

Effectiveness of ultrasonography in detecting intraosseous vascularization: an *in-vitro* study

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ABSTRACT | Ultrasonography is useful to diagnose lesions, insofar as it detects the type of injury, and to assess the degree of vascularization of tumors. However, intraosseous lesions may represent a challenge, since the surrounding bone thickness could prevent ultrasound signal capture. The aim of this study was to evaluate the influence of surrounding bone thickness on the ability of ultrasonography in capturing the echo signal of blood vessels. Macerated porcine hemimandibles (n = 20) with different buccal bone thicknesses were prepared and adapted to receive CFlex-type rubber tubes connected to a glass capillary through which pump-driven water was conducted to simulate blood vasculature. Doppler ultrasonography was used to assess the blood flow in the region of the mandibular canal at the level of the molar teeth. Student's t-test was used to assess differences between the bone thicknesses of hemimandibles with a negative and with a positive ultrasound signal. The presence of the echo signal in the simulated vasculature was assessed by ultrasonography. Reproducibility and reliability were confirmed for the analyses. The simulated flow signal was captured in cortical bones with a thickness in the 0.2–1.0 mm range (0.59 ± 0.42 mm), but was not captured in those with a thickness greater than 1.0 mm (1.39 ± 0.59 mm). In conclusion, ultrasonography can be used to investigate intraosseous vascularization in mandibular areas with a buccal bone thickness up to 1.0 mm.

DESCRIPTORS | Ultrasonography; Ultrasonography, Doppler; Diagnostic Imaging; Bone and Bones / blood supply.

RESUMO | **Eficácia da ultrassonografia na detecção de vascularização intraóssea: um estudo *in vitro*** • A ultrassonografia é um recurso de imagem para a finalidade de diagnosticar lesões e para avaliar o grau de vascularização intraóssea de tumores. No entanto, lesões intraósseas podem representar um desafio devido à espessura de osso circundante que poderá impedir a captura do sinal de ultrassom. O objetivo deste estudo foi avaliar a influência da espessura óssea na captura do sinal de eco dos vasos utilizando a ultrassonografia. Hemimandíbulas maceradas suínas (n = 20) com espessuras ósseas diferentes foram adaptadas para receber tubos de borracha tipo CFlex ligados a um capilar de vidro, por onde água foi conduzida por meio de uma bomba para simular a vascularização sanguínea. A ultrassonografia Doppler foi usada para avaliar o fluxo de sangue na região do canal mandibular ao nível dos dentes molares. O teste t de Student foi utilizado para avaliar as diferenças entre as espessuras de osso das hemimandíbulas por meio de sinal negativo e sinal positivo do ultrassom. A reprodutibilidade e a confiabilidade foram confirmadas para as análises. O sinal de fluxo simulado foi capturado em ossos corticais com espessura na faixa de 0,2 a 1,0 mm (0.59 ± 0.42 mm), mas não foi capturado a uma espessura superior a 1,0 mm (1.39 ± 0.59 mm). Concluindo, a ultrassonografia pode ser usada para investigar a vascularização intraóssea em áreas mandibulares com uma espessura óssea vestibular de até 1,0 mm.

DESCRITORES | Ultrassonografia; Ultrassonografia Doppler; Diagnóstico por Imagem; Osso e Ossos / irrigação sanguínea.

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INTRODUCTION

Ultrasonography is an imaging examination using sound waves with a frequency above the human audible threshold.¹ The image of anatomical structures is formed by real-time emission and capture of ultrasound wave echoes.² This method allows assessment of subcutaneous body structures, and has been used in the oral cavity to identify alterations of soft and hard tissues,³ blood flow and vascularization.^{4,5}

The capability of ultrasonography to detect blood vessels has validated it as a useful tool in the diagnosis of neoplasms. Accordingly, the differentiation of malignant lesions can be made on the basis of the vascularization pattern of the lesion.^{6,7} In addition, ultrasonography is a non-invasive method, which causes little discomfort to the patient, and is affordable and relatively easy to use.⁸

According to the literature, it is possible to employ ultrasonography to detect signals of simulated blood flow, using macerated porcine jaws and rubber vessels with flexibility and stiffness comparable to those of a real artery.^{9,10}

An ultrasonic signal is not detected in healthy mandibular bone tissues, since dense cortex acts as a shield that blocks sound waves.⁹ However, if the bone tissue has suffered thinning due to any type of injury, trauma resorption, or an intraosseous lesion, the ultrasound device can capture the signal from this tissue.⁵ This property enables the clinician to detect the intraosseous content and extent of a mandibular lesion, thus allowing for a precise surgical plan. However, little is known regarding the limit of bone thickness allowing ultrasound signal capture, and subsequent diagnosis of the degree of vascularization using ultrasonography.¹⁰ Thus, the purpose of this study was to evaluate capture of ultrasonography signals in porcine hemimandibles of different thicknesses to determine the thickness limit in which this imaging method can be used.

MATERIALS AND METHODS

The procedures of the study involving porcine jaws were submitted to and approved by the Research Ethics Committee (Subcommittee for Animal Bioethics) of the School of Dentistry, University of São Paulo (Protocol no. 147).

Macerated porcine mandibles (n = 10) were used, insofar as their bone structure is similar to that of humans in the region of molar teeth.¹⁰ Each mandible was sectioned in the region of incisor teeth and numbered, generating 20 hemimandibles, which composed the study material.

The hemimandibles were randomly eroded with a common electric plaster cutter in the region of third molars and assessed with a Terason t3000 ultrasound system (Terason Division, Teratech Corporation, New York, NY), set at Doppler mode, and using an adapted vascular simulator. The vascular simulator was installed in the anatomical region of the mandibular canal. It consisted of a glass capillary tube, connected on both ends to CFlex-type rubber tubes supported by nylon clamps, according to previously described methodology.¹¹⁻¹³ Thus, blood plasma was simulated using water, whereas erythrocytes were simulated using graphite powder. In all cases, blood flow simulation was run using the same regular aquarium pump connected to the capillary tube, in order to simulate blood flow under the same pressure conditions (170 mmHg; Figure 1).

The mandibles were sectioned twice, at the respective region of both mental foramina. Only the parts posterior to the mental foramina were considered. The bone thickness of all hemimandibles was measured at the buccal aspect of the mandibular canal, in millimeters, using a caliper, 5 mm distally to the mental foramen (Figure 2). The 0.5 mm corresponding to the glass capillary was subtracted from the total to obtain a value considered as the bone thickness measurement.

Digital images for the negative (Figure 3A) and

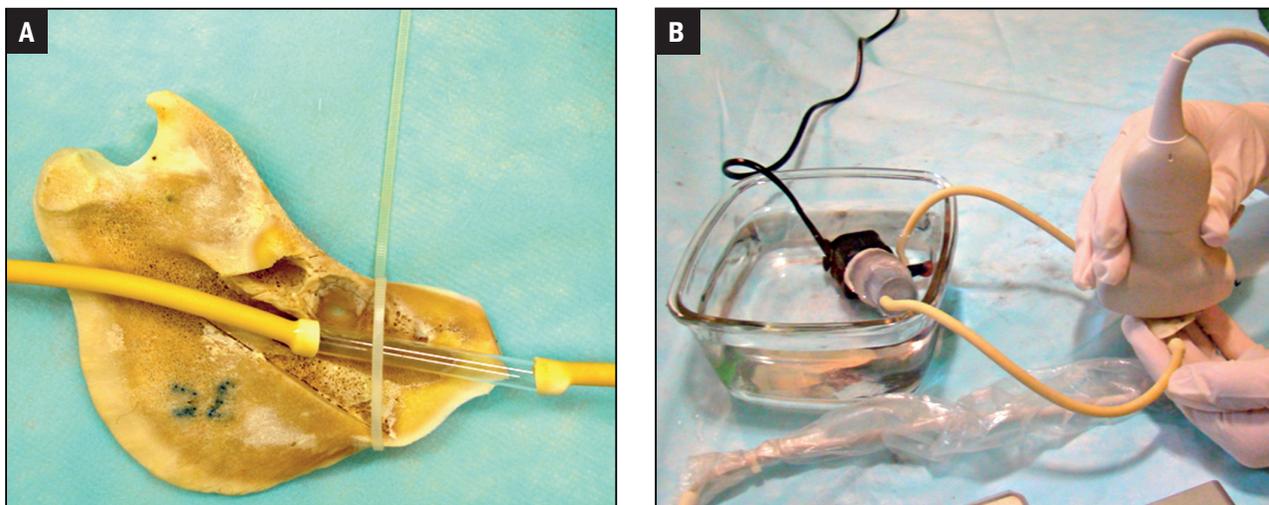


Figure 1 | Methodology used in the present study; **A:** ultrasound vascular simulator; **B:** capture of the ultrasonic signal.

positive signals (Figure 3B) were displayed in the monitor of the ultrasound system. Positivity of the ultrasonic signal was recorded for all hemimandibles in random order by two trained observers (i.e. dentists with expertise in oral radiology). Intraobserver reliability was assessed between measurements performed 2 weeks apart to eliminate memory bias. Intra- and interobserver agreement was assessed using the kappa test. Student’s t-test was used to assess differences between bone thicknesses of hemimandibles with negative and positive ultrasound signals.

RESULTS

The echo signals of the simulated flow in porcine hemimandibles were captured in the cortical bones with a thickness in the 0.2–1.0 mm range (0.59 ± 0.42 mm). However, signals in jaws with a thickness greater than 1.0 mm (1.3 ± 1.2 mm) could not be captured (Figure 4). No false positive results were recorded, leading to a sensitivity of 100% for Doppler ultrasonography as a diagnostic method.

Intraobserver reproducibility and interobserver reliability were confirmed for the ultrasound analysis, according to the kappa index result (0.93,

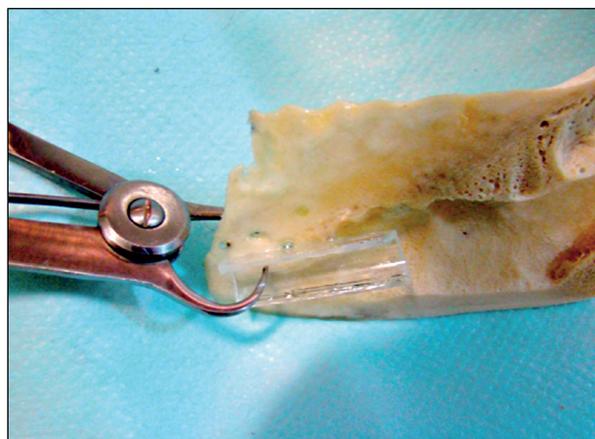


Figure 2 | Measurement using the thickness gauge (caliper).

$p = 0.001$ and 0.88 , $p = 0.001$, respectively). The results for Student’s t-test showed statistically significant differences between mean values for buccal bone thickness in cases with positive and negative signals (Table 1).

DISCUSSION

As supported by the present results, Doppler ultrasonography has been regarded as a useful method to assess the degree of vascularization of a lesion in real time. This attribute is considered the

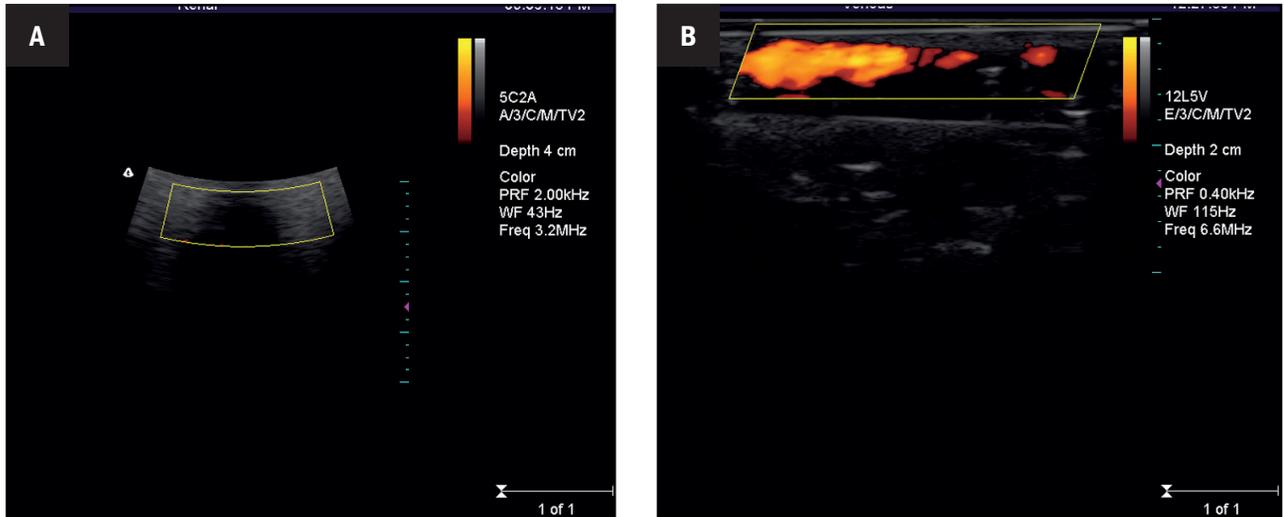


Figure 3 | Digital images of ultrasonography; **A**: example of an image with a negative signal; **B**: ultrasound images showing the positive signal for blood vasculature, captured during examination of hemimandibles.

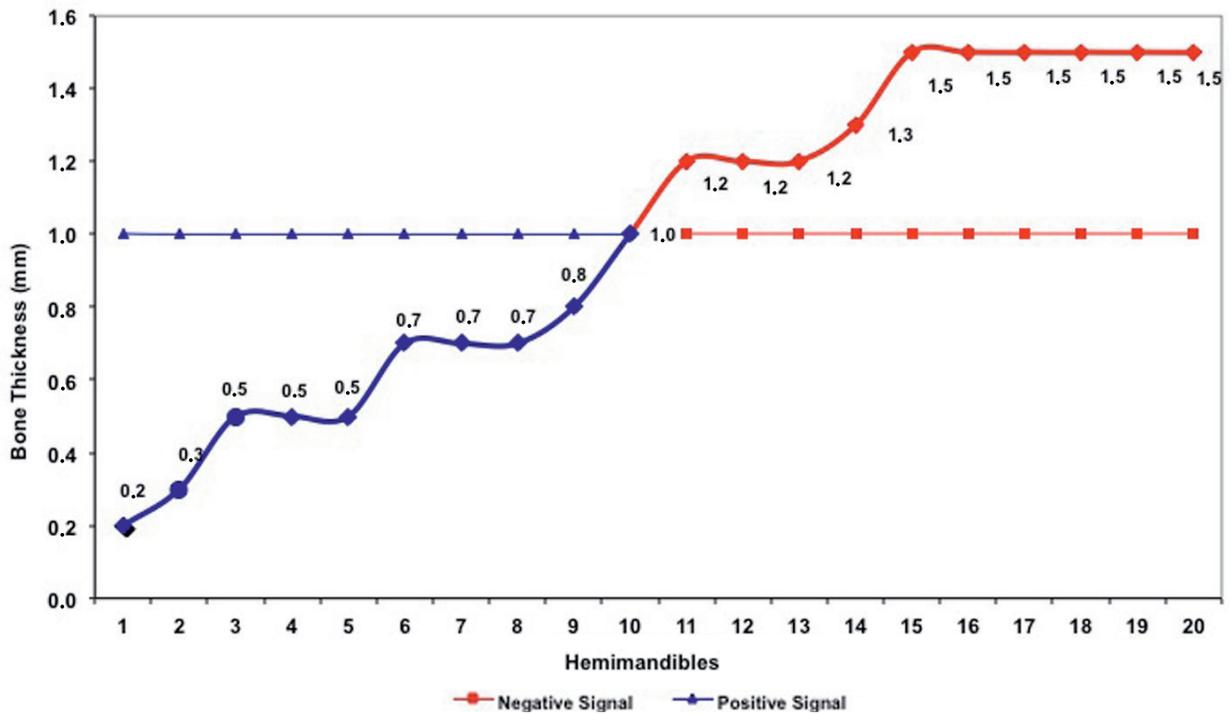


Figure 4 | Relationship between thickness of hemimandibles and capture of the signal obtained.

distinct feature of this method, because it enables a detailed evaluation of the activity and aggressiveness of the change.^{7,10,14} To our knowledge, this is the first study assessing reliability and reproduc-

ibility for ultrasonography as a method to detect intrasosseous blood flow in the oral cavity. Ultrasonography does not use radiation or contrast dyes. Furthermore, compared to computed tomography

Table 1 | Descriptive measurements of bone thickness in relation to capture of signal.

Capture of total signal	Bone thickness (mm)					Student's T test
	Minimum values	Maximum values	Mean values	Standard deviation	Variance	P value
Negative	1.20	1.50	1.39	0.14	0.02	0.001
Positive	0.20	1.00	0.59	0.45	0.20	

and magnetic resonance imaging, ultrasonography offers advantages such as lower cost, shorter scan acquisition time and wider availability, although it produces less detailed images.¹⁵

The ultrasound technique has important characteristics not only for assessing bone tissues but also for detecting blood flow alterations in soft tissues, thus helping to diagnose oral lesions and tumors, and plan related surgical treatments.^{3-5,9} Some authors have sought to understand bone resistance to sound waves, and have found that it is possible to assess the degree of vascularization and blood flow of lesions surrounded by cortical bone, provided this bone has anatomical alterations (i.e., irregularity or discontinuity).^{3,7-9} Thus, Doppler-mode ultrasonography can be used to provide information on the intraosseous content of osteolytic lesions before surgical procedures are performed.^{6,7,9} Our results support the aforementioned statement, insofar as there was a statistically significant difference between the groups with positive and negative capture of echo signals from mandibles with different bone thicknesses. Furthermore, two other studies on oral pathologies confirmed the usefulness of ultrasonography to detect bone healing after surgical removal of intraosseous lesions,^{16,17} confirming the clinical relevance of the present study.

Studies in the literature on computational models have validated ultrasonography simulation in

evaluating pathological processes, emphasizing that this method can be useful in assessing the conditions of complex outflow and the regions where the shear stress of pulsatile flow occurs, thus providing accurate diagnosis.^{5,8,10-13} In the present study, blood flow was observed in a mandible with a thickness in the 0.2–1.0 mm range. This imaging diagnosis result is of fundamental clinical interest for the analysis of certain mandibular intraosseous injuries, such as median dermoid cyst and aneurysmal bone cyst, in order to detect the degree of vascularization using Doppler ultrasound examination.^{6,9,10}

In conclusion, ultrasonography can be applied in studying the intraosseous vascularization of lesions surrounded by cortical bone with a thickness in the 0.2–1.0 mm range, using the Doppler mode. However, this was an *in-vitro* study. Further clinical assessments are required to confirm the validity of this method in diagnosing osteolytic lesions.

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