RESEARCH ARTICLE

Shear-wave sonoelastography for assessing masseter muscle hardness in comparison with strain sonoelastography: study with phantoms and healthy volunteers

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Objectives: Shear-wave sonoelastography is expected to facilitate low operator dependency, high reproducibility and quantitative evaluation, whereas there are few reports on available normative values of *in vivo* tissue in head and neck fields. The purpose of this study was to examine the reliabilities on measuring hardness using shear-wave sonoelastography and to clarify normal values of masseter muscle hardness in healthy volunteers.

Methods: Phantoms with known hardness ranging from 20 to 140 kPa were scanned with shear-wave sonoelastography, and inter- and intraoperator reliabilities were examined compared with strain sonoelastography. The relationships between the actual and measured hardness were analyzed. The masseter muscle hardness in 30 healthy volunteers was measured using shear-wave sonoelastography.

Results: The inter- and intraoperator intraclass correlation coefficients were almost perfect. Strong correlations were seen between the actual and measured hardness. The mean hardness of the masseter muscles in healthy volunteers was 42.82 ± 5.56 kPa at rest and 53.36 ± 8.46 kPa during jaw clenching.

Conclusions: The hardness measured with shear-wave sonoelastography showed high-level reliability. Shear-wave sonoelastography may be suitable for evaluation of the masseter muscles.

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Introduction

Biomechanical evaluation of muscles is important in basic and clinical research, including neuromuscular diseases, muscle injuries and training.^{1,2} When muscle fibres are damaged, muscles show shortening and hardening.^{3–6} Exercise of muscles induces fluid accumulation and an increase in the intramuscular pressure,

and, consequently, changes muscle hardness and thickness.^{3,4} For these reasons, the hardness of skeletal muscles is widely used as an index in the fields of orthopaedics and sports medicine.^{7–10} Similarly, the hardness of the masticatory muscles can aid in the diagnosis and treatment of myalgia in patients with temporomandibular disorders.^{11–14}

Available non-invasive methods for measuring muscle hardness are diverse, including a hardness meter^{11,15} and sonoelastography.^{12–14} Although hardness meter

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Figure 1 Phantom used in this study. The size of phantoms was set at a length of 110 mm, width of 60 mm and thickness of 40 mm. The Young's moduli of the phantoms were created ranging from 20 to 140 kPa, with 20-kPa increments. As the references, the acoustic coupling agents with two kinds of Young's moduli (7 and 40 kPa) were put on the surface of the phantoms. The size of coupling agents was set at a length of 110 mm, width of 60 mm and thickness of 5 mm.

has been verified to be sufficient for clinical use,¹¹ it cannot evaluate total change because the measurements were performed only at several points in the muscle. In this regard, sonoelastography is more effective in assessing a wide area of the objective muscle.^{12–14} Our previous study experimentally verified the utility of elasticity index (EI) ratio obtained by a strain-type sonoelastography for evaluating muscle hardness with simultaneous use of a coupling agent as a reference.¹⁶ However, the hardness expected based on the EI ratio at clenching in the human masseter muscle was over the range of muscle phantom hardness that ensured measurement accuracy. Moreover, strain-type sonoelastography has some disadvantages, including operator dependency, a low reproducibility and a relative evaluation of stiffness,^{7,17–21} because it displays images based on the distortion of the tissue caused by manual compression.^{22,23}

A new technique, shear-wave sonoelastography, has recently been commercialized.^{10,24–33} Shear-wave sonoelastography provides data on tissue stiffness by measuring the propagation speed of shear wave produced by an acoustic radiation force impulse. This technique is expected to lead to a reduction in operator dependency, high reproducibility and quantitative evaluation.^{28,34–38} However, there are few reports on available normative values of *in vivo* tissue in head and neck fields.²⁴

The first purpose of this study was to experimentally clarify the reliability on measurement using shear-wave sonoelastography compared with strain sonoelastography with the use of muscle phantoms that sufficiently covered the actual hardness of the human masseter muscle at clenching. The second was to provide

quantitative normative values of masseter muscle hardness at rest and during jaw clenching.

Methods and materials

Phantom study

Commercially produced phantoms were prepared, simulating the masseter muscles (OST Co., Ltd., Kashiwa-shi, Chiba, Japan) (Figure 1).^{7,16} The materials were made from agar, talc, ticking agent and low-molecular-weight gel. The size of the phantoms was set at a length of 110 mm, width of 60 mm and thickness of 40 mm. Young's moduli of the phantoms were created ranging from 20 to 140 kPa, with 20-kPa increments. As references for measuring hardness on sonoelastography, the acoustic coupling agents with two kinds of Young's moduli (7 and 40 kPa) were put on the surface of the phantoms. The size of the acoustic coupling agents was set at a length of 110 mm, width of 60 mm and thickness of 5 mm. The reason for using two kinds of coupling agents was to examine which can be employed as the reference on strain sonoelastography.

Sonoelastography was performed using a LOGIQ® E9 and a 9-l transducer (GE Healthcare, Tokyo, Japan). A 9-l transducer has been developed for shear-wave sonoelastography, and it provides a frequency of $5.25 \text{ MHz} \pm 20\%$. Sonoelastography of each phantom was obtained using a 4.0-cm image depth, 55 echo gain and 66-dB dynamic range. The autofocus was adapted, and the focus was at about a depth of 2 cm.

Shear-wave sonoelastography displays tissue hardness in colours from blue to red. The tissue hardness is automatically expressed as Young's modulus (kPa) or shear-wave velocity (m s^{-1}) (Figure 2). In this study,

Figure 2 Shear-wave sonoelastogram of a phantom with Young's modulus of 20 kPa equipped with a coupling agent of 40 kPa. The hardness in the region of interest of the phantom was expressed as Young's modulus (13.85 kPa) or shear-wave velocity (2.15 m s^{-1}).

Figure 3 Strain sonoelastogram of a phantom with Young's modulus of 60 kPa with a coupling agent of 40 kPa. The regions of interest (ROIs) were set in the phantom and superficial coupling agent, and the elasticity indices (EIs) in the respective ROI were measured: pEI and cEI. The EI ratio of the phantom was calculated as follows: EI ratio = pEI/cEI.

Young's modulus (kPa) was adopted. A transducer was held on the phantom for at least 3 s. This time was sufficient to be a 3-time irradiation of the push pulse and the shear wave reception. A region of interest (ROI) of 200 mm² was set on a shear-wave sonoelastogram of the phantom. The tissue hardness was measured using the software provided with the machine.

Strain sonoelastography also displays the elasticity in colours from red to blue. The EI is defined as the strain value of each area compared with the average strain value (EI = 1) of the whole area of interest. A softer EI is assigned as 0–1, and a harder EI is assigned as 1–6 (Figure 3). In this study, the optimum compression pressure was applied while monitoring the elastic scale on the left edge of the sonoelastogram. Measurements were performed using the software Elasto Q Analysis (BT11) (GE Healthcare, Tokyo, Japan). ROIs of 270 and 40 mm² were set on each phantom and coupling agent, respectively. The respective EIs were measured. The EI ratio of the phantom was determined as follows: EI ratio = (EI of the phantom)/(EI of the coupling agent).

Three examiners (YA, MN and WN) performed shear-wave and strain sonoelastography of each phantom and measured the Young's modulus (kPa) and EI ratio. The examiners had 4 years' of experience in performing strain sonoelastography. They performed shear-wave sonoelastography after receiving an operating explanation and training well. One examiner (YA) performed them for three sessions; two sessions on Day 1 and one session 3 days later. The interoperator and intraoperator reliabilities [intraclass correlation coefficient (ICC)] were calculated. The relationships between the actual and measured hardness of the phantoms were examined.

Healthy masseter muscle study

30 healthy volunteers (21 males and 9 females) were enrolled in this study. The age of the participants ranged

from 26 to 63 years, with a median age of 31.5 years. All participants had no symptoms of masticatory muscles and temporomandibular joints and had no pathologies such as tumours or inflammation.

The study was performed with the approval of the Ethics Committee of the Aichi Gakuin University (No. 217), based on the guideline in the Declaration of Helsinki. All participants were informed of the purpose of the study and provided consent before participating.

Shear-wave and strain sonoelastography of the masseter muscle was performed by one examiner (YA), using the same machine and transducer and under the same conditions. The participants underwent sonographic examination in a supine position on a bed. The bilateral masseter muscles were scanned perpendicular to the anterior border of the muscle and to the surface

Figure 4 Shear-wave sonoelastogram of the masseter muscle in a 31-year-old female. The region of interest was set on the masseter muscle, and Young's modulus of the masseter muscle was measured (56.23 kPa).

Figure 5 Strain sonoelastogram of the masseter muscle in a 29-year-old male. A coupling agent of 40 kPa was put on the surface of the masseter muscle. The regions of interest (ROIs) were set in the masseter muscle and superficial coupling agent, and the elasticity indices (EIs) in the respective ROI were measured: mEI and cEI. The EI ratio of the masseter muscle was calculated as follows: EI ratio = mEI/cEI.

of the underlying mandibular ramus at 2.0 cm above the inferior border of the mandible. As the reference, the coupling agent with Young's modulus of 40 kPa was put on the surface of the masseter muscle. The scan was obtained at rest and during jaw clenching with maximum force. Each measurement was performed twice for each type of elastography and under each condition, and the two measurements were averaged.

On shear-wave sonoelastogram of the masseter muscle, ROIs of the arbitrary sizes were set on the masseter muscle and Young's modulus (kPa) was measured (Figure 4). On strain sonoelastogram of the masseter muscle, ROIs of 70–220 and 40 mm² were set on the masseter muscle and coupling agent, respectively. The respective EIs were measured. The EI ratio of the masseter muscle was determined as follows: EI ratio = (EI of the masseter muscle)/(EI of the coupling agent) (Figure 5). The measurement error of the Young's module on shear-wave sonoelastography was 3.91% of the coefficients of variation of the five repeated measurement on one muscle made by one examiner (YA) while that of the EI ratio on strain sonoelastography was 4.10%.

Based on the relationship between the actual hardness and EI ratio in the phantom experiment using strain sonoelastography, the approximated curve was described with Microsoft[®] Excel[®] 2010 (Microsoft Japan Co., Ltd, Tokyo, Japan). The predictive hardness (kPa) was calculated from the measured EI ratio of the masseter muscle. The standard deviation of the predicted value was 2.42. The predictive hardness (kPa) on strain sonoelastography was compared with the hardness (kPa) measured on shear-wave sonoelastography.

Statistical analysis

Wilcoxon signed-rank test was used for comparison between the hardness with the use of the two types of

coupling agent, between the left and right hardness, between the hardness at rest and during jaw clenching and between the measured hardness on shear-wave sonoelastography and the predictive hardness on strain sonoelastography. The Mann–Whitney *U* test was used for comparison of the hardness between the males and females. The relationships between the actual and measured hardness were examined by the least squares method. SPSS[®] statistics v. 22 (IBM Corp., Tokyo, Japan; formerly SPSS Inc., Chicago, IL) was applied for statistical analysis. Values of *p* < 0.05 were considered to indicate significance.

Results

Phantom study

The results of the interoperator and intraoperator ICCs on measuring the hardness of the phantoms are shown in Table 1. ICCs on measuring hardness on shear-wave sonoelastograms were 0.999 or 1.000, despite the hardness of the coupling agents. ICCs on measuring EI ratios on strain sonoelastograms ranged from 0.979 to 0.993, and these values were sufficiently high, although they were slightly lower than those of shear-wave sonoelastograms.

Table 1 Intraclass correlation coefficient (ICC)

	Coupling agent (kPa)	Interoperator ICC	Intraoperator ICC
Measured tissue modules on shear-wave sonoelastography	7	0.999	1.000
	40	0.999	0.999
Measured EI ratios on strain sonoelastography	7	0.983	0.993
	40	0.979	0.991

EI, elasticity index.

Figure 6 Relationship between the actual hardness and measured Young's moduli on shear-wave sonoelastography (SWSE). Strong correlations were seen between the actual hardness and measured values, using both coupling agents (CAs). The two approximate curves were in good agreement. ■, use of phantoms equipped with CA of 7 kPa; △, use of phantoms equipped with CA of 40 kPa.

The relationships between the actual and measured hardness of the phantoms on shear-wave sonoelastograms are shown in Figure 6. Strong correlations were seen between the actual and measured hardness, using either coupling agent ($R^2 = 0.9588$ with the use of the 7-kPa coupling agent and $R^2 = 0.9731$ with the use of the 40-kPa coupling agent). Using both coupling agents, two approximate curves showing the relationships between the actual and measured hardness revealed favourable agreement.

The relationships between the actual hardness and measured EI ratios of the phantoms on strain sonoelastograms are shown in Figure 7. Strong correlations were seen between the actual hardness and EI ratios ($R^2 = 0.9802$ with the use of the 7-kPa coupling agent and $R^2 = 0.9564$ with the use of the 40-kPa coupling agent). The values with the use of the 7-kPa coupling agent were higher than those with 40 kPa ($p = 0.0180$, Wilcoxon signed-rank test). The approximate curve with the use of the 7-kPa coupling agent was located above that with the 40-kPa coupling agent.

Healthy masseter muscle study

The results of the hardness of the masseter muscles in the healthy volunteers are shown in Table 2. The mean hardness on shear-wave sonoelastography was 42.82 ± 5.56 kPa at rest and 53.86 ± 8.26 kPa during jaw clenching. The mean EI ratio on strain sonoelastography was 1.43 ± 0.30 at rest and 3.32 ± 1.01 during jaw clenching. On both sonoelastograms, there were no significant differences between the left and right values and between males and females. There were significant

differences between the values at rest and during jaw clenching (Table 3).

Based on the relationship between the actual hardness and EI ratio in the phantom experiment on strain sonoelastography, the predictive equation for determining masseter muscle hardness from the EI ratio was calculated. The predictive hardness calculated from the EI ratio is shown in Figure 8. The hardness measured on shear-wave sonoelastography and the predictive hardness calculated on strain sonoelastography were compared. Hardness at rest on shear-wave sonoelastography was greater than on strain sonoelastography (Table 4). Hardness during jaw clenching demonstrated similar values on both sonoelastograms.

Discussion

Shear-wave sonoelastography is a new technique expected to facilitate a reduction in operator dependency and high-level reproducibility. In the phantom experiment of this study, we confirmed its ability to yield high interoperator and intraoperator ICCs (about 1.00). The present results were slightly higher than in previous studies showing an interoperator ICC of 0.72–0.97 and intraoperator ICC of 0.78–0.98.^{28,36–38} On the other hand, the reproducibility of strain sonoelastography is considered to be dependent on the operators' skill. Previous studies examining *in vivo* the muscle, kidney and heart indicated an interoperator ICC of 0.72–0.89 and intraoperator ICC of 0.47–0.96.^{7,18–21} Our previous study using the same phantoms with

Figure 7 Relationship between the actual hardness and measured elasticity index ratio on strain sonoelastography (SSE). Strong correlations were seen between the actual hardness and measured values, using both coupling agents (CAs). However, the two approximate curves were not completely consistent. ♦, use of phantoms equipped with CA of 7 kPa; ○, use of phantoms equipped with CA of 40 kPa; EI, elasticity index.

Table 2 Hardness of healthy masseter muscles measured with shear-wave sonoelastography (SWSE) and strain sonoelastography (SSE)

			Hardness on SWSE (kPa)	Hardness on SSE	
				EI ratio	kPa
Rest	Male	Right	40.82 ± 6.59	1.36 ± 0.34	36.29 ± 3.33
		Left	44.11 ± 6.67	1.41 ± 0.35	36.75 ± 3.39
	Female	Right	44.47 ± 8.07	1.66 ± 0.35	39.19 ± 3.32
		Left	43.47 ± 7.84	1.56 ± 0.38	38.21 ± 3.67
	All	Right	41.79 ± 6.68	1.42 ± 0.32	36.86 ± 3.13
		Left	43.85 ± 6.87	1.44 ± 0.32	37.01 ± 3.13
Clenching	Male	Average	42.82 ± 5.56	1.43 ± 0.30	36.94 ± 2.88
		Right	51.62 ± 9.72	3.23 ± 1.10	53.58 ± 9.78
		Left	54.77 ± 8.33	3.27 ± 1.22	53.83 ± 10.57
	Female	Right	57.31 ± 12.61	3.70 ± 1.23	57.67 ± 10.82
		Left	59.07 ± 12.68	3.76 ± 1.52	58.02 ± 12.14
	All	Right	52.33 ± 10.05	3.30 ± 1.06	54.17 ± 9.41
		Left	55.39 ± 9.45	3.34 ± 1.20	54.46 ± 10.45
		Average	53.86 ± 8.26	3.32 ± 1.01	54.36 ± 8.96

EI, elasticity index.

elasticity of 20–60 kPa also showed lower ICCs for the interoperator (0.80 to 0.89) and intraoperator (0.74–0.86) agreements in strain sonoelastography.¹⁶ This study was subject to the tissue-mimicking phantom with a wider range of elasticity (20–140 kPa), and scanned by experienced examiners, and, therefore, high interoperator ICC (0.98) and intraoperator ICC (0.99) of strain sonoelastography could be obtained.

A significant correlation between the actual and measured hardness was seen on shear-wave sonoelastography. The approximate curve based on the relationship between both values using the 7-kPa coupling agent was in good agreement with that with the use of 40 kPa. On the other hand, strain sonoelastography showed a significant correlation between both values, but the two approximate curves using the two kinds of coupling agents were in disagreement. This discrepancy may be caused by the reasons that strain sonoelastography displayed relative values of the

phantom against the coupling agent and that the EI was determined for the softer tissue as 0–1 and the harder tissue as 1–6; that is, it would be due to the degree of difference in the hardness of the subject and reference.^{13,16} The results of the phantom experiment would be helpful for developing coupling agents used *in vivo*.

The hardness of skeletal muscles is widely used as an index for diagnosis and treatment in the fields of orthopaedics and sports medicine.^{28,34–38} Regarding the masseter muscle, its hardness in patients with temporomandibular disorder accompanied by myofascial pain has been clarified to be greater than those of healthy volunteers.^{11,12,14} The difference between the left and right hardness of the masseter muscles in patients was greater.^{11,12} Furthermore, masseter muscle hardness in patients decreased with massage treatment together with a reduction in pain; therefore, we suggest that it may be useful as a predictor of therapeutic efficacy.^{11,14}

Table 3 Results of statistical analysis of the masseter muscle hardness

		Hardness on SWSE (kPa)	EI ratio on SSE
Comparison between right and left values (Wilcoxon signed-rank test)			
Rest	Male	$p = 0.125859$	$p = 0.286235$
	Female	$p = 0.575062$	$p = 0.202622$
	All	$p = 0.171376$	$p = 0.965505$
Clenching	Male	$p = 0.135357$	$p = 0.386925$
	Female	$p = 0.878482$	$p = 0.593955$
	All	$p = 0.171376$	$p = 0.681890$
Comparison between males and females (Mann–Whitney <i>U</i> test)			
Rest	Right	$p = 0.110499$	$p = 0.081672$
	Left	$p = 0.567374$	$p = 0.877545$
Clenching	Right	$p = 0.378923$	$p = 0.179363$
	Left	$p = 0.537949$	$p = 0.596566$
Comparison between rest and clenching (Wilcoxon signed-rank test)			
Male	Right	$p = 0.000892^a$	$p = 0.000103^a$
	Left	$p = 0.000103^a$	$p = 0.000103^a$
Female	Right	$p = 0.012515^b$	$p = 0.005062^a$
	Left	$p = 0.012515^b$	$p = 0.005062^a$
All	Right	$p = 0.000026^a$	$p = 0.000002^a$
	Left	$p = 0.000004^a$	$p = 0.000002^a$

EI, elasticity index; SSE, strain sonoelastography; SWSE, shear-wave sonoelastography.

^a $p < 0.01$ significant difference.^b $p < 0.05$ significant difference.

Figure 8 Hardness of healthy masseter muscles (kPa). It shows the hardness measured on shear-wave sonoelastography (SWSE), and the predictive hardness calculated from elasticity index ratios on strain sonoelastography (SSE). L, left; R, right.

If shear-wave sonoelastography can provide the normative values of the hardness of masseter muscles, the values would be a reference for exploration of the causes of muscle pain and the selection of treatment methods for patients.

In previous studies, the hardness of skeletal muscles was variable.^{10,24–31} Regarding the hardness of muscles of the upper limbs, the supraspinatus muscle has been reported as 31.2²⁴ or 32.7–40.0 kPa,³¹ and the biceps muscle as 5.1 kPa.²⁸ Concerning the hardness of muscles of the lower limbs, the quadriceps femoris has been reported as 12.7³⁰ or 11–16,²⁹ the tibialis anterior muscle as 5.8²⁷ or 40.6,¹⁰ and the gastrocnemius muscle as 8.0,²⁶ 11.1,²⁴ 16.5¹⁰ or 27.0–32.0 kPa.²⁵ In all of these reports, an Aixplorer[®] machine (Supersonic Imaging, Aix-en-Provence, France) was used.

Concerning the masseter muscles, Arda et al²⁴ measured the hardness of the masseter muscles of 127 healthy volunteers (89 females and 38 males, mean age of 37.72 ±

Table 4 Comparison between the hardness measured with shear-wave sonoelastography and the predictive hardness with strain sonoelastography

			Wilcoxon signed-rank test
Rest	Male	Right	$p = 0.0040^a$
		Left	$p = 0.0022^a$
	Female	Right	$p = 0.0284^b$
		Left	$p = 0.0125^b$
	All	Right	$p = 0.0002^a$
		Left	$p = 0.0001^a$
Clenching	Male	Right	$p = 0.6542$
		Left	$p = 0.433$
	Female	Right	$p = 0.6465$
		Left	$p = 0.5751$
	All	Right	$p = 0.5577$
		Left	$p = 0.8130$

^a $p < 0.01$ significant difference.

^b $p < 0.05$ significant difference.

9.11 years, 17–63 years) using Aixplorer and a 6- to 13-MHz transducer. The result was 10.4 kPa. In this study, the mean hardness of the masseter muscle of healthy volunteers using shear-wave sonoelastography was 42.82 ± 5.56 kPa at rest and 53.86 ± 8.26 kPa during jaw clenching. The discrepancy between the two studies would be due to the differences in the device, software, and race and sex distribution of the subjects, and the measurement site and methods. The current study showed no significant difference between the left and right values, or between males and females. The latter result was also confirmed in the report by Arda et al.²⁴ Since the number of subjects in this study was small (30 cases), it is uncertain whether we can provide precise data. The correlation between muscle hardness and patient's age was not analyzed, because age distribution in this study was not uniform. In the future, it will be necessary to increase the number of cases and examine changes in hardness caused by ageing.

In conclusion, the high-level reliabilities for measuring the hardness of the tissue-mimicking phantoms on shear-wave sonoelastography were confirmed. The actual and measured hardness showed a strong correlation. The mean hardness of the masseter muscles in healthy volunteers was 42.85 ± 5.56 kPa at rest and 53.36 ± 8.46 kPa during jaw clenching.

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