Assessing the Reliability of Echo Intensity of Craniovertebral Muscle Group using B-Mode Ultrasound: A Technical Note

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ABSTRACT
An acceptable reliability is needed for each scale and a valid decision-making process. Ultrasonography is a simple, cost-effective, and accessible tool compared to magnetic resonance imaging (MRI) to assess echo intensity (EI) as a biomarker of muscle function in neck musculoskeletal problems. However, no evidence is available regarding the reliability of neck muscle echogenicity according to rehabilitative ultrasonography in clinical studies on forward head posture (FHP). We determined the reliability of neck muscles EI in individuals with and without FHP. Transverse images of deep neck flexors (Longus Coli) and suboccipital (Rectus capitis posterior minor) muscles were acquired from 20 individuals with FHP and 20 controls in one session. The intraclass correlation coefficient (ICC), minimum detectable change (MDC), and standard error of measurement (SEM) for EI were measured in this study. The ICC, SEM, and MDC ranges were 0.50 - 0.51, 2.73 - 3.41, and 7.56 - 9.46 for the Longus colli muscle and 0.48 - 0.49, 3.29 - 4.98, and 9.13 - 13.81 for the rectus capitis posterior minor (RCPm) muscle, respectively. Based on the present findings, EI showed acceptable reliability; therefore, it can be used for assessment of neck muscle morphology.

Keywords
Neck Muscles; Ultrasonography; Posture

Introduction
Today, forward head posture (FHP) is commonly seen in postural deviation in the shoulder girdle and neck region of individuals with sedentary jobs [1]. In FHP, there is hyperextension in the upper neck vertebrae, in addition to a smooth arc in the lower vertebrae. FHP seems to be linked to alterations in the length and strength of neck muscles. The evidence suggests that it can shorten the posterior muscles, such as the rectus capitis posterior minor (RCPm) and lengthen the anterior neck muscles, including the Longus colli (LCo) muscle [2]. According to previous studies, LCo muscle stabilizes and flattens cervical lordosis [3], while suboccipital muscles are involved in the stabilization and better movement of muscles, especially RCPm, which crosses the upper cervical joints [4].

In recent years, the importance of muscle echo intensity (EI) in de-
determining the muscle status and function has been emphasized [5]. In fact, grey-scale analysis of muscles can be used to measure EI for the assessment of fibrous composition and intramuscular fat content. On the other hand, ultrasound (US) is a less expensive and more accessible imaging modality compared to MRI for the analysis of changes in the morphological characteristics of muscles [6]. In addition, intra-rater reliability is measured to evaluate the data stability, which is documented by a single person in two trials or more. Overall, adequate reliability of all clinical measurements and decisions must be verified, and an acceptable reliability is needed for each scale and a valid decision-making process [7].

So far, few studies have reported acceptable inter- and intra-reliability for muscle thickness or cross-sectional areas (CSA) of the neck region [8, 9]. The reliability of assessing echogenicity using ultrasound has not been widely considered in the literature, and morphological changes in subjects with FHP have insufficiently been embedded in evidence-based practice. Thus, we evaluated the reliability of muscle EI in LCo and RCPm muscles of individuals with and without FHP.

Material and Methods

Design

Fifty women (20 women with FHP and 20 women without FHP) within the age range of 30 - 45 years participated in this study. In both groups, female subjects, aged 30 - 45 years, were recruited. The craniovertebral angle (CVA) exceeded 48° in the non-FHP group, while in the FHP group, CVA was < 48°. It should be noted that in this study, the intersection of a horizontal line in the C7 spinous process as well as a line that connects the tragus midpoint to the overlying skin of C7 spinous process was defined as CVA [10].

Many factors can influence the EI and even CSA of the muscles, like patients’ age and level of activity. Hence, all participants completed Tegner physical activity questionnaire and both group were matched in age, Body mass index (BMI) and physical activity.

The exclusion criteria were radicular pain in upper extremities or previous neck/shoulder surgery or pain in the past six months, severe thoracic kyphosis, history of cervical surgery and trauma or neuromuscular/musculoskeletal disorders, current or previous neck/shoulder damage, temporomandibular joint disorders, and cardiorespiratory conditions.

Instrumentation

The morphometric muscle parameters such as CSA and EI were examined using HS-2100 rehabilitative US imaging (RUSI) system (Honda; Japan), attached to a linear probe (7 cm; 7.5 MHz). It is a simple, cost-effective, and accessible tool compared to MRI to assess EI, as a biomarker of muscle function, in neck musculoskeletal problems [11]. US is appropriate for detecting and visualizing soft tissues, including nerves, subcutaneous fat, vessels, muscles, fasciae, and tendons in longitudinal/ transverse scan via high resolution [12]. Gain and dynamic range were adjustable according desired measurements. EI was considered as the average pixel intensity of the muscle and its value was calculated through the gray scale analysis by the standard histogram levels from 0 to 255, in which the hypo echo (i.e. black color) is 0, whereas the hyper echo (i.e. white color) is equal to 255 [13]. In this way, the Digital Imaging and Communications in Medicine (DICOM) images were stored for analyzing by an offline computer through the Image J software (– National Institutes Health; U.S.). Echotexture values were achieved using the histogram and EI was considered as reference for the average value of the grayscale pixel distribution, and echo variation (EV) was calculated via the relationship between standard deviation (SD) and the average pixel distribution by Eq. (1):

$$EV = \frac{\sigma}{\mu} \times 100$$
σ shows the image SD, and μ indicates the average pixel intensity in the ROI of the three obtained images [14].

Protocol
The Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran approved the study protocol. Informed consent was received from all patients and informed about the aims and objectives before enrolling in the study. The experiments were performed by a Ph.D. candidate in physical therapy trained by a musculoskeletal radiologist for a one year. Within-day intra-rater reliability of US measurements of EI was assessed in one session within 30-minute intervals for all samples. The probe was pulled away from the target site after each scan and placed on the same area again for the following scan. All scans were acquired bilaterally with a transverse view. In all statistical analyses, we calculated the average of three US measurements. To determine EI in a US image, the average pixel intensity was evaluated in the target muscle based on gray levels in the region of interest. The CSAs of RCPm and LCo muscles were traced in this study. An HS-2100 rehabilitative ultrasound imaging (RUSI) system (Honda, Japan), attached to a linear probe (7 cm; 7.5 MHz), was used in B-mode to acquire images of the cervical muscles. We fixed the gain at 48% of the range, dynamic range at 93 dB, and time compensation was adjusted to a neutral position for all depths of the images. We also adjusted the depth setting for the muscles for visualizing their superior and inferior margins.

To visualize the cervical muscles, the examinees sat comfortably during imaging. Afterwards, they were asked to keep their hips and knees at 90° flexion, while keeping the neck and head neutral. They kept their upper arms in a resting position, while held their hands on the thighs. The probe was placed transversely on the C2 spinous process, images were acquired from the RCPm muscles using the RUSI system. Following that, the examiner changed the probe position vertically and laterally to identify the C1 and C2 laminae, respectively. Furthermore, the probe was moved upward or downward for identifying the RCPm muscle.

For visualizing the LCo muscle, the examiner placed the probe transversely at C6 vertebral level; this level was selected considering the absence of overlap between the longus capitis muscle and LCo muscle [15].

Data Analysis
Statistical analyses were performed by SPSS 24. Intraclass correlation coefficient (ICC) was measured to determine the intra-session reliability of the average EI of three measurements (two-way, mixed consistency ICC). Overall, ICC of smaller than 0.4, ICC = 0.4 - 0.75, and ICC of greater than 0.75 showed poor, fair to good, and excellent reliability, respectively [16]. In addition, measurement accuracy was indicated based on SEM values using Eq. (2):

\[
2) \quad \text{SEM} = \sqrt{1 - \text{ICC}}
\]

Moreover, minimum detectable change (MDC) describes the least significant change in an individual’s score; it is indicative of an actual change above the measurement error. MDC was calculated based on the following Eq. (3) [17]:

\[
3) \quad \text{MDC} = 1.96 \times \sqrt{2} \times \text{SEM}
\]

Discussion
We can gather information about the composition of muscles by measuring EI [18]. Table 1 presents the participants’ demographic information, CVAs and activity level.

To measure EI, the grayscale analysis of image pixels is necessary. For this purpose, all pixels in a considered muscle area are classified according to a standard histogram function, which can be highly found in several image editing software programs. Previous studies demonstrated that the quantitative grayscale assessment was superior to the visual analysis of US images alone [19]. Nevertheless, it is indispensable to establish reference values for
the quantitative grayscale assessment, which is a slightly more time-consuming process. The moderate reliability of EI was observed in both groups. Table 2 presents the ICC, SEM, and MDC values for the between-day and within-day reliability in both groups. A moderate ICC was reported in the two groups for the within-day reliability.

According to our literature review, the current study is the first one, investigating the muscle EI reliability in FHP and control subjects. Moderate reliability of EI may be attributed to differences in probe placement and possible image variations in terms of background brightness, which can influence the absorption of echo signals [20]. To overcome this, we fixed the gain at 48% of the range, kept dynamic range at 93 dB, and time compensation was adjusted at the neutral position for all depths of images. We also adjusted the depth setting for the muscles for visualizing their superior and inferior margins. Moreover, observer-dependent factors (e.g., US probe adjustment) significantly affect echogenicity measurements. Additionally, other factors like hydration balance may be influential [21].

Considering the complexity of cervical muscles and the variable anatomy of individuals, variations in consistent anatomical landmarks posed a challenge in the present study. Assessments were performed only at one spinal level (consistent for every muscle). Furthermore, it was impossible to visualize the whole muscle as the images were two-dimensional. Moreover, reproduction of muscle images in the same plane was impossible. It was also challenging to accurately document the anatomical landmarks and tissue boundaries due to the blurred transition between different muscle layers or difficulty in distinguishing the thickened fascia from aponeuroses.

In this study, we could not find these differences/alterations due to the reasons as follows: (1) there can be changes in muscle elasticity rather than muscle quality; thus, more investigations are needed to assess this possibility by sono-elastography measurement of the muscles for exploring elasticity alterations between the groups. (2) Average echo intensity might not be vulnerable for detecting the alterations in the muscle fat.

The use of ultrasonography is growing in assessment of patients with musculoskeletal disorders because of its noninvasive and

| Groups | Age (year) | BMI (kg/m²) | CVA (degree) | Activity Level |
|--------|------------|-------------|--------------|----------------|
| Control | 37.15 ± 4.8 | 23.8 ± 3.77 | 53.34 ± 1.88 | 3±0.12 |
| FHP     | 36.65 ± 4.11 | 23.46 ± 3.12 | 43.18 ± 1.55 | 3±0.14 |

FHP: Forward head posture, BMI: Body mass index, CVA: Craniovertebral angles

| Groups | EI of RCPm muscle | EI of LCo muscle |
|--------|--------------------|------------------|
|        | ICC | SEM | MDC   | ICC | SEM | MDC   |
| Control | 0.49 | 13.44 | 37.22 | 0.51 | 11.13 | 30.83 |
| FHP     | 0.48 | 11.24 | 31.13 | 0.50 | 12.56 | 34.79 |

EI: echo intensity, RCPm: rectus capitis posterior minor, LCo: Longus colli, ICC: intraclass correlation coefficient; SEM: standard error of measurement, MDC: minimum detectable change, FHP: forward head posture.
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safety characteristics. However, its reliability in detecting the changes in musculoskeletal variables, which could lead us to a proper diagnosis of the disorders and the best plan and effective rehabilitation program, is under question and is considered with suspicion. This study could provide the evidence on the credit of this method in assessment of these group of patients. Besides, EI as a reliable measure will be useful for future studies.

The first limitation of this study is that we only assessed within-day intra-rater reliability as changes in muscle relaxation, posture, and hydration in different sessions can influence between-day reliability. Therefore, more studies with a larger sample size, including both sexes are necessary to evaluate the morphometric characteristics of muscles in longitudinal scans for better visualization.

Conclusion
This study showed the acceptable reliability of EI measurement in the assessment of LCo and RCPm muscles in individuals with and without FHP.

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Conflict of Interest
None

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