Applicability of the masseter muscle as a nutritional biomarker

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Abstract
Nutritional assessment is feasible with computed tomography anthropometry. The abdominal muscle at the L3 vertebra is a well-known nutritional biomarker for predicting the prognosis of various diseases, especially sarcopenia. However, studies on nutritional assessment of the brain using computed tomography are still scarce. This study aimed to investigate the applicability of the masseter muscle as a nutritional biomarker.

Patients who underwent simultaneous brain and abdominopelvic computed tomography in the emergency department was retrospectively analyzed. We assessed their masseter muscle 2-cm below the zygomatic arch and abdominal muscle at L3 via computed tomography anthropometry. The skeletal muscle index, prognostic nutritional index, and other nutritional biomarkers were assessed for sarcopenia using the receiver operating characteristic curve analysis.

A total of 314 patients (240 men and 72 women) were analyzed (mean age, 50.24 years; mean areas of the masseter and abdominal muscles, 1039.6 and 13478.3 mm², respectively), Masseter muscle areas significantly differed in sarcopenic, obese, and geriatric patients (P < .001). The areas under the curve of the masseter muscle in sarcopenic, geriatric, and obese patients were 0.663, 0.686, and 0.602, respectively. Multivariable linear regression analysis showed a correlation with the abdominal muscle area, weight, and age.

The masseter muscle, analyzed via computed tomography anthropometry, showed a statistically significant association with systemic nutritional biomarkers, and its use as a nutritional biomarker would be feasible.

Abbreviations: AUC = area under curve, BMI = body mass index, CT = computed tomography, ICD = international classification of disease, MMA = masseter muscle area, PNI = prognostic nutritional index, ROC = receiver operating characteristic, SMI = skeletal muscle index, WBC = white blood cell count.

Keywords: anthropometry, computed tomography, masseter muscle, nutrition assessment, sarcopenia

1. Introduction
The nutritional status of patients affects the clinical outcome, and proper nutritional support is crucial.[1-3] Therefore, appropriate nutritional assessment is essential for patient management. Studies have focused on nutritional assessment of patients.

Many biomarkers are used to analyze nutritional status. Basic biomarkers based on body anthropometry are height and weight.[4,5] Body mass index (BMI) is a traditional nutritional biomarker. It is used to define several properties, from obesity to underweight. However, few studies do not present a statistical association between BMI and clinical outcomes.[6-7] Additionally, there are several assessment tools that make use of patient history, physical examination, and anthropometric measurements (BMI), for example, the Subjective Global Assessment and Nutrition Risk Screening 2002.[8,9] Sometimes, history-taking or anthropometric assessment of the body might be difficult or inaccurate because of the patient’s condition, such as decreased mental status and shock. Additionally, some laboratory biomarkers reflect the nutritional status, such as albumin and hemoglobin levels.[10,11] However, many studies have shown that albumin is associated with systemic inflammation, and it cannot be considered a reliable nutritional biomarker.[12,13] Besides albumin, the prognostic nutritional index (PNI), which is calculated from albumin levels and total lymphocyte count, has also been studied.[14] PNI has demonstrated the ability to predict the prognosis of several diseases, from malignancy to cardiovascular disease.[15-17] Furthermore, skeletal muscle is a good biomarker of nutritional status, and its importance has been emphasized. Sarcopenia is characterized by loss of skeletal muscle mass and function and is associated with prognosis in patients; it is considered an independent condition by the International Classification of Disease, 10th Clinical Modification (ICD-10-CM).[18-21]
Several methods can be used to check muscle mass and diagnose sarcopenia. Anthropometry with computed tomography (CT) is one of the recommended methods. According to a meta-analysis, many studies have assessed abdominal muscle mass at the 3rd lumbar vertebra (L3) and diagnosed sarcopenia. Sarcopenia is associated with several disease parameters from incidence to prognosis, including morbidity and mortality. This association is confined not only to systemic disease but also to head and neck cancer and brain hemorrhage. Despite this, reports on the efforts to find a nutritional biomarker from head and neck CT are scarce, and most studies of CT anthropometry have analyzed only abdominal CT.

Authors focused on the masseter muscle, because the masseter muscle is the muscle for mastication, and mastication is one of the main processes of oral nutrition. We hypothesized that well-nourished individuals would eat well and have large masseter muscles. Therefore, we aimed to compare the area of the masseter muscle with well-known nutritional biomarkers to prove its applicability as a nutritional biomarker.

2. Methods

2.1. Data collection

This retrospective study was approved by the institutional review board (UC18RESI0065), and the requirement for informed consent was waived.

The retrospective review included patients who underwent trauma and visited the emergency room between January 2017 and December 2017. All patients aged >17 years who underwent head CT and abdominal CT simultaneously were included in our analysis. Patients were excluded if CT images were not available to measure the cross-sectional area of muscles owing to trauma or artifacts. Additionally, patients with missing data were excluded.

Medical records were reviewed to collect the basic information of patients, including sex, age, weight, height, and BMI, and laboratory data, including hemoglobin and serum albumin levels and white blood cell count (WBC). WBC differentiation was also performed to determine segmented neutrophil and lymphocyte counts.

Patients older than 64 years were classified as the geriatrics group. Additionally, patients with BMI >25.0 were classified as the obesity group according to Asian recommendations. PNI was calculated via the following equation: [(10 × serum albumin [g/dL]) + (0.05 × total lymphocyte count)], and patients were classified in the nutritional risk group based on the PNI. Anemia was defined as a hemoglobin concentration <12 g/dL.

2.2. Evaluation of body composition

Measurement of the skeletal muscle area using the head and abdominal CT was performed using a 3D slicer (version 4.10.0, www.slicer.org). The cross-sectional area of the masseter muscle (MMA) was measured on head CT bilaterally 2 cm below the zygomatic arch (Fig. 1). The cross-sectional area of the abdominal skeletal muscles (including psoas, paraspinal, transversus abdominis, rectus abdominis, internal oblique muscles, and external oblique muscles) was measured on the abdominal CT bilaterally at the level of the third lumbar vertebral body; this was a common site of measurement in previous studies.

Sarcopenia was defined using sex-specific cut-off points for skeletal muscle index (SMI). SMI was calculated as the total cross-sectional area of the abdominal skeletal muscles (cm²) divided by height squared (m²). The cut-off points of SMI were 44.6 cm²/m² for men and 34.1 cm²/m² for women.

2.3. Statistical analysis

All continuous variables are expressed as mean ± standard deviation. Categorical variables and MMA were compared using the independent t test. Additionally, receiver operating curve analysis was performed to the MMA and abdominal muscle area. The association between continuous variables and MMA was determined using the Spearman’s correlation analysis. Additionally, multivariable linear regression analysis with a forward stepwise procedure was performed to identify factors affecting MMA. P values <.05 and area under curve >.6 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, V 25.0 (IBM Corp., Armonk, NY).

3. Results

A total of 533 patients visited the hospital, and 314 were included in the analysis after reviewing their CT images. Their mean age was 50.2 years, and there were 240 male patients (76.4%). The mean MMA was 1039.60, and the mean SMI was 47.14. There were 192 patients with sarcopenia (61.1%), 87 patients with obesity (27.7%), 30 patients with anemia (9.6%), and 7 patients with nutritional risk according to PNI (2.3%). The cut-off value of PNI for the nutritional risk group was 36.083. Other baseline characteristics are summarized in Table 1.

Figure 1. Muscle cross-sectional area measurement. The masseter muscle cross-sectional area (MMA) is estimated by tracing it on the head CT scan sectioned 2 cm below the zygomatic arch. The abdominal skeletal muscle is measured on abdominal CT scan at the level of the third lumbar vertebral body.
MMA showed a statistical difference on qualitative analysis. Sarcopenic \( \left( P < .001 \right) \), anemic \( \left( P = .001 \right) \), and geriatric \( \left( P < .001 \right) \) patients had less MMA (Table 2). In contrast, obese patients showed a significantly greater MMA \( \left( P < .001 \right) \). However, patients with a low PNI showed no statistically significant difference. The results of the ROC analysis are summarized in Table 3 and Figure 2. MMA showed an area under curve (AUC) >0.6 in sarcopenic \( \left( 0.685 \right) \), geriatric \( \left( 0.686 \right) \), anemic \( \left( 0.654 \right) \), and obese \( \left( 0.658 \right) \) patients and in those with a nutritional risk \( \left( 0.602 \right) \) and proved its validity. The abdominal muscle area showed an AUC higher than 0.6 for every parameter.

### Table 1
Baseline characteristics of analyzed patients.

| Parameters       | Sex          | Male     | Female     | Age (yr) | Height (cm) | Weight (kg) | BMI       | Obesity |
|------------------|--------------|----------|------------|----------|-------------|-------------|-----------|---------|
|                  | Male         | 240 (76.4) | 74 (23.6)  | 50.24 ± 17.81 | 168.22 ± 8.04 | 67.01 ± 11.99 | 23.61 ± 3.42 | 27.7    |
|                  | Female       | 1156.16 ± 236.45 | 879.74 ± 261.05 | 1056.49 ± 274.91 | 941.12 ± 212.65 | 87.07 ± 15.79 | 30.57 ± 14.50 | 36.4    |

### Table 2
Summary of masseter muscle analysis in qualitative parameters.

| Parameters       | Masseter muscle area          | P  |
|------------------|------------------------------|----|
| Sarcopenia       | No                           | 1093.37 ± 27.03 | .001 |
|                  | Yes                          | 913.77 ± 243.65 | .001 |
| Geriatrics       | No                           | 1076.63 ± 276.81 | .001 |
|                  | Yes                          | 889.11 ± 236.43 | .001 |
| Nutritional risk | No                           | 1038.86 ± 279.10 | .358 |
|                  | Yes                          | 941.12 ± 212.65 | .001 |
| Anemia           | No                           | 1056.49 ± 274.91 | .001 |
|                  | Yes                          | 879.74 ± 261.05 | .001 |
| Obesity          | No                           | 994.93 ± 253.54 | .001 |
|                  | Yes                          | 1156.16 ± 305.93 | .001 |

### Table 3
Results of receiver operating characteristics analysis.

| Parameters       | Muscle area  | Area under curve | 95% confidence interval |
|------------------|--------------|------------------|-------------------------|
| Sarcopenia       | Abdomen     | 0.652            | 0.812–0.892             |
|                  | Masseter    | 0.685            | 0.621–0.740             |
| Geriatrics       | Abdomen     | 0.703            | 0.741–0.854             |
|                  | Masseter    | 0.686            | 0.604–0.751             |
| Obesity          | Abdomen     | 0.658            | 0.602–0.738             |
|                  | Masseter    | 0.654            | 0.536–0.772             |
| Nutritional risk | Abdomen     | 0.654            | 0.409–0.796             |
|                  | Masseter    | 0.654            | 0.547–0.762             |

MMA also showed statistical correlation on qualitative analysis. Table 4 shows the results of the Spearman’s analysis. MMA showed a correlation with abdominal muscle area \( \left( \rho = 0.518, P < .001 \right) \), SMI \( \left( \rho = 0.478, P < .001 \right) \), age \( \left( \rho = -0.330, P < .001 \right) \), weight \( \left( \rho = 0.498, P < .001 \right) \), BMI \( \left( \rho = 0.366, P < .001 \right) \), and hemoglobin level \( \left( \rho = 0.346, P < .001 \right) \). Additionally, multivariable linear regression analysis was performed. The results are shown in Table 5. Abdominal muscle area \( P < .001 \), weight \( P < .001 \), and age \( P = .021 \) showed statistical significance.

### 4. Discussion
In this study, we adopted CT anthropometry and retrospectively analyzed the data of 314 patients to investigate the applicability of the masseter muscle as a nutritional biomarker. We found that the MMA showed a statistical difference in sarcopenic, geriatric, and obese patients. It also showed correlations with abdominal muscle area, weight, and age on multivariable linear regression analysis.

Muscles have several functions and play an important role in nutrition and metabolism. They can make us move and have a major role in function, physical status, and quality of life. Additionally, they store energy as glycogen, which is an important fuel for exercise. The energy could be metabolized during sepsis and multiorgan failure. There are several tools for measuring muscle mass. Dual energy X-ray absorptiometry and bioelectric impedance analysis are popular tools for muscle assessment. They can estimate body composition. However, they need additional equipment, which is not popular in general medical treatment. They also require additional practice in medical management and thereby could increase medical costs. In contrast, CT is another very popular tool, and CT images obtained during usual medical processes can be used as a nutritional assessment tool without increasing medical costs. Furthermore, the European Working Group on Sarcopenia in Older People considered CT the gold standard for assessment of muscles.

The masseter muscle is the main structure for mastication. It is a powerful superficial quadrangular muscle located between the zygomatic arch and mandible. It is responsible for elevation...
and protraction of the mandible. Studies have reported that it is associated not only with chewing ability but also with other systemic parameters. Yamaguchi et al reported that it is associated with BMI, grip strength, and walking speed. Gaszynska et al reported a relationship with chewing ability, hand grip strength, and other activities of daily living. Therefore, the masseter muscle could represent activities, such as daily food ingestion as well as physical status and quality of life and act as a potential nutritional biomarker.

Many studies have analyzed the head and neck muscles. Swartz et al reported a correlation between the head and neck muscle and the abdominal muscle. They checked the cross-sectional area of the C3 and L3 vertebrae via CT anthropometry and showed the feasibility of using head and neck muscle measurements in predicting skeletal muscle mass. However, the C3 vertebra is far from the brain, and the usual brain CT might not detect the lesion. In addition, many muscles are located in the paravertebral area; however, we thought that they have less nutritional importance compared to the masseter muscle. Hu et al reported that the masseter area could predict mortality after severe traumatic brain injury. Similar to the present study, they checked the masseter muscle at 2 cm below the zygomatic arch via CT and could prove its significance. However, they checked only the masseter muscle and did not compare it with other parameters, such as abdominal muscle. In contrast, Wallace et al analyzed the masseter and psoas muscles in trauma patients and reported that the MMA and psoas muscle were correlated and could predict mortality in trauma patients. Similarly, MMA showed a positive correlation with the abdominal muscle and negative correlation with age in the present study.

Additionally, there are basic anthropometric methods available, such as BMI. It can be used with many nutritional assessment tools, such as the Subjective Global Assessment and Nutrition Risk Screening 2002. It does not increase radiation exposure or medical cost, but only requires height and weight values, which are simple and easy to measure. However, the measurement might be difficult for trauma patients or critically ill patients, because some of them are unconscious, bed-ridden, or

| Parameters             | Correlation coefficient | P    |
|------------------------|-------------------------|------|
| Abdominal muscle area  | 0.518                   | <.001|
| Age                    | -0.330                  | <.001|
| Height                 | 0.366                   | <.001|
| Weight                 | 0.498                   | <.001|
| Body mass index        | 0.366                   | <.001|
| Skeletal muscle index  | 0.478                   | <.001|
| Prognostic nutritional index | 0.096               | .95  |
| Hemoglobin             | 0.346                   | <.001|

| Parameters             | Unstandardized coefficient | Standardized coefficient | P    |
|------------------------|----------------------------|--------------------------|------|
| Abdominal muscle area  | 0.20                       | 0.261                    | <.001|
| Weight                 | 6.518                      | 0.284                    | <.001|
| Age                    | -1.975                     | -0.127                   | .021 |
under skeletal traction, external or internal fixation, extracorporeal membrane oxygenation, and continuous renal replacement therapy. Thus, it might be inaccurate to check the height and weight of these patients. However, in these patients, performing CT might be inevitable if medically required.

All enrolled patients had injury from blunt trauma. Previous studies recommended checking whole body scan with CT of patients who sustained blunt trauma but were suspected to have major trauma. [34,35] Blunt injury, such as injury sustained in a car accident or falls, can occur to any individual. Consequently, we could investigate masseter and abdomen muscular images simultaneously.

The present study has a unique value in that it analyzed the masseter muscle, while focusing on the nutritional parameters. Quantitative and qualitative analyses of the masseter muscle showed its statistical validity. This might mean that the masseter muscle, which is an external muscle, could reflect systemic nutritional status. However, the AUC of MMA was less than that of the abdominal muscle area, although it showed a reliable AUC for sarcopenic, geriatric, obese, and anemic patients. In contrast, for patients with a previous operation, disease, or trauma in the abdomen or lumbar vertebra, MMA could be used as an alternative method to check for sarcopenia.

The study has limitations. Although MMA proved its statistical association with nutritional biomarkers, its statistical significance was less than that of the abdominal muscle area. However, a study reported that the masseter muscle area had equal or better association with clinical outcome than did the psosas muscle area. [30] Therefore, further study to analyze the clinical and nutritional aspects of the masseter and abdominal muscles is required. Second, we could not show the prognosis or other clinical outcomes because we enrolled patients who visited the emergency department for various conditions, ranging from minor contusion to major trauma. Patients with minor trauma were not checked for severity-related factors, such as Glasgow Coma Scale score or subjected to arterial blood gas analysis because they were assumed to be normal. In addition, they had a short follow-up period, and some of them had no follow-up data. Therefore, analysis for prognosis was impossible in this study.

Additionally, the functional status was not analyzed in this study. A previous study recommended checking both muscle quality and quantity. [14] However, it was difficult to analyze the functional status because various trauma patients were included in the study, for example, unconscious patients owing to brain injury and immovable patients owing to spinal cord injury or extremity injuries. Further study including functional status would be desirable.

In conclusion, MMA demonstrated potential as a nutritional biomarker by showing a statistical correlation with previously known nutritional biomarkers. Further studies with additional parameters or various conditions would yield important information on the masseter muscle and nutrition and may provide more evidence of its applicability as a biomarker.

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