Surgical periodontal therapy

The description of periodontal surgical proced1ures was also reported in detail by Carvalho Dutra *et al.* (2019). Briefly, periodontal surgical procedures were performed through a full thickness open flap with intracrevicular buccal and lingual incisions to allow a complete visualization of the bone defect and being conducted by one single operator. Periodontal sites were randomly assigned by lottery to receive either gel A or B blind to the operator. No adverse effects were observed at any of the recall visits, independent of the gel used.

Cone-beam computed tomography (CBCT)

All tomographic images (T0 and T2) were analyzed by two independent, trained, and calibrated specialists that were blinded to study groups. Intra and inter-examiner agreement for the bone fill measurements retrieve weighted kappa values and intra-class correlation coefficients ≥0.95. The tomographic images were measured directly using the Kodak Dental Imaging Software KDIS (Kodak Dental Systems, Rochester, NY, USA) on a computer with a GeForce 9500 GT graphics card, an LED monitor with 1920x1080 pixel resolution, and default brightness and contrast levels.

The electronic measuring tool included in the CBCT software allowed measurements to the nearest hundredth of a millimetre. The cross-sectional slice thickness was reconstructed to 1 mm for all measurements, and the location of each slice was identified using the location of the gutta-percha. All measurements were performed using only one computer, with unaltered screen settings. The examiners were allowed to modify bone density and image size to enable optimal viewing. Three measurements were taken for each defect based on Misch *et al.* (2006): (a) the length from the CEJ (cement-enamel junction) to the base of the bony defect; (b) the length from the CEJ to the crest of bone adjacent to the defect; and (c) the width of the defect; thus obtaining a measurement of the bone defect in mm^3.

Statistical analysis

A descriptive characterization of the sample was first performed. Periodontal clinical parameters were compared at the 3 examination times (sites were the unity of analysis). ANOVA and Bonferroni post-hoc tests were used equal variances were assumed. Welch and Tamhane post-hoc test were used when equal variances were not assumed. The Generalized Estimating Equations (GEE) method was used for inter- and intra-group comparisons in relation to examination times and CAL and PD parameters. Marginal logistic models were obtained and directly incorporated the correlation between the measurements of the same sample unit. A marginal regression was adjusted for the PD and CAL with the variables examination time and type of treatment, as well as their interactions. Assumptions for model errors were verified by the Poisson distribution for the dependent variables CAL and PD and by the normal distribution for the subtraction measurements. The effects of each treatment on the subtraction measurements and the inter-group differences were evaluated through the Fischer, Friedman or Kruskal-Wallis tests, when appropriate. The results of bone filling obtained by CT were compared in two stages using the Wilcoxin test. Assumptions for all analyzes were verified: normality of the residuals, equal variances and identification of possible outliers. All analyses were performed through statistical software R (Software R originally created by Ross Ihaka and Robert Gentleman, University of Auckland, New Zealand (version 3.6.1).

Results

The sample comprised 32 individuals, mean age of 45.6 years old, being 19 women and 13 men. Of the 64 treated sites, 81.2% were posterior teeth, 34 in the maxilla and 30 in the mandible.

Clinical periodontal parameters and DSR overtime and between treatment groups were previously reported by Carvalho Dutra *et al.* (2019). Summarizing these previous findings, both placebo and ALN groups showed significant improvements in PD, CAL, and BOP after surgical procedures at T1 and T2. However, intergroup analysis (ALN versus placebo) showed a significantly better result for the ALN group, with higher CAL gain and PD reductions. Through DSR, ALN group demonstrated higher T2-positive effects (white regions = higher mineral density) and lower scores of no effects when compared to the placebo group ($p<0.001$).

In this study, groups were similar in intrabony defect measurements obtained by CBCT at T0. CBCT revealed that both groups significantly reduced the intrabony defect from T0 to T2 [ALN 1.92 ± 0.77mm ($p<0.001$) and placebo 1.21 ± 0.49mm ($p=0.045$)]. However, in the intergroup comparison, the ALN group showed a significantly greater reduction in the intrabony defect through CBCT compared to the placebo group ($p<0.001$; Table 1).

Additionally, the reduction in the bone defect showed a strong positive correlation with the reductions in PD and CAL in both groups (Table 2).

Table 3 shows the stratification of bone filling gains at three cutoff points: ≤ 0.5mm; > 0.5 < 1.0mm and > 1.0mm. The ALN group showed significantly greater bone filling than the placebo group at the cutoff points > 0.5 < 1.0mm (p=0.037) and > 1.0mm (p=0.028). In contrast, the placebo group showed greater gains in the < 0.5mm range than the ALN group (p<0.001).

The comparison between the two imaging techniques (DSR versus CBCT) at T2, demonstrated that when CBCT revealed gains ≤ 0.5mm, the DSR pointed 100% of negative effects in gains of > 0.5 < 1.0 mm; DSR pointed positive effects (> mineral density) in 68% and absence of effects in 32%; for gains >1.0mm, DSR showed 100% positive effects. Thus, the sensitivity of DSR in relation to CBCT increases with the increase in bone filling gain, i.e., small filling gains are not detected by DSR (Table 4).

Table 1. Comparative analysis between ALN and placebo groups in relation to CBCT measurements (reduction of intrabony defect - mm³).

Examination times	ALN group		Placebo group		p (intergroup comparison)
	Mean	s.d.	Mean	s.d.	
T0 (baseline)	5.95	2.47	5.91	2.18	0.818
T2 (180 days)	4.03	1.72	4.70	2.55	0.031
(intragroup comparison)	<0.001		0.045		
Reduction of intrabony defect (mm³)	1.92	0.77	1.21	0.49	<0.001

ALN= 1% sodium alendronate ; CBCT (cone-beam computed tomography); s.d. (standard deviation); Wilcoxin test.

Table 2. Correlation between CAL and PD measures with the reduction of intrabony defect at T2 (180 days) (CBCT measurements- mm³).

Variables	Intrabony defect ALN group		Intrabony defect Placebo group	
	r*	p	r*	p
CAL	0.275	0.001	0.269	0.021
PD	0.281	0.001	0.231	0.029

*Spearman Correlation. CAL= clinical attachment level; PD = probing depth; ALN = 1% sodium alendronate; CBCT (cone-beam computed tomography).

Table 3. Comparison between the 1%-ALN and placebo groups in relation to intrabony filling stratified by T2 (180 days) cutoff points (CBCT).

CBCT [intrabony filling (mm³)]	ALN group (n=32 sites)		Placebo group (n=32 sites)		p
	n	(%)	n	(%)	
≤ 0.5	5	21.8	21	65.6	<0.001
> 0.5 < 1.0	17	53.1	8	25.0	0.037
> 1.0	10	25.1	3	9.4	0.028

ALN= 1% sodium alendronate; CBCT (cone-beam computed tomography).

Table 4. Comparative analysis of DSR measurements in relation to intrabony filling evaluated through CBCT (64 sites).

CBCT [intrabony filling (mm³)]	Digital Subtraction Radiograph (DSR)		
	Positive effecct (n=30)	Non effect (n=26)	Negative effect (n=8)
≤ 0.5 (n=26)	0	0	26 (S = 100%)
> 0.5 < 1.0 (n=25)	17 (S = 68%)	8 (S = 32%)	0
> 1.0 (n=13)	13 (S = 100%)	0	0

S=sensitivity; C.

Discussion

In the present study, topical application of 1% ALN gel in periodontal intrabony defects showed, after 6 months, better clinical results and significantly greater bone filling detected through CBCT compared to a placebo gel.

Thus, corroborating the findings of the few studies with the use of ALN in surgical procedures (Reddy *et al*., 2005; Veena *et al*., 2010). Additionally, this is the first study to present bone filling results through CBCT and still comparing it with DSR.

Some studies (Reddy *et al*., 2005; Sharma and Pradeep 2012; Pradeep *et al*., 2015; Sharma *et al*., 2017; Sheokand *et al*., 2019) have also demonstrated that ALN gel induced a greater filling of bone defects. Interestingly, all previous studies have used periapical radiographies, which have lower sensitivity than CBCT (Misch *et al*., 2006). It is also relevant to mention that the improvements identified in the present study were associated with surgical procedures.

Findings from the present study demonstrated that, after 6 months of therapeutic procedures, the topical application of ALN provided additional benefits similar to those by Sheokand *et al*. (2019) and Sharma and Pradeep (2012) with similar follow-up time and clinical evaluation, except from the CBCT analysis. Therefore, the use of CBCT increased the sensitivity and specificity of the images in the present study (Brägger 2005; Misch, 2006; Anter *et al*., 2016).

CBCT has the great advantage of provides variable fields of view. Therefore, an optimum field of view can be selected for each individual based on the task for which CBCT is used and the region of interest. Moreover, it provides a submillimeter isotropic voxel resolution (Vandenberghe *et al*., 2007; Scarfe and Farman, 2008; Noujein *et al*., 2009).

Previous studies demonstrated that CBCT images: provided more accurate information on periodontal bone levels in three dimensions when compared to photostimulated phosphor plates images; provided better morphological description of periodontal bone defects, while the images obtained by charged coupled device sensor provided more bone details; CBCT images were better in detection of periodontal defects when compared to periapical radiographies and medical computed tomography (Mol and Balasundaram 2008); CBCT technique has better diagnostic accuracy than periapical films in the detection of interradicular periodontal bone defects (Noujein *et al*., 2009); CBCT provided useful information regarding linear and volumetric measurement of periodontal defects in vitro. It was emphasized that the size and volume of periodontal bone defects are directly correlated to the prognosis (Tayman *et al*., 2019).

However, contrary to the studies previously reported, it was stated that CBCT and conventional periapical radiographies differed on measuring the height of the alveolar bone crest but there was not a significant difference between the two methods in detecting the depth of bone defects (Anter *et al*., 2016).

Acar and Kamburoglu (2014) have discussed CBCT merits and limitations, as well as its role in diagnosing periodontal conditions such as furcation involvement, periodontal ligament space, alveolar bone defects, soft-tissue assessment and outcomes of regenerative periodontal therapy and bone grafts. Finally, they concluded that "CBCT has obvious benefits in periodontology, but its use should be kept only when necessary to avoid radiation hazards" or in research on bone regeneration.

In the present study, significant positive effects on bone repair was registered by DSR only in the ALN group. Possibly, this positive effects can be associated with the decreased alveolar bone resorption due to osteoclastic inhibition, the increased bone matrix deposition at the sites treated with ALN, or the lower ability to detect bone gain from this technique compared to CBCT.

DSR in Periodontology, basically allows the detection of small changes in alveolar bone, which might otherwise go undetected. Studies can focus on specific sites, such as furcations or intrabony defects and can show favourable outcomes of therapy in terms of bone behavior (Corbet *et al*., 2009). Mol (2004) discusses how it is often proposed that the time and effort involved in producing subtraction images of high quality to detect small changes is prohibitive in clinical practice but a good option in clinical research in the impossibility of performing the CBTC.

Another interesting finding of our study was that the sensitivity of DSR in relation to CBCT increases with the increase in bone filling gain, i.e., small filling gains are not detected by DSR. In this scenario, DSR could be the first choice of image for studies of bone repair assessing mineral density in periodontal defects that exceed a repair expectation> than 1.0 mm due to technical ease and lower cost and radiation.

DSR is highly precise and accurate for assessing bony changes associated with periodontitis (Reddy 2005). Thus, advanced digital-imaging systems may be more cost-effective in a clinical trial because the level of precision will have an effect on study length and sample size. However, CBCT remains primarily a research tool for clinical trials and effecting and maximizing the validity of scientific evidence (Cosso *et al*., 2014; Kumar *et al*., 2017).

In accordance with our findings, previous studies showed no adverse effects with low-dose topical ALN administration (Veena *et al*., 2010; Killen *et al*., 2012;

Pradeep *et al.*, 2015; Dutra *et al.*, 2017; Sharma *et al.*, 2017). Nonetheless, the external validity of the results should be considered with caution. Future studies are necessary to evaluate the potential cumulative effects of topical ALN use since the use of systemic bisphosphonates and the incidence of osteonecrosis associated with surgical procedures in the oral cavity is debated.

In accordance with Cosso *et al.* (2014) the present data suggest that using CBCT before periodontal regenerative surgery could result in accurate measurement of height and volume of alveolar bone defects. In addition, we suggest for the purpose of evaluating the effectiveness of regenerative surgery that CBCT should be performed 6 months after surgery.

Thus, CBCT applications provide evident benefits in periodontal research. It should be used when two-dimensional radiographies are unsatisfactory or ther is a need for greater accuracy in detecting changes in bone behavior in clinical research, considering the latent radiation threats of the examination.

However, it is important to emphasize the limited evidence supports the use in clinical practice of CBCT for the detection and characterization of furcation and intrabony defects. Further research is needed to determine the utility of CBCT imaging in supporting minimally invasive therapies, in assessing periodontal regenerative outcomes, and in determining the necessity of combination therapy (orthodontics, guided periodontal tissue regeneration, soft tissue grafting) in complex cases. In addition, the development of new, cost-effective approaches to CBCT imaging is also indicated. As noted above, since the long-term radiation hazards of effective dose accumulation are unknown, adherence to judicious principles of radiation exposure is imperative to minimize patient risk (Mandelaris *et al.*, 2017).

In this research, it was shown that the topical application of 1% ALN demonstrated higher PD reductions and CAL gains. It also confirmed the efficiency of ALN in inducing positive 3D volumetric changes in the filling of periodontal intrabony defects through CBCT, proving to be a greater sensitivity assessment method. Additionally, the results obtained by the DSR are very interesting for its indication of use in bone repair studies that aim to assess mineralization in periodontal intrabony defects, considering the limitations of the use of CBCT in Periodontology.

Thus, these results present two important signaling points: effectiveness of ALN in periodontal repair and alternatives for the use of DSR or CBCT in periodontal research.

Conclusion

Topical application of 1% ALN gel in intrabony defects showed better clinical results and significantly greater bone filling detected through CBCT compared to a placebo gel, after 6 months. Additionally, CBCT should be used as a relevant tool to measure periodontal bone volumetric alterations in clinical research and DSR can be considered very interesting for its indication in bone repair studies aiming to assess mineral density in periodontal intrabony defects.

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