Reproducibility of Anterior Scalene Stiffness Measurement with Shear Wave Elastography: An Inter-Examiner Reliability Study

Introduction

The scalene muscles, which are located on the antero-lateral aspect of the neck, consist of up to four muscles: anterior, medium, posterior, and minimus [1]. These muscles run from the transverse processes of the cervical vertebrae to the first and second ribs, and their main functions include lateral flexion of the cervical spine and, controversially, cervical spine rotation if activated unilaterally [2]. When activated bilaterally, they contribute to cervical flexion [3]. The scalene muscles also serve as an accessory inspiratory muscle group [4].

Despite the fact that the attachments, surrounding structures, nerve supply, and actions of these muscles have been extensively described in the literature, numerous anatomical variations have been observed [5-7]. This region's inter-scalene triangle is particularly relevant from a clinical perspective because it serves as the pathway for the roots and trunks of the brachial plexus and the subclavian artery [8].

The anterior scalene (AS) muscle has been the subject of previous studies, and it has been found to be a significant structure associated with neck pain [9]. In patients with chronic neck pain, there was a greater conversion of slow-twitch type-1 fibers to fast-twitch type-2B fibers, as well as greater electromyographic activity during low-load tasks, which may explain the greater muscle fatigue specific to the pain side [10]. Although the anterior scalene muscle is clinically relevant and has been the subject of several studies on its morphology and function, there is a lack of research using US to investigate this muscle compared to other muscles in the neck area, such as short rotators, cervical multifidus, semispinalis, upper trapezius, levator scapulae, or longus colli [11,12].

Ultrasound imaging (US) is a diagnostic tool that is commonly used in both clinical and research settings due to its speed, ease of use, safety, and cost-effectiveness compared to other imaging modalities [13]. This imaging technique provides real-time information, making it particularly useful for dynamic assessments of musculoskeletal structures [14]. In addition to the US metrics using the bidimensional mode which includes muscle size, shape and brightness and quality [15], recent advances allow the muscle stiffness measurement [16].

Shear-wave elastography (SWE) is a non-invasive imaging technology designed to provide quantitative and objective stiffness data with absolute values (in contrast with strain elastography, which provides relative values within the acquired image at one point) by calculating the Young's modulus (measured in kPa) and local shear wave speed (measured in m/s) [17]. Previous studies have used SWE to assess both general muscle stiffness and specific locations within the muscles such as myofascial trigger point (MTrP) [18]. Although SWE demonstrated to be a reliable, valid, and objective [19], up to date the evidence assessing the clinical relevance of US for assessing the anterior scalene muscle is limited.

As clinicians value the use of objective tools with reliable indices of effectiveness, such as validity, reliability, specificity, and sensitivity [20], it is crucial to evaluate the diagnostic precision of SWE at this location before proceeding with studies analyzing the correlation between the anterior scalene muscle stiffness with clinical severity indicators or assessing stiffness differences between asymptomatic subjects and clinical populations. Hence, this study aims to establish a simple and reproducible US protocol for identifying and measuring the anterior scalene muscle's stiffness to determine its reproducibility in a sample of healthy individuals.

Methods

Study Design

Between October 2022 and March 2023, a diagnostic accuracy designed cross-sectional observational study was conducted at *BLINDED*. To improve the quality of this report, the study adhered to the Reporting Reliability and Agreement Studies (GRRAS) guidelines [21] and the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) guidelines [22]. The Ethics Committee of *BLINDED* provided oversight and approval for the study protocol prior to data collection.

Participants

After posting local announcements around the campus, a sample consisting of healthy volunteers was recruited through convenience sampling. Volunteers between the ages of 18 and 65 who had not experienced any neck pain symptoms in the past year were considered eligible for participation. Participants were excluded if they reported a history of whiplash, took medication that affected muscle tone (such as muscle relaxants), underwent any surgical procedure, had any neuropathic condition (such as radiculopathy, thoracic outlet syndrome, or myelopathy), or had severe degenerative radiologic findings. Eligible participants were required to read and sign an informed written consent before being included in the data collection.

Sample Size Calculation

The minimum sample size for this study was estimated according to the guidelines presented by Walter et al., [23] which are based on intraclass correlation coefficients (ICCs). The sample size calculation for this study is based on the only previous study assessing the test-retest reliability of SWE measurements in healthy subjects, considering

ICC=0.80 as their indicative values obtained measuring 30 AS muscles. Thus, ICC values greater than 0.80, which are considered indicative of good reliability [24], were deemed minimally acceptable.

Given that: 1) an expected ICC value of 0.9 was hypothesized; 2) a power of 80% and a significance level of 5% were established; and 3) 10% losses were assumed due to the longitudinal nature of the study (participants were examined twice with a considerable time difference between trials), the minimum sample size required for this study was determined to be 65 SWE images.

Examiners

For conducting this study, one examiner with over 10 years of experience if musculoskeletal ultrasound imaging and 10 years of clinical experience focused on musculoskeletal conditions and chronic neck pain and one novel examiner with 1 year of experience in this field acquired and codified all the images. For enhancing the quality of the study, the imaging acquisition was conducted randomizing the volunteers' participation order and the sides firstly examined. The first session was conducted from 9:00 AM to 1:00 PM and the second trial was conducted from 3:00 PM to 17:00 PM. Both examiners were isolated using this schedule for avoiding communication and agreement, alternating turns each day.

Participants were asked to attend two appointments with a 24-hour interval between each appointment (alternating the examiner conducting the assessment). To ensure blinding, an independent rater with similar experience conducted all the images measurements in a randomized order. This rater was not aware of the examiner, participant or side evaluated.

Ultrasound Imaging Acquisition Protocol

The US device used for collecting all the images was a Logiq E9 device, using a linear transducer 6-15 MHz ML-6-15-D (General Electric Healthcare, Milwaukee, WI, USA). The console settings were also standard for all the acquisitions (Frequency=12 MHz, Gain=65 dB and Depth=4.5 cm).

Participants were positioned in the supine position with a pillow placed under their knees to minimize lumbar lordosis. They were instructed to relax their neck muscles during the procedure to reduce muscle stiffness changes attributable to muscle contraction [25].

After administering acoustic coupling gel on the transducer and placing it on the supraclavicular region beside the cricoid cartilage, a lateral gliding was performed until locating the carotid artery in the lateral border of the image. Then, the transducer was glided in the cranial and caudal directions until locating the C6 transverse process in a short-axis view. This image was used as a reference since is C6 is characterized by a prominent the anterior tubercle and a smaller posterior tubercle [26]. After locating C6, the probe was caudally glided until locating the transverse process of C7, which is characterized by a prominent posterior tubercle [27], to freeze the image, codify it and save for latter analyses. In order to blind the side examined, the posterior tubercle was consistently orientated to the left side of the image and the AS muscle to the right side of the image. The region of interest selected for assessing muscle stiffness had enough width and height to cover completely the AS muscle.

Measurement of Muscle Stiffness

All images were analyzed using the US device measuring software. The process consisted of a careful contouring of the anterior scalene perimeter, avoiding the inclusion of bone, nerve roots or surrounding fascia as shown in **Figure 1**. Then, the Young's Modulus and the Shear Wave Speed measurements were automatically calculated.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS v.27, Armonk, NY, USA) for Mac OS was used for conducting all data processing and analysis, setting the two-tailed significance level cut-off at $p<0.05$. Initially, histograms and Shapiro-Wilk tests were used to assess the distribution of continuous variables. If the statistical analysis resulted in $p<0.05$ were regarded as non-normally distributed, while $p > 0.05$ was deemed normally distributed.

Next, the characteristics of the overall sample were described using descriptive statistics. For categorical data, the frequency and percentage of each category were reported (e.g., the number and percentage of men and women). Meanwhile, central tendency metrics such as mean and median, and dispersion metrics such as standard deviation and interquartile range were used to report continuous variables, depending on whether they were normally or non-normally distributed. In addition, demographic features were reported independently for men and women, whereas muscle morphology and quality features were reported by gender and side. The Student's T-test was used to analyze between-group differences in the mean difference with a 95% confidence interval.

Inter-examiner reliability analyses was based on the calculation of 1) the mean average and standard deviation of each SWE metric, 2) the absolute error between examiners 3), the intraclass correlation coefficients ($ICC_{3,2}$, which is a 2-way mixed model consistency type appropriate for this setting calculated as $ICC = \frac{MSR-MSE}{MSR}$, being MSR the mean square for rows and MSE the mean square for error [24]), 4) the standard error of measurement (SEM, calculated as $SEM = Standard\ Deviation\ of\ Absolute\ Error \times \sqrt{1 - ICC}$, and 5) the minimal detectable changes calculated as $MDC = 1.96 \times SEM \times \sqrt{2}$) [24].

Results

Out of the 42 individuals who expressed interest in participating in the study, 3 were excluded due to a history of clinically significant neck pain episodes within the previous year and 4 were lost for the second appointment. As a result, 35 asymptomatic volunteers were included in the data collection, analyzing left and right sides from all participants. This led to a total of 70 anterior scalene muscles being examined, obtaining a total of 140 US images.

Table 1 summarizes the sociodemographic characteristics of the sample (and compared by gender) and the US characteristics of the anterior scalene muscle (reported by gender and side). Males and females had comparable age and BMI (both, p>0.05), but males were significantly taller and heavier (both, p<0.01). Regarding the anterior scalene muscle, results showed no side-to-side size nor stiffness asymmetries (p>0.05). Only muscle size (cross-sectional area, p<0.01) was statistically significant different between genders, with larger areas in males.

Inter-examiner reliability estimates for assessing AS muscle stiffness are summarized in **Table 2**. These results showed good-to-excellent reproducibility estimates for assessing Young's modulus (ICC=0.757-0.942) and good-to-excellent for assessing the shear wave speed (ICC=0.704-0.927). In addition, **Table 2** summarizes the SEM (3.1 kPa for Young's Modulus and 0.21 m/s for shear wave speed), MDC (8.5 kPa for Young's Modulus and 0.60 m/s for shear wave speed) and CV (29.6% for Young's Modulus and

14.7% for shear wave speed). Limits of agreement are illustrated using Bland-Altman plots in **Figure 2**.

Discussion

Up to the authors' knowledge, this is the first study investigating the inter-examiner reliability of a US procedure for assessing the anterior scalene stiffness, finding good to excellent reliability for both metrics (Young's modulus and shear wave speed). However, this is not the first study conducted for determining the diagnostic accuracy of SWE for assessing the anterior scalene stiffness. Bedewi et al., [25] conducted a preliminary study including a sample of 15 asymptomatic subjects for evaluating the test-retest reliability of a similar procedure to calculate the Young's modulus. As the authors recognize, this study included a small sample size, with no minimum sample size calculation.

For this reason, their results should be interpreted cautiously, as there is a considerable risk of bias attributable to type-II errors. In addition, the imaging acquisition procedure was not sufficiently detailed. For instance, the authors only defined the patients' position as supine position and the probe placement was described to be beside the thyroid lobe. Since the average length of this gland was reported to be around 4.22 to 4.32 cm [26] and considering that a cross sectional area was attempted, the chance of determining the same placement point is relatively low.

Focusing on the reliability estimates differences between the studies, the authors reported an average ICC of 0.80 while this procedure obtained slightly better estimates (ICC≥0.85). This difference could be attributable to the location specificity proposed in this procedure. Additionally, it should be noted that intra-examiner reliability is generally better than inter-examiner reliability for assessing neck muscles in asymptomatic populations [27], and therefore the expected intra-examiner reliability differences between procedures may be substantial and needs further research to confirm the hypothesis. No further comparisons between studies are possible as Bedewi et al. [25] did not provide any score differences between test and retest trials or any other data (i.e., SEM, MDC or CV).

The only second study analyzing the AS muscle stiffness available [28] consisted of an assessment of the shear wave speed (but not the Young's modulus) in a sample of 20 asymptomatic subjects. Regarding their methodology, the probe placement was described at the lower fourth of the anterolateral aspect of the neck. This description may involve a considerable risk of low reproducibility due to lack of details [29]. However, this hypothesis cannot be confirmed as the authors did not provide any reliability data.

Although this study primarily aimed to assess the procedure reliability and not to provide normative values as the sample size calculation may not be adequate for this descriptive design objective (even if this study has the largest sample), a discussion about the AS stiffness scores obtained among the studies may be of interest. This comparison is feasible despite the demographic differences among the studies as Bedewi et al., [25] and Kuo et al., [30] found that age, heigh, weight and BMI are not significantly correlated with the AS stiffness. Mean Young's modulus scores and its dispersion indicate that this metric is relatively consistent between Bedewi et al., [25] and the results obtained in this study (18.83 ± 5.32 kPa and 16.1 ± 9.3 kPa for the right side and 21.71 ± 4.8 kPa and 14.4 ± 8.9 kPa for the left side, respectively). However, the shear wave speed was substantially different between the values provided by Kuo et al., [30] and our results (mean of 1.12 ± 0.17 m/s and 2.17 ± 0.56 m/s, respectively).

Although these differences could be attributable to the differences between the procedures, descriptive studies including adequate sample sizes should elucidate if these differences are explained by measurement errors or inter-subjects variability, discuss which stiffness metric is most sensitive and specific and explore differences among asymptomatic populations including a wider range of age, BMI and other body composition features, between clinical populations and asymptomatic subjects and analyze the association between stiffness metrics with clinical severity indicators such as central sensitization (e.g., central sensitization inventory), neurophysiological status (e.g., pressure pain thresholds), psychological status (e.g., anxiety and depression), function (e.g., neck pain disability), and neurologic deficits (e.g., radiculopathy) [18].

Limitations

It's important to acknowledge certain limitations of this study. One such limitation is our restriction of the sample to asymptomatic individuals. As a result, we cannot be certain whether the reliability estimates we obtained would apply to patients experiencing neck pain symptoms, especially since certain clinical populations have demonstrated histological changes that may impede the visualization of muscle boundaries. Secondly, we only assessed one cervical level and employed a single US device with two examiners conducting a single measurement. As such, additional research that includes multiple cervical levels, various US devices and more examiners is required to validate our findings. Additionally, we only conducted a single measurement per examiner, therefore future studies could explore whether an increased number of trials and calculating a mean average of these measurements would enhance the inter-examiner reliability.

Conclusion

This ultrasound procedure for locating and measuring the anterior scalene muscle stiffness is acceptably reliable in asymptomatic subjects based on the results obtained in this study. Considering that one examiner was experience while the other examiner was novel, the use of SWE for measuring the AS stiffness is supported (independently if the examiners are not experienced). In addition, this paper proposes technical considerations for future studies using this protocol for reporting normative values of AS stiffness in asymptomatic populations and also proposals for assessing its discriminative capacity between asymptomatic and clinical populations and the SWE association with clinical severity indicators.

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