Detection of simulated osteoporosis in human anterior maxillary alveolar bone with digital subtraction

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The purpose of this in vitro study was to examine radiographic changes in human anterior maxillary alveolar bone during simulated osteoporosis (decalcification) and to determine the minimal amount of generalized decalcifications that can be detected under optimal radiographic conditions with the use of digital subtraction. Five samples of human anterior maxillary alveolus were progressively decalcified at timed intervals with 0.1 N hydrochloric acid solutions, and the percentage of calcium lost during each interval was quantified with calcium assays. Sets of four radiographs were exposed at 70 kVp initially and after each decalcification interval. The radiographs were digitized and digitally filtered, and bone profiles (scan lines) were generated between selected points on lead markers. To further reduce corrupting film-grain noise each set of four profiles were superimposed and averaged on a pixel-by-pixel basis. The averaged profile from each stage of decalcification was subtracted from the averaged initial profile on a pixel-by-pixel basis, and the mean profile intensity change for each decalcification stage calculated. Statistical analysis was performed with repeated measures analysis of variance. Results indicate that generalized decalcification less than or equal to 5.3% was detected in all samples of human anterior maxillae with the use of digital subtraction. **(ORAL SURG ORAL MED ORAL PATHOL 1994;78:655-61)**

Osteoporosis is an immense health care problem that affects more than 24 million Americans and is responsible for 1.3 million bone fractures each year.[1] Its effects are devastating in terms of health care costs, loss of quality of life, and increased mortality. The most common form of this disease is postmenopausal osteoporosis that results from an accelerated phase of bone loss in women with estrogen deficiency.

Beginning about age 30 to 35 skeletal mass declines in all populations of men and women.[2] Trabecular bone is lost earliest and at a more rapid rate than cortical bone.[2, 3] Estrogen deficiency superimposed on the usual age-related bone loss is associated with an overall accelerated loss up to three times normal, particularly of trabecular bone.[2] Over their lifetimes, women lose about 50% of their trabecular bone and 35% of their cortical bone, whereas men lose two thirds these amounts.[4]

Anatomic location of this bone loss varies with the type of bone. Cortical bone is located primarily in the shafts of long bones, whereas high concentrations of trabecular bone are found in the vertebrae, pelvis, ribs, and ends of long bones. Postmenopausal osteoporosis is classically characterized by vertebral fractures followed by those of the wrist.[2]

Although symptoms of osteoporosis are preceded by decades of silent bone loss, it is striking that there are no suitable tests for early detection and mass screening. The time of menopause is often when a woman is evaluated for her risk of the disease to determine the need for bone density tests.[2] Risk factors include the female sex, early menopause, Caucasian or Oriental race, positive family history, small build, chronic low calcium intake, lack of physical activity, nulliparity, alcohol abuse, cigarette smoking, high caffeine intake, and high sodium intake.[2] These risk factors may help to identify those patients who require the most careful screening. In addition, contributing factors related to specific drugs or illnesses are associated with an increased incidence of osteoporosis.[2] However, it is not possible to predict the occurrence of osteoporosis on the basis of risk factors alone. In addition, significant loss of bone can occur before menopause[2] and with estrogen deficiency, yearly loss of vertebral trabecular bone can approach 5% to 10%.[3] Each 10% decrease in spinal bone density is accompanied by a two- to threefold increase in fracture risk.[5, 6] Consequently, osteoporosis often is not detected until a fracture has occurred and only one half of existing vertebral fractures are diagnosed.[3, 7] This is particularly disheartening because prevention of osteoporosis may be much more successful than treatment of overt disease.[2]

Bone density measurements are useful in evaluating patients at risk for osteoporosis. Measurement at sites in the appendicular long bones are less expensive and performed more easily, but they are directed at sites composed of large amounts of cortical bone and do not correlate well with axial (for example, spinal) measurements. The most practical method of mea-

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suring the density of axial bones is dual x-ray absorptiometry that, unlike single photon absorptiometry, does not require a uniform layer of soft tissue.[5] Quantitative computed tomography allows for a three-dimensional density assessment and separate calculations of trabecular and cortical compartments without superimposition of other tissues.[7] However, the routine use of these bone density measurements in the perimenopausal population is controversial, no single measurement is adequate, and costs preclude mass use.

The literature suggests links between oral bone loss and systemic osteoporosis.[2, 8-16] Some investigators have studied dental radiographs in an attempt to distinguish between normal and osteoporotic patients.[17, 18] However, there is a lack of information defining the specific relationship between suggested oral age-related bone density changes and systemic osteoporosis. This is complicated by the lack of a commercially available instrument to measure bone density of specific areas of the jaws[19] and the need to evaluate current methods of oral bone imaging for sensitivity and clinical applicability.[8]

We are studying the feasibility of using dental radiographs of alveolar bone to monitor oral bone density changes as a possible screening tool for osteoporosis. Dental radiographs, routinely taken during periodic dental examinations, may provide a longitudinal, quantitative window into the status of systemic and oral bone density with minimal additional exposure or risk. Of particular interest is the maxillary anterior alveolar region, which contains a relatively high concentration of trabeculae with relatively thin cortices. These types of areas should show the earliest radiographic evidence of decalcification.[20, 21] Anterior maxillary alveolar bone is also readily accessible with minimal soft tissue superimposition (lips retracted) for routine standardized radiographs.

In a previous study[22] we used digital subtraction to determine the minimal amount of generalized decalcification (simulated osteoporosis) that could be detected in samples of dog alveolar bone. One-dimensional (1 x n pixel) profiles or scan lines were analyzed instead of bone areas because tooth roots are frequently separated by as little as 1 to 2 mm, and this narrow region includes not only the trabecular bone of interest but also periodontal ligament space and lamina dura. Such anatomy favors analysis of single or multiple parallel profiles as opposed to larger, fixed-width areas. The purpose of the present in vitro study was to examine changes in dental radiographs of samples of human maxillary anterior alveolar bone that were subjected to progressive simulated osteoporosis and to quantify the minimal amount of generalized decalcification that can be detected using digital subtraction.

MATERIAL AND METHODS

Specimen preparation and decalcification

One sample of alveolar bone was obtained from the anterior segment of five dry human maxillae. Each sample consisted of an interproximal segment of bone approximately 18 mm long and bounded by lamina dura from adjacent teeth. All samples were rinsed three times in distilled water with an ultrasonic cleaner and dried.

Samples were securely fastened on a plastic mount with a central window for the area of interest so that identical radiographs could be made by placing the mount directly on radiographic film. Small reference lead markers were placed on the mount at opposite ends of the length of the sample.

All samples were subjected to simulated osteoporosis by decalcifying them in 30 ml of 0.1 N hydrochloric acid solution. External (periosteal) surfaces of cortical bone and lamina dura were protected with a thin layer of wax to simulate the preferential trabecular and endosteal demineralization seen in osteoporosis.[23] Specimens were progressively partially decalcified in fresh solutions at cumulative timed intervals equal to 3, 5, 10, 15, 20, 25, 30, 40, 50, 70, and 90 minutes. After each decalcification period, solutions were collected and specimens were washed in distilled water and dried.

Radiographs

The bone samples, along with a six-step (0.5 to 7.5 mm) aluminum step-wedge, were radiographed initially and after each decalcification cycle under identical conditions with D-speed periapical film (Kodak DF-58, Ultraspeed, Eastman Kodak Co., Rochester, N.Y. expiration date 2/95). Four radiographs of each sample were exposed (70 kVp, 2 mA, 2 seconds) using a Faxitron x-ray unit (model #807-010, Hewlett Packard Company, McMinnville, Ore.).

Variation in x-ray geometry was minimized by rigidly fixing the bone sample to a flat plastic mount. The mounted bone sample and step-wedge were placed directly over the radiographic film, and all were centrally positioned on the floor of the Faxitron x-ray unit.

All radiographs for each sample were developed in one batch at the same time in an automatic processor (Dent-X Model 8-DE, Stamford, Conn.) with Picker developer and fixer solutions. The developer was maintained and monitored for strict quality control. This included daily test processing of films that were

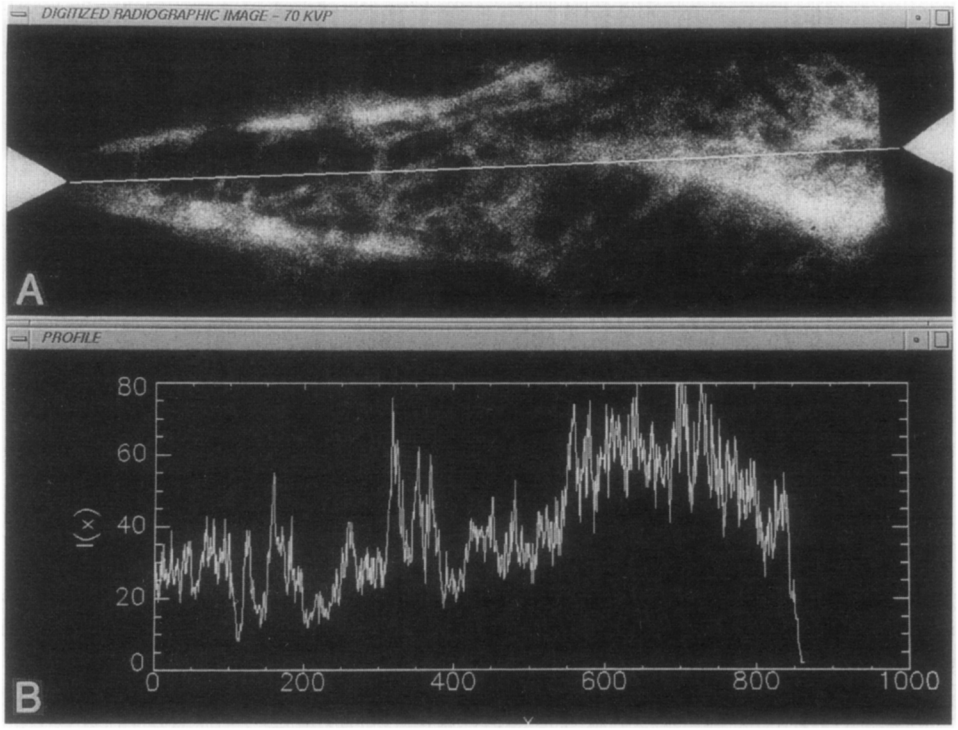

Fig. 1. Representative human maxillary alveolar radiographic image. **A,** Digitized image with the bony profile (scan line) selected between points on lead markers. **B,** Corresponding profile intensity value, I(x), plot.

preexposed at specified settings on a given x-ray machine. The test films were visually inspected for contamination and checked with daily logged densitometry readings for strict film density control. Processing chemicals were monitored daily, and the processor was maintained according to regularly scheduled cleanings.

Calcium determinations

After each decalcification interval, the hydrochloric acid solution was collected for determination of calcium concentration. After the last interval of partial decalcification, the bone samples were completely decalcified in a solution of 60 ml of 0.1 N hydrochloric acid, and these final solutions were collected. Complete decalcification was confirmed by trace (negligible) amounts of calcium in a final assay.

Calcium assays were performed at the University of Iowa Hospitals and Clinics Department of Pathology with a colorimetric (calcium-cresolphthalein complexone) determination. The percentage of decalcification at each interval was calculated on the basis of the total calcium released from each sample. For the purpose of data analysis, the percentage of decalcification was divided into five stages ($\leq$5.3%, $\leq$8.7%, $\leq$16.2%, $\leq$20.4%, $\leq$23.3%).

Radiograph digitization and digital subtraction

All radiographs were identically illuminated and digitized with an Imapro QCS 1260 flat bed scanner (Imapro Corp., Ogdensburg, N.Y.) with a pixel spatial resolution of 0.02 mm and an 8-bit grey scale depth. The mean pixel intensity or grey level of every step-wedge step was calculated in each radiographic image. A set of baseline step intensities for each bone sample was calculated by averaging the respective step grey levels of the four initial radiographic images. To correct for density differences that resulted from varying exposure and developing conditions, these baseline step intensities were used to correct pixel grey levels in all images by linear expansion, compression, and shifting.[24] It should be noted that the range of the baseline step intensities bounded the range of tissue intensities for each bone sample.

To reduce image film-grain noise, a digital smoothing filter (neighborhood averaging filter) with a window width of 0.14 mm was applied to all images. This filter can reduce film-grain noise in D-speed film images of maxillary alveolar bone by over one half.[25] Intensity profiles (scan lines) were generated between selected points on markers that had been attached to the plastic mounts (Fig. 1). These profiles were image arrays of dimension 1 x n pixels, where n is the profile

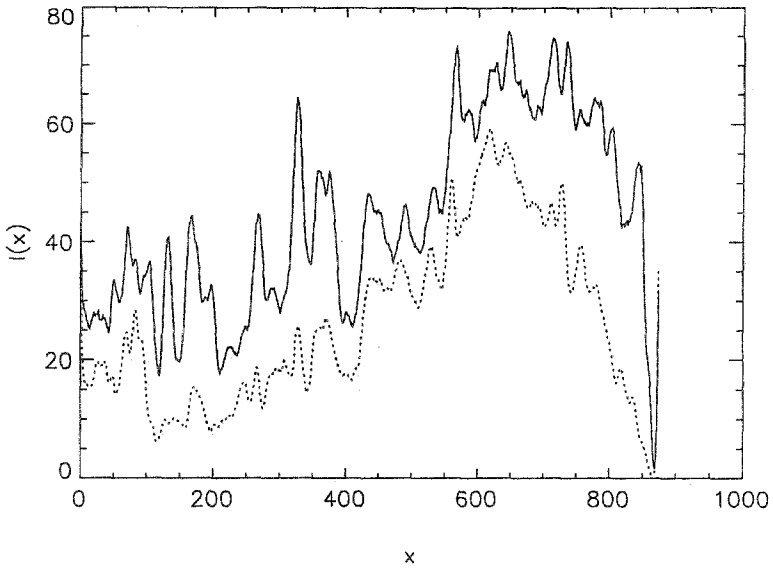

Fig. 2. I(x) plots of sample shown in Fig. 1 after digital filtering and image averaging. The solid line illustrates the initial bone profile intensity, and the dotted line illustrates the same profile after 34% generalized decalcification of the sample.

length. Filtering, profile selection, and all subsequent image processing was performed with the PV-wave version 3.1 command language (Precision Visuals Inc., Boulder, Colo.) on an SG Indigo Elan computer system (Silicon Graphics Computer Systems, Mountain View, Calif.).

To further suppress contaminating image film-grain noise, each set of four profiles was superimposed and averaged on a pixel-by-pixel basis. Averaging to reduce image film-grain noise is a standard technique in image analysis[26, 27] and has been previously reported in the dental literature.[28] The averaged profile from each subsequent stage of decalcification was subtracted from the baseline profile on a pixel-by-pixel basis (Fig. 2) and the mean intensity change for the profile calculated.

Statistical analysis

Statistical analysis, performed with the SAS statistical software package (SAS Institute, Inc., Cary, N.C.), consisted of a repeated measures analysis of variance. This statistical test is indicated when the measurements are taken on the same experimental unit[29] and was used because the mean intensity change measurements were repeated on the same bone sample over stages of decalcification. All comparisons were made to the baseline of zero intensity change. The null hypothesis is that there is no difference between the initial intensity profile and profile intensities after decalcification.

RESULTS

The relationship between the percentage of alveolar calcium loss and the corresponding mean change in profile intensity after digital subtraction is graphically illustrated in Fig. 3 for each of the five alveolar samples. As presented in Table I the mean intensity changes were all statistically significant from the baseline bony profile. In other words, radiographic digital subtraction clearly demonstrated alveolar bony changes with no greater than 5.3% alveolar calcium loss.

DISCUSSION

Traditional gross estimates of bone decalcification that must exist before detection is possible with conventional radiographs range from 30% to 60%.[3, 9, 30, 31] In contrast, the results of this study demonstrate that under the given in vitro conditions, generalized decalcification that simulates osteoporosis of no greater than 5.3% can be detected in samples of human anterior maxillary alveolar bone with the use of digital subtraction.

Several factors played a role in this difference. First, we intentionally chose optimal conditions for digital subtraction with fixed lead markers to ensure reproducible profile registration. A difficult although not insurmountable problem in applying this technique clinically is the need for accurate superimposition between initial and subsequent images. Ruttiman et al.[32] described a spatial registration technique with

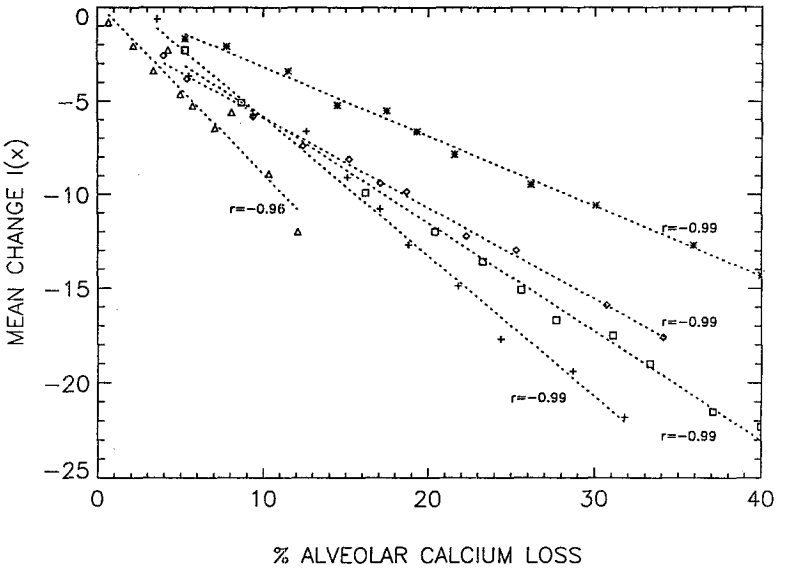

Fig. 3. Least-squares (regression) line and corresponding product-moment correlation coefficient (r) for each of the five alveolar samples demonstrating the relationship between percentage of calcium loss and mean profile intensity change calculated with digital subtraction.

Table I. Radiographic profile intensity changes during decalcification (n = 5)

Decalcification stage	*Cumulative mean percentage intensity change*	*Standard deviation*	*F value (Pr > F)*
≤5.3%	−2.36	1.47	12.86 (0.0230)
5.4-8.7%	−4.23	1.64	33.12 (0.0045)
8.8-16.2%	−6.11	2.36	33.35 (0.0045)
16.3-20.4%	−8.05	2.60	47.87 (0.0023)
20.5-23.3%	−9.68	3.19	45.88 (0.0025)

an estimated precision of ± 1 pixel. Presently we are investigating the use of other features to describe bone density change that may be less sensitive than digital subtraction to imprecise spatial registration. In addition to having reproducible profile registration provided by the lead markers, clean and dry bone samples were used. In life, bone consists of osseous tissue and soft tissues composed of blood vessels, connective tissue, and fat. The presence of any soft tissues including gingiva and lip or cheek superimposition will tend to obscure bony changes and confound detection of minimal bone loss. These effects will need to be studied and quantified. Finally, tolerance for other clinical errors such as variation in radiographic exposure and processing must be examined.

The clinical application of any radiographic dental imaging technique to study oral bone density change must be able to discriminate between bone loss as a result of metabolic disease and bone loss as a result of periodontal disease. We are currently investigating methods to perform this discrimination. Of course if dental imaging techniques are ever to be used to screen for systemic disease, extensive testing would be necessary to determine correlations (if any) between oral and systemic bone density loss.

The results of the present study compare favorably with our previous findings. Using samples of dog alveolar bone and two sets of radiographic exposures from two different x-ray sources, we detected minimal levels of generalized decalcification that ranged from less than 7.5% to less than 19%. We attribute the difference in our results with human maxillary anterior alveolar bone to improved technique and differences in the bony samples.

Although most studies that use radiometric techniques to detect or to quantify oral bone loss have dealt with localized lesions, a few investigators have made in vitro assessments of oral bone as it relates to generalized demineralization. Ruttiman et al.[32] examined changes in osseous fractal dimension from radiographs of mandibular bone after generalized decalcification. The change in this feature was exam-

ined before and after specimens were partially decalcified. In 25 of 30 cases the fractal dimension increased after decalcification. Brägger et al.[24] compared radiographic density changes, assessed with computer-assisted densitometric analysis, with actual calcium loss of five dry human mandibular cortical bone specimens. The cortical specimens were placed on strips of 0.1 to 0.4 mm thick copper foil and radiographed after partial decalcification. Correlation coefficients ranged from r = 0.723 to 0.975. Hildebolt et al.[16] studied mandibular alveolar bone in vitro with the use of dual energy radiography (dual x-ray absorptiometry) and bite-wing radiographs to determine correlations between loss of bone mineral content and region of interest gray scale histograms. They then applied the histogram technique to a small population of patients to study correlations with measured densities of forearm, femur, and spine.

As previously mentioned, we are particularly interested in studying the anterior region of maxillary alveolar bone as a possible site for osteoporosis screening and study of oral age-related bone density loss. In part, this is because of the highly trabecular nature of this region. Bones that are predominately trabecular may be preferred sites for assessing mineral density because of the metabolic responsiveness,[7] the higher incidence of fracture in trabecular bones,[5] and the earliest radiographic evidence of change.[20, 21]

Some authors have attempted to use dental radiographs to discriminate between normal and osteoporotic women. Kribbs[17] used a microdensitometric technique and intraoral mandibular radiographs and found significant differences in mandibular mass but with a large overlap between the two groups. Similarly, Mohajery and Brooks[18] made multiple densitometric point readings of mandibular premolar periapical radiographs of normal and osteoporotic women. They found that the difference in the densities approached statistical significance ($p = 0.0534$). Such results that demonstrate a lack of clear distinction between normal and osteoporotic groups is not surprising considering the modest correlations that exist for the different techniques of assessing bone density at different sites[33] and the need for multiple measurements to confirm disease.[5]

It is interesting to note that quantitative computed tomography, which allows separate calculations of vertebral trabecular density and better discrimination between osteoporotic and normal women in cross-sectional studies,[7, 33] has relatively poor correlations with other density measurements.[34] However, some investigators feel that when similar parameters are measured, no real discrepancy exists.[34] For example, when lumbar spine measurements with dual x-ray absorptiometry are made with a lateral scan as opposed to a posteroanterior scan, an almost selective measurement of trabecular bone can be made without superimposition of cortical elements, osteophytes, aortic calcifications, etc. With these lateral measurements, estimated cross-sectional age-related bone loss is higher, correlations with quantitative computed tomography is higher, and discrimination between normal and osteoporotic women improves.[7] We feel that this underscores the need to investigate the most trabecular regions of bone.

In summary, five samples of human anterior maxillary alveolus were progressively decalcified in a manner that simulates osteoporosis. Digital subtraction was used to demonstrate that decalcification no greater than 5.3% was detected with D-speed dental radiographs exposed at 70 kVp. This study illustrates that, given these in vitro conditions, it is technically feasible to detect generalized levels of decalcification lower than the widely accepted levels of 30% to 60% using conventional radiography of human alveolar bone.

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