# Comparison of two methods for alveolar bone loss measurement in an experimental periodontal disease model in rats

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**Abstract:** There are many studies that evaluate possible risk factors for periodontal diseases in animals. Most of them have focused only on the biological aspects of disease occurrence; therefore, it has been difficult to compare studies of the different methodological approaches. The aim of the present study was to compare different methods – linear and area – of the evaluation of morphometrical alveolar bone loss. Sixty hemimaxillae, defleshed and stained with 1% methylene blue to delineate the cementoenamel junction and alveolar bone crest, were obtained from a previous study that induced periodontal disease by means of ligatures in two groups of fifteen Wistar rats during 9 weeks. Ligatures were placed around the right upper second molars, and the contra-lateral teeth remained as intra-group controls. Digital photographs were taken from the specimens and submitted to a single, calibrated, blind examiner who performed the morphometrical evaluation of alveolar bone loss using both linear and area methods. Mean values of linear and area measurements were obtained from each side – buccal and palatal – of the specimens. The degree of association between the two methods was determined by Pearson's Correlation Coefficient. An almost perfect association (0.98) was determined between the linear and area evaluations. A mathematical formula was subsequently created to estimate the total area of alveolar bone loss, from linear mean measurements. Both methods were suitable for detecting bone level alterations. The results of the present study allow for the transformation of data and better compilation of results from different studies.

**Descriptors:** Alveolar Bone Loss; Periodontitis; Models, Animal; Methods

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## Introduction

Periodontitis is defined as a chronic inflammatory disease affecting tooth supporting tissues, involving bacteria as the primary etiologic factor and culminating in the destruction of the dental attachment apparatus. Host defense, genetic and environmental factors are responsible for different periodontal conditions that can be observed in humans.[1] Sometimes, identification of these modifying factors in humans is limited by confounding variables or ethical considerations.[2] In rats, different models to induce periodontal disease have been proposed.[3] The similarity of the rats to humans makes them an attractive model for the study of the pathogenesis of periodontitis.[4]

Regarding methodological issues in the study of induced periodontal disease in rats, comparisons among investigations turn out to be almost impossible. This is because different methods for periodontal disease induction and evaluation of alveolar bone loss have been applied, and also because of the different durations of studies. Recent studies have induced periodontal disease in rats by means of ligatures,[4-8] LPS injection[9] and infection with periodontopathogenic bacteria.[10,11] Considering the experimental period, Kuhr *et al.*[12] have concluded that bone loss after placement of ligatures occurs mainly in the first 15 days and recommends this model for short observation periods. Susin and Rosing[8] observed no differences between analyses performed after 29, 43 or 57 days. They have suggested that periods shorter than 29 days in induced periodontal disease observations should be considered. On the other hand, particularly regarding studies involving the influence of alcohol on bone loss, periods shorter than eight weeks are not recommended. This is because a previous study observed a positive effect of ethanol on bone metabolism only after this period of time.[13]

Different methods for measuring alveolar bone loss have also been proposed in the literature. Li and Amor[14] have observed no differences in the accuracy of Morphometric, Histometric and Micro-Computed Tomographic analyses for quantifying alveolar bone loss. Fernandes *et al.*[4] compared histometric and morphometric (linear) methods and concluded that both are capable of detecting differences in bone height in rats. Kuhr *et al.*[12] have proposed a comparison between linear and area methods for evaluating alveolar bone loss morphometrically. They have measured areas in teeth with ligatures and in the adjacent teeth. When long periods of time were used, the area and linear measurements did not correlate as well. The authors then concluded that the linear method should only be used when slight differences in bone loss exist between experimental groups. Recently, Fine *et al.*[15] have proposed a direct visual method for evaluating alveolar bone loss. Linear and area measurements were compared and no differences were detected. Considering that the literature still does not support a pattern of induced-periodontitis evaluation, the aim of the present study was to compare two methods for measurement of alveolar bone loss, using pictures taken of the defleshed maxillae of Wistar rats, and trying to propose an estimate of area measurements by means of the linear data.

# Material and Methods

## Specimen preparation

Sixty hemi-maxillae, defleshed and stained with 1% methylene blue, were obtained from a previous study that induced alveolar bone loss by means of ligatures in two groups of fifteen Wistar rats during 9 weeks. These hemi-maxillae were used in the present study. Ligatures were placed around the right second maxillary molars, and the contra-lateral side remained as intra-group controls. 4.0 cotton ligatures (Ethicon®, Johnson & Johnson®, São Paulo, Brazil) were used. Thus, the present study comprised 60 specimens (30 with and 30 without the presence of ligatures). Data in the present study was collected in order to determine the effect of alcohol on periodontal breakdown. To clarify, groups 1 and 2 represent exposure or not to alcohol intake, respectively, which is not part of the aim of this study.

A digital camera (Nikon D100®, Ayuthaia, Thailand) attached to a tripod with medical lenses, using minimal focal distance, was used to take standardized pictures from the buccal and palatal aspects of each specimen. A millimeter ruler was photographed together with all specimens to validate measurement conversions. The examiner was unaware of either the group distribution, or of ligature presence or absence. Alveolar bone loss was estimated using two different measurement methods (linear and area), and also with image analysis computer software (Image Tool 3.0, UTHSCSA, San Antonio, USA).

## Linear measurement of alveolar bone loss

Five linear measurements (in mm) were made from the cemento-enamel junction to the alveolar bone crest in each specimen (buccaly and palatally).

Periodontal bone loss was defined as the mean of ten measurements performed on the buccal and palatal aspects. Figure 1 illustrates the linear measurements.

## Area measurement of alveolar bone loss

The area of alveolar bone loss was measured from the cementoenamel junction to the alveolar bone crest, limited by the distal aspect of the distal root and the mesial aspect of the mesial root. Area was defined as the average measurements of buccal and palatal aspects. Figure 2 illustrates the area measurements.

## Reproducibility

Before performing alveolar bone loss measurements, the examiner was trained and calibrated. Double measurements of 20 specimens were performed within the period of one week. A very high correlation was obtained between the 2 measurements, and verified by intra-class correlation coefficient (ICC = 0.98).

## Statistical analysis

In groups 1 and 2, linear alveolar bone loss was calculated as the mean of ten measurements performed on the buccal and palatal aspects. Total area bone loss was measured on the buccal and palatal aspects, and a mean of the two observations was calculated. The degree of association between the two methods utilized for measuring alveolar bone loss was calculated by Pearson's Correlation Test. The alpha level was set at .05.

# Results

Descriptive results obtained from the morphometric measurements of mean alveolar bone loss in groups 1 and 2 (linear and area) are shown in Table 1.

Mean linear measurements of alveolar bone loss are expressed in mm. In teeth without ligature, alveolar bone loss was 0.37 ± 0.07 and 0.32 ± 0.07 for groups 1 and 2, respectively. In teeth with induced periodontal breakdown, groups 1 and 2 exhibited alveolar bone loss of 0.84 ± 0.18 and 0.78 ± 0.14, respectively.

Mean area measurements of alveolar bone loss are expressed in mm². In teeth without ligature, groups 1 and 2 presented area alveolar bone loss of 0.70 ± 0.15 and 0.61 ± 0.16, respectively. In teeth with ligature, alveolar bone loss was 1.50 ± 0.33 for group 1 and 1.37 ± 0.27 for group 2.

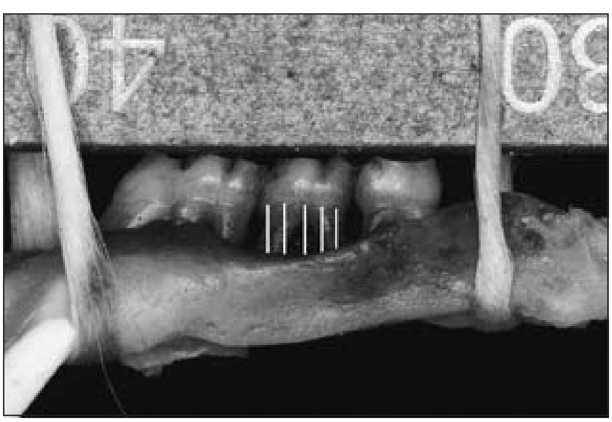

**Figure 1 -** Linear measurements on the buccal aspect.

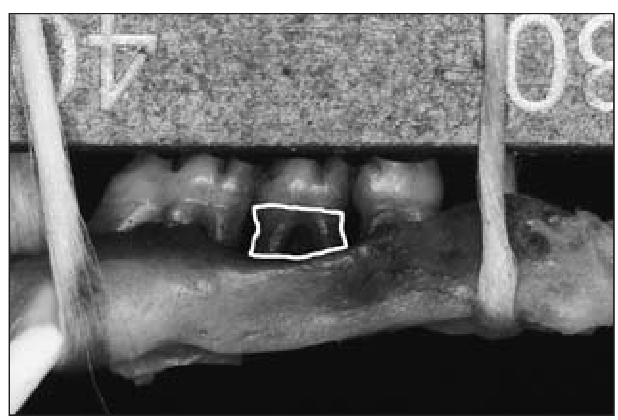

**Figure 2 -** Area measurements on the buccal aspect.

**Table 1 -** Results obtained from the linear and area measurements in a morphometric evaluation.

| | Linear Measurements (in mm) | | Area Measurements (in mm$^2$) | |
|---|---|---|---|---|
| | Without ligature | With ligature | Without ligature | With ligature |
| Group 1 | 0.37 ± 0.07 | 0.84 ± 0.18 | 0.70 ± 0.15 | 1.50 ± 0.33 |
| Group 2 | 0.32 ± 0.07 | 0.78 ± 0.14 | 0.61 ± 0.16 | 1.37 ± 0.27 |

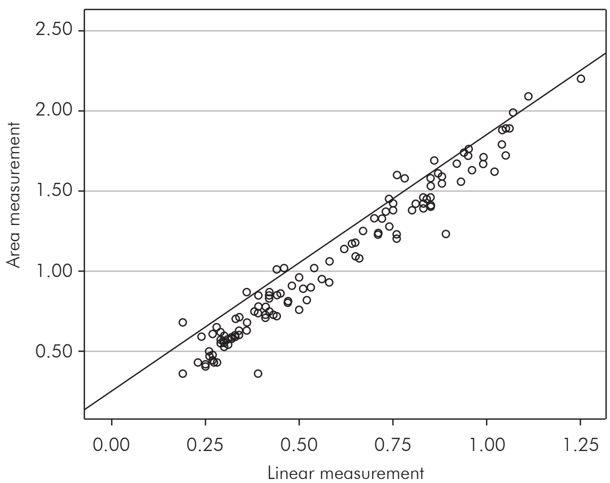


**Figure 3 -** Pearson's Correlation between linear and area measurements.

The Pearson's Correlation Coefficient between linear and area measurements is shown in Figure 3. A correlation of 0.98 ($p < 0.001$) was observed, which means an almost perfect correlation between the two methods of analyzing alveolar bone loss.

A mathematical formula for estimating the area of alveolar bone loss, taking only linear measurements, was created:

$$A = 0.25 + (L \times 1.6)$$

where A means estimation of area and L means linear measurement.

## Discussion

The present study evaluated two different methods for measuring alveolar bone loss morphometrically. Linear and area measurements were performed using standardized digital pictures, taken of defleshed maxillae (buccaly and palatally). Person's correlation showed evidence of a strong correlation between the two methods.

Recently, animal studies have been used extensively for evaluating the impact of risk factors that might, eventually, influence the onset of periodontal disease.[5,8,16,17] They mainly have been concerned with studying the biological plausibility of the events. Nevertheless, very few of them have focused on methodological aspects that can influence the results. Some authors have conducted studies concerning an accurate and easy method for evaluating alveolar bone loss.[4,12,14,15] Park *et al.*[11] have suggested using Micro-Computed Tomography for the assessment of alveolar bone loss because of the significant agreement between examiners, reliability and reproducibility (ICC > 0.99). On the other hand, Li and Amar[14] have compared techniques of Micro-Computed Tomographic with morphometric and histometric measurements for assessment of alveolar bone loss, and have reported accuracy in all three methods.

In the present study, we compared different morphometric methods for evaluating alveolar bone loss. The present findings disagree with those of a previous, similar study.[12] Kuhr *et al.*[12] perceived differences between linear and area methods when evaluating alveolar bone loss in second molars and adjacent teeth, during the longest period (60 days) of evaluation. The study concluded that the area method can assess bone loss better, when higher discrepancies are present between groups. In our investigation, both methods were reliable for detecting bone loss changes, even during a similar period of evaluation (63 days). Moreover, morphometric analysis seems to be an easy, accurate and reproducible method for this purpose as long as Micro-Computed Tomography remains expensive and histological analysis requires substantial tissue preparation steps and effort.[14] Comparing the results of the present study with those of the study performed by Fine *et al.*,[15] no important discrepancies could be identified.

Based on the findings of the present study, we can assume that morphometrical analysis provides an easier method when evaluating only alveolar bone loss. The accuracy and the reproducibility of this method make it less prone to errors in bone loss measurements. Despite the fact that linear measurements consist of an easier way to detect alterations in bone level, as well as being less time consuming, both area and linear methods are reliable for the evaluation of alveolar bone loss.

One highlight of the present study is the possibility of estimating area measurements from linear data, and vice versa. This would be of interest since discussions about which measurement should be considered the "gold standard" still exists. This highlight makes it evident that one can, in fact, convert measurements when interpreting different studies, thus allowing more realistic comparisons.

Further studies, for evaluating different methodological aspects in experimental models, are warranted for better understanding of the advantages and limitations of methods used for bone loss assessment.

## Conclusion

Within the limitations of the present study, we can assume that linear and area methods are suitable for detecting changes in alveolar bone loss even during longer periods of time.

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