Influence of soft tissues on mandibular gray scale levels

Influência dos tecidos moles sobre os níveis de cinza mandibulares

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ABSTRACT: The purpose of this study was to analyze the gray levels, expressed in pixels, of the mandibular retromolar region, with regard to the influence of muscular and fat soft tissues near this region. Fifteen dry mandibles were X-rayed with the presence of soft tissue simulators. The radiographs were digitized and evaluated by Digora® software. A one cm thick layer of wax was used as a simulator of the muscular soft tissue. Animal fat samples of different thicknesses – 0.5, 1.0, 1.5 and 2.0 cm – were used as a simulator of the fat soft tissue. Results showed that the fat soft tissue simulator influenced the gray level values in pixels of the mandibular retromolar region when analyzed in different thicknesses using the Digora® digitized image software.

DESCRIPTORS: Soft tissues; Digora; Bone density; Mandible; Radiography, dental.

RESUMO: O presente trabalho foi desenvolvido com o objetivo de analisar os níveis de cinza, expressos em "pixels", da região retromolar mandibular, considerando a influência dos tecidos moles muscular e, principalmente, adiposo adjacentes a essa região. Para o estudo, 15 mandíbulas secas foram tecnicamente radiografadas, sendo que as radiografias obtidas foram escaneadas e avaliadas pelo programa de imagens digitalizadas Digora®. Como simuladores dos tecidos muscular e adiposo, foram utilizadas cera utilidade na espessura de 1,0 cm e amostras de gordura animal em espessuras diferentes de 0,5; 1,0; 1,5 e 2,0 cm. Os resultados mostraram que o tecido adiposo foi capaz de influenciar a análise dos valores de níveis de cinza em "pixels" da região retromolar mandibular quando estudado em espessuras diferentes pelo programa de imagens digitalizadas Digora®.

DESCRITORES: Tecidos moles; Digora; Densidade óssea; Mandíbula; Radiografia dentária.

INTRODUCTION

The evaluation of mandibular bone density through *in vitro* digitized images renders a more comprehensive evaluation, which is not possible with radiographic film sensitizing, mainly due to the absence of muscular and fat soft tissues that can interfere in this sensitizing. These soft tissues compromise directly the gray level mensuration in digitized radiographs and consequently the evaluation of bone density expressed in pixel values.

Concern over the importance of bone density studies, regarding the presence of muscular and fat soft tissues, comes from researches such as the those conducted by Henderson *et al.*[10] (1995) and Baumgartner *et al.*[4] (1996), who investigated the possible correlation between an increase in fat tissue and a lower degree of osteoporosis in women during menopause or between the mechanical action of muscular force and higher bone density found in people who exercise regularly.

In a effort to simulate muscular and fat soft tissues in *in vitro* studies, authors such as Buhl *et al.*[6] (1991) and Laskey *et al.*[11] (1992) utilized tissues of animal origin to estimate the effect of their thickness and composition on bone density (BD), the total bone mineral quantity (BMD) and the bone mineral content (BMC) through dual energy X-ray absorptiometry (DEXA). On the other hand, a number of scientific studies[1,2,7,9,12,21,22] have utilized material of different compositions such as acrylic resin, plastic and water as soft tissue simulators.

Some studies[3,13,15,20] have also investigated mandibular bone density, however, through gray level analysis in pixels utilizing dry mandibles and considering the presence of soft tissue simulators in their studies.

Therefore, the main objective of this study was to evaluate the influence of different thicknesses of fat soft tissue simulators on the analysis of gray levels in the mandibular retromolar region, using

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Ultraspeed film and the Digora® digitized image program.

METHODOLOGY

This study was submitted to and approved by the Committee of Ethics and Scientific Research of the School of Dentistry, Pontifical Catholic University, Rio Grande do Sul.

In order to obtain the fat soft tissue simulators, four plastic containers were constructed, with different thicknesses of 0.5 cm, 1.0 cm, 1.5 cm and 2.0 cm. For the muscular tissue simulator, 1.0 cm thick wax was chosen, according to the study of Souza *et al.*[20] (1999). The fat tissue samples were of bovine origin and similar to the ones found in the subcutaneous tissue of the mandibular retromolar region. Afterwards, the samples were kept inside each container.

Finally, five simulators were obtained from the soft tissues of the mandibular retromolar region with the utilization of plastic containers, wax, and fat tissue samples of bovine origin, classified as follows:

• Fat tissue simulators (FT)
  - Container 1 – Simulator FT1 –
    Dimensions: 4.0 cm x 3.0 cm x 0.5 cm.
  - Container 2 – Simulator FT2 –
    Dimensions: 4.0 cm x 3.0 cm x 1.0 cm.
  - Container 3 – Simulator FT3 –
    Dimensions: 4.0 cm x 3.0 cm x 1.5 cm.
  - Container 4 – Simulator FT4 –
    Dimensions: 4.0 cm x 3.0 cm x 2.0 cm.
• Muscular tissue simulator (MT)
  - Utility wax (Epoxiglass, Epoxiglass
    Ind. Com. de Produtos Químicos Ltda.,
    Diadema, SP, Brazil) – Simulator MT –
    Dimensions: 4.0 cm x 3.0 cm x 1.0 cm.

A simulation criterion of muscular and fat soft tissues of the mandibular retromolar region was established to obtain radiographs, in such way that MT and FT1 simulators, duly positioned as described above, were in a closer position than they would be in a study with human beings. Mandible radiographs in this condition were considered as controls for a later comparison with the other radiographs taken in which only fat tissue simulators were substituted during the taking of radiographs. A previous test was also performed, following the same conditions of the study, with the objective of standardizing the time of radiographic exposure, considering the utilization of Kodak Ultraspeed film. As per the manufacturer's directions for the adjustment of exposure time and after the evaluation of the radiographs by a radiology specialist, the exposure time of 0.40 s for the utilized film was obtained. In order to achieve a standardization of radiographs, a positioner device was made with the objective of keeping radiographic films parallel and near the area to be radiographed.

Each radiographic film was placed in the positioner in such way that it would enclose the analyzed retromolar regions. Afterwards, simulators were positioned in juxtaposition with the vestibular cortical area of the lower molar region, including the retromolar area of interest. In this way, a set composed of radiographic film, mandible and simulators, parallel among themselves, was obtained (Figure 1). The central beam of X-rays was directed perpendicularly to the film and at a standardized distance of 25 cm.

Three radiographs were taken for each specific situation. All taken radiographs were made utilizing periapical film Ultraspeed Lot 3100720 (Kodak Ultraspeed, size 2, DF-58, Rochester, USA). For each radiography, an X-ray device (Spectro 70 X – Dabi Atlante, Ribeirão Preto, SP, Brazil) with a voltage of 70 kVp and a current of 8 mA was used and a 2.5 mmAl filter. The processing was executed in an automatic processor machine (automatic processor Air Technics Inc. AT 2000, Hicksville, New York, USA) for 4.5 minutes, at a temperature of about 28ºC. A total of 360 periapical radiographs were obtained, and they were handled with special gloves to avoid digital impressions that could harm further analysis of gray levels. Before the optic analysis of gray levels through the Digora® software (Digora for Windows 1.51, Soredex, Helsinki, Finland), all radiographic film

FIGURE 1 - Radiography taken with the set composed by radiographic film, mandible and simulators, parallel among each other.

was scanned, and the corresponding images were filed in a computer in tiff format (tagged image file format). For this purpose, an appropriate flat-bed scanner (Scanjet 6100 C/T, Hewlett Packard, San Diego, USA) was used, through which the groups of radiographic films that correspond to each retromolar region were positioned on the scanner bed. The respective projections of the identifying trade marks were turned toward the bed face and aligned in three columns of eight films each. This way, the HP Desk Scan II program allowed the image to be adjusted for size, mode and resolution according to the functions: type (sharp black and white photo), path (screen), scaling (475%) and size (242 KB).

A specific file was created for the gray level optic analysis in the retromolar regions by means of the Digora® program, so that the previously scanned images could be located on the hard drive, according to the soft tissue simulator type (MTFT 1, 2, 3, 4) and imported to the program. Each image was measured according to the gray levels expressed in pixels, through the "density measurement" function in delimited areas inside the retromolar image (Figure 2).

For this purpose, an informative table "cursor" was used in order to make an orientation of these areas possible through x (horizontal) and y (vertical) axis coordinates. Therefore, the areas were selected trying, at first, to avoid some anatomic structures such as the mandibular canal and the cortical strips in the oblique and mylohyoid orientation, so that the exact area could be identified, according to the coordinates found and visualized in the function "start", regarding the
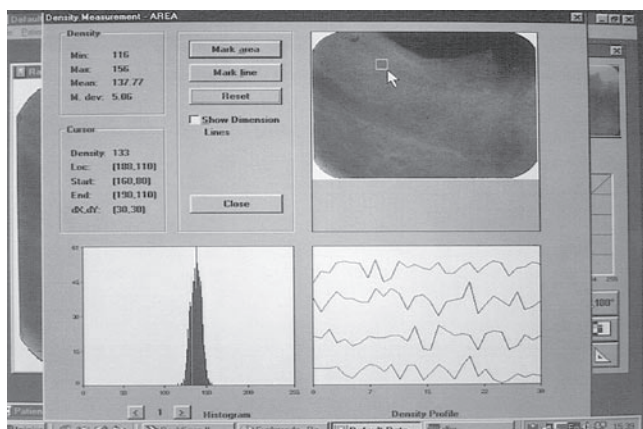

FIGURE 2 - The Digora® program screen, presenting the functions necessary for the mensuration of gray levels.

initial point of demarcation. On the other hand, the size of the area was of approximately 2 mm², which corresponds to 30 x 30 pixels, identified by the coordinates dX and dY, considering that each pixel measured 70 μm. For each analyzed area, the initial coordinates were taken note of and the average values of gray levels were described by the function "mean".

RESULTS

The results of the present study, according to gray level values in pixels, were obtained for thicknesses of soft tissue simulators evaluated by Anova and the Tukey's statistical tests for variance analysis.

Tables 1 to 4 show the values obtained from thicknesses of soft tissue simulators, in the Digora® program using Ultraspeed film.

DISCUSSION

The influence of muscular and fat tissues, described by the study of Brot *et al.*[5] (1997) on bone density alterations, without a doubt, is better explored with the utilization of specific equipment, such as dual energy X-ray absorptiometry (DEXA)[4,10,16,17]. It is a technology that is able to distinguish different degrees of photonic or radiographic attenuation, originated in tissues with different molecular structures, such as bones or fat and muscular soft tissues, adjacent to the studied

TABLE 1 - Comparison between gray level values, in pixels, obtained in each thickness of soft tissue simulators, analyzed in the Digora® program using Ultraspeed film. FO-PUCRS, 2001.

Simulator	n	Gray level (pixels)	p
MTFT1(control)	90	116.83	0.05*
MTFT2	90	125.16	0.000
MTFT3	90	130.58	0.000
MTFT4	90	136.62	0.000

∗Statistically significant level; n: sample size.

TABLE 2 - Comparison between gray level values, in pixels, obtained between thicknesses MTFT2 and MTFT3 of soft tissue simulators, analyzed in the Digora® program using Ultraspeed film. FO-PUCRS, 2001.

Simulator	n	Gray level (pixels)	p
MTFT2	90	125.16	0.020*
MTFT3	90	130.58	0.020

∗Statistically significant value for p = 5%; n: sample size.

TABLE 3 - Comparison between gray level values, in pixels, obtained between thicknesses MTFT2 and MTFT4 of soft tissue simulators, analyzed in the Digora® program using Ultraspeed film. FO-PUCRS, 2001.

Simulator	n	Gray level (pixels)	p
MTFT2	90	125.16	0.000*
MTFT4	90	136.62	0.000*

*Statistically significant for p = 0.5%; n: sample size.

TABLE 4 - Comparison between gray level values, in pixels, obtained between thicknesses MTFT3 and MTFT4 of soft tissue simulators, analyzed in the Digora® program using Ultraspeed film. FO-PUCRS, 2001.

Simulator	n	Gray level (pixels)	p
MTFT3	90	130.58	0.006*
MTFT4	90	136.62	0.006*

*Statistically significant for p = 0.5%; n: sample size.

region. As bone density, in general, is derived from this attenuation degree, its quantification becomes more precise when one considers the influence of these tissues.

The present study tried to confirm the need for reproducing, as closely as possible, *in vivo* studies[1,2,6,7,9,11,12,21,22]. In these studies the authors made use of materials and samples of animal origin, which would serve as a model to simulate bone, muscle, and fat, with a view to obtaining a better representation of the real situation.

Consequently, this research searched for a better representation of an *in vivo* study, utilizing utility wax according to the study of Souza *et al.*[20] (1999) and fat tissue samples as suggested by Buhl *et al.*[6] (1991) and Laskey *et al.*[11] (1992). Besides this aspect, the study also questioned the influence of different thicknesses related to fat tissue, which assumes distinct standards according to each individual or nutritional condition on mandibular bone density analyzed according to gray levels in digitized images. The results obtained in tables one, two, three and four confirmed that, in fact, small variations of about 5.0 mm in the thickness of fat tissue are able to generate different results, statistically corroborated, of mandibular bone density assessed by an optic analysis of gray levels.

This fact allows the inference that more obese individuals could present action synergism of the fat tissue in relation to its mandibular bone structure, when evaluated as in the present study, resulting from the metabolic action of the tissue itself, according to the studies of Reid *et al.*[16,17] (1992), Baumgartner *et al.*[4] (1996), Brot *et al.*[5] (1997), as well as a larger attenuation of the radiographic beam, which, on the other hand, would sensitize the film in smaller proportions, which would be shown, in the final analyses, in higher pixel values. This larger attenuation of the X-ray beam confirms that the fat tissue, even if it does not have a high atomic number in its molecular structure, as occurs in bone tissue, for example, is able to hinder the passage of the beam, affecting, therefore, the sensitizing of films as well as the optic reading of gray levels in pixels.

On the other hand, the optic analysis of gray level in pixels or in equivalent millimeters of aluminum (mmAl) of face bones, has been increasingly investigated. Based on this context, this research study establishes a continuity of the research[3,8,13-15,18,19] carried out mainly by Souza *et al.*[20] (1999), who in their study investigated the influence of the muscular tissue on the optic analysis of mandibular retromolar bone density in digitized images. This bone density evaluation method, in spite of not being very precise compared to the dual energy X-ray absorptiometry (DEXA), is presented as another important alternative of diagnosing alterations and pathologies which affect the bone tissue.

CONCLUSION

Based on the developed methodology and the obtained results in the present study, it was possible to conclude that the presence of muscular soft tissue and chiefly the fat soft tissue simulators adjacent to the studied region had an important role in the determination of gray levels for the mandibular retromolar region.

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