



## Ultrasonographic Evaluation of the Masseter Muscle in Skeletal Malocclusions (Class I, II, and III)

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### Abstract

**Background:** There is limited research on the sonographic view of people with skeletal malocclusions. Therefore, this study aimed to evaluate the sonographic findings of the masseter muscle in patients with skeletal malocclusions.

**Methods:** In this descriptive study, 48 patients aged 15-20 years with skeletal class I, II, and III malocclusions (n = 16) who were referred to Mashhad Dental School for treatment were selected. The masseter muscle was evaluated by ultrasound, including transverse and longitudinal scans on both sides of the face in resting and contraction states. The age, gender, muscle thickness, muscle pattern (Malocclusion were classified based on A-point, nasion, B-point (ANB):  $0 < ANB < 4$  as class I,  $ANB > 4$  as class II,  $ANB < 0$  as class III), side of chewing, and body mass index (BMI) parameters were measured for each patient. Paired t-tests compared masseter muscle states; ANOVA assessed differences among malocclusion groups.

**Results:** The most commonly observed pattern in the masseter muscle of patients with class III skeletal malocclusions was type II, and in people with class II malocclusions was type I. There was a positive and significant correlation between the thickness of masseter muscle and BMI in each group separately ( $P < 0.001$ ). However, the masseter muscle pattern did not show a significant correlation with BMI, gender, and age. A significant difference was observed between the thickness of the masseter muscle in the resting and contracted states in each group ( $P < 0.001$ ).

**Conclusion:** This study showed that skeletal malocclusions can affect the pattern and internal structure of the masseter muscle in the anterior-posterior dimension of the face. Ultrasound can be a suitable diagnostic tool for these patients.

**Keywords:** Ultrasound, Skeletal Malocclusions, Masseter Muscle, Thickness, Muscle Pattern

**Conflicts of Interest:** None declared

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### Introduction

The masseter muscle is one of the strong muscles and primary elevators of the mandible, morphologically being the largest structure with significant chewing forces (1). Abnormally generated forces by masticatory muscles, including the masseter muscle, cause damaging effects on

teeth, periodontal tissues, and temporomandibular joints (TMJ) (2). Masseter muscle thickness is deemed a valuable diagnostic criterion for its health. Recent years have witnessed diverse techniques employed to examine muscle functions and dimensions, such as electromyography

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#### ↑What is “already known” in this topic:

Ultrasound imaging is a safe, inexpensive, and non-invasive technique, making it highly beneficial in clinical examinations with its dynamic images. Research gaps exist in sonographic evaluations of individuals with skeletal malocclusions, particularly regarding the masseter muscle. This study aimed to assess the sonographic findings of the masseter muscle in these patients.

#### →What this article adds:

Ultrasound images showed that the pattern and internal structure of the masseter muscle altered in skeletal malocclusion people. Then, it can be a suitable diagnostic tool for patients with Skeletal Malocclusions (Class I, II, and III). So, clear results of pattern and muscle thickness can be effective in the stability and durability of orthodontic treatment.

(EMG; surface and depth), computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (3). Ultrasound imaging is an accurate, reliable, safe, inexpensive, fast, and non-invasive technique, making it highly beneficial in clinical examinations due to its ability to provide dynamic images of the region under investigation (4-6). The masseter muscle appears as a black area (hypoechoic area) containing internal echogenic (hyperechoic area) bands—related to tendons or fascia within the muscle and often referred to as septa—in ultrasound images (7). Muscle fibers appear as hypoechoic regions, while echogenic lines show up as white areas. Measurements of the masseter muscle thickness occur from the external fascial layer of the muscle to the buccal surface of the ramus of the mandible (7). Additionally, research indicates that masseter muscle thickness may influence jaw growth (8). Due to limited studies regarding sonographic views and pattern alterations of the masseter muscle in patients with skeletal class I, II, and III malocclusions, and because ultrasound imaging provides clear visualization of muscle morphology, this study aimed to assess the sonographic findings of the masseter muscle in these patient groups. The results of this study may help guide treatment strategies before and during orthodontic therapy.

## Methods

### Study population

This descriptive study was conducted on 48 patients aged 15-20 years who had skeletal class I, II, and III malocclusions. According to the study by Park et al. (9), each group (Class I, II, and III) consisted of 16 people. All patients who had malocclusion classes I, II, and III were included in the study from the beginning of 2021 to the end of 2022 using a census sampling method with simple selection based on the inclusion criteria. The study was carried out at the Department of Oral and Maxillofacial Radiology of Mashhad Faculty of Dentistry. Also, the Mashhad University of Medical Sciences Ethical Committee approved the study (No. IRMUMS DENTISTRY.REC.1398.122). Written consent was obtained from the patients before entering the study.

The inclusion criteria were considered into account when selecting the population: no history of previous facial muscle disease (such as Bell's palsy, tumors, and muscle hypertrophy), no history of head and neck radiotherapy, no history of Botox injections in the facial muscles, no history of functional orthodontic or surgical treatments, no history of suffering from inflammatory diseases (Parafunction disorders or bruxism) or TMJ disorders. having an age 15-20 years, having lateral cephalometric radiography at starting the study, having a vertical dimension in the normal range ( $25 < \text{FMA} < 30$ ), having a complete dental system (regardless of wisdom teeth), having a confirmed skeletal malocclusion (class I, II and III) based on A point, nasion, B point (ANB) in the lateral cephalogram. Skeletal malocclusion (Class I, II, and III) was categorized into three groups according to ANB in the lateral cephalogram: a)  $0 < \text{ANB} < 4$  as class I malocclusion. b)  $\text{ANB} > 4$  as class II malocclusion. c)  $\text{ANB} < 0$  as class III malocclusion.

Exclusion criteria included the patient's unwillingness to continue cooperation during the study and the use of drugs that affect the function and structure of the facial muscles. Also, patients with restrictions in opening their mouth were excluded.

### Sample size calculation

This study aims to compare the pattern of the masseter muscle of patients with skeletal malocclusions in three classes I, II, and III. The study of Park et al. (9) investigated the relationship between the masseter muscle thickness and the facial profile. Based on the results of the Park et al. study (9) and considering alpha (0.05) and beta (0.2), the sample size of the present study is equal to:

$$n = \frac{(Z_{1-\alpha/2} - Z_{1-\beta})^2 (S_1^2 + S_2^2)}{(M_1 - M_2)^2} = \frac{(1.96 - 0.84)^2 (1.5^2 + 1.4^2)}{(11.4 - 10.77)^2} = 13$$

Based on this formula, the sample size in each group (I, II, and III) was 13 people, and considering a drop-out rate of 20%, the total number needed was 16 people per class of malocclusions.

### Study protocol

At first, the orthodontist manually traced the malocclusion class. Also, drug use history, orthodontics, surgery in the masseter muscle area, and having special habits such as biting foreign objects were checked. A clinical examination of the TMJ area was then performed, and the necessary conditions for study entry were checked. Patients were also asked about their dominant side of chewing.

Then, the patient's demographic details such as gender, age, weight, height, and body mass index (BMI), were recorded.

Finally, all patients were examined using an ultrasound imaging system (E-CUBE7, South Korea) with a linear probe that had a multi-frequency range of 2-12MHz. The examination was conducted in two states: rest and maximum voluntary contraction of the masseter muscle. A total of 384 ultrasound measurements of the masseter muscle were performed on each side, including transverse and longitudinal scans in both states (8 scans per patient). The hand probe was easily adjustable to obtain the best view of the masseter muscle. Sonographic information, including the thickness of masseter muscles (Left and right) in both rest and contraction states and the type of internal pattern based on the visibility, width, and echogenicity of the bands (10), were recorded in a checklist for each patient. All ultrasound images were conducted by an experienced oral and maxillofacial radiologist with over 11 years in the ultrasound field. Procedures took place with patients positioned sideways, slightly rotating their heads and necks to facilitate probe access to the masseter muscles. Then, the patient was asked to clench their teeth to make the muscle contract, and the muscle thickness was recorded in the maximum contraction state (10). The internal pattern of the masseter muscle in ultrasound images was divided into 3 groups based on the visibility, width, and echogenicity of the echogenic bands. The ultrasound images of each patient, based on real-time observation, were placed in one of these three groups according to a study by Imanimoghaddam et al. (10). These groups in-

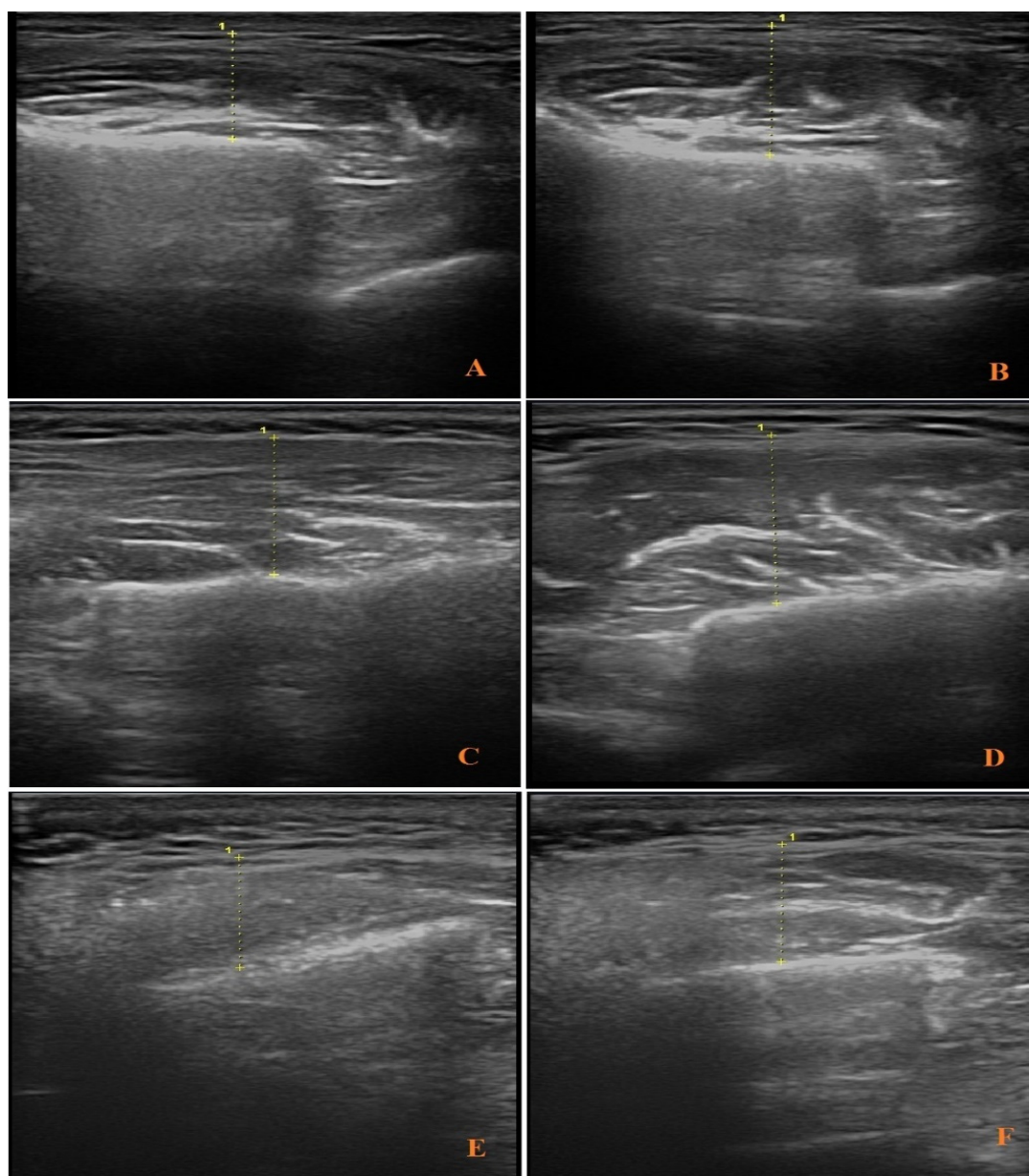
cluded the following:

Group I: In this type, the masseter muscle pattern is normal, with thin and thick hyperechoic bands clearly visible (Figure 1 A-B). Group II: In this type, the number of thin and thick bands is reduced, and the remaining bands have increased thickness and show a decrease in echogenicity (Figure 1 C-D). Group III: This type itself is divided into two subgroups, including type IIIa, in which a sharp reduction in the number of bands is observed, and type IIIb, in which the bands have disappeared (Figure 1 E-F). Group III represents the abnormal state of the muscle. This classification system provides a detailed framework for assessing the internal pattern of the masseter muscle using ultrasound imaging, allowing for a compre-

hensive understanding of its structural changes.

### Statistical analysis

Data analysis was conducted using SPSS version 22 software. The results were reported using graphs and tables. Quantitative data were reported as mean  $\pm$  standard deviation (SD). Also, qualitative data was reported as frequency (percentage). The one-sample Kolmogorov-Smirnov test was utilized to evaluate the normality of quantitative variables, demonstrating a normal distribution of the thickness of the right and left masseter muscles in both the rest and contraction states across all three malocclusion groups. Paired sample t-tests were conducted to compare the masseter muscle in the rest and contraction



**Figure 1.** Ultrasound view of the masseter muscle. A-B: Normal view (group I internal pattern) in a 16-year-old boy with CL II malocclusion: thin and thick hyperechoic bands are well visible. A: Muscle sonogram at rest: muscle thickness: 1.06 cm. B: Muscle sonogram in contraction state: muscle thickness: 1.33 cm. C-D: Sonographic view (group II internal pattern) in a 17-year-old female with CL III malocclusion: reduction of fine bands and reduction of residual echo bands. C: Muscle sonogram at rest: muscle thickness: 1.42 cm. D: Muscle sonogram in contraction state: muscle thickness: 1.78 cm. E-F: Sonographic view of group III (abnormal state) in an 18-year-old female with CL I malocclusion: severe reduction of muscle bands. E: Muscle sonogram at rest: muscle thickness: 1.15 cm. F: Muscle sonogram in contraction state: muscle thickness: 1.22 cm.

states within each group. Pearson correlation coefficient was utilized to measure the linear relationship between two quantitative variables. Additionally, an Analysis of Variance (ANOVA) test was conducted to identify differences among the malocclusion groups (I, II, and III). A significance level of  $P < 0.05$  was established.

## Results

In this study, the paired samples t-test revealed statistically significant differences in masseter muscle thickness between resting and contracting states within each group (as shown in Tables 1–3;  $P < 0.001$ ).

Based on the data from Table 4 and Figure 2, it was observed that there is a substantial connection between masseter muscle thickness and patient BMI in classes I and III, particularly in most scenarios, as well as in class II when considering the right transverse, right longitudinal, and left transverse positions (at rest). Notably, this correlation appears stronger in classes I and III compared to class II malocclusions, as depicted in Figure 2 A-C.

## Chewing side

According to Table 5, which displays the masseter muscle thickness based on chewing sides, there were statistically significant differences in muscle thickness for both the right transverse and right longitudinal (at rest) as well as the right transverse during contraction ( $P < 0.05$ ). In contrast, no significant differences were observed in any of the other conditions ( $P > 0.05$ ).

## Age and gender

The statistical results demonstrated that the masseter muscle thickness in various scenarios did not exhibit any substantial relationship with the sex or age of patients ( $P > 0.05$ ).

## Right and left masseter muscle pattern in three classes of malocclusion in ultrasound images

In this study, we examined the patterns of the right and left masseter muscles in ultrasound images of three classes of malocclusion. In the transverse plane, we found that in the malocclusion I group, the highest frequency was related to pattern type I (56.3%). In the malocclusion II group,

Table 1. Comparison of the left and right masseter muscle thickness in the rest and contraction states in the malocclusion I group

Group	Difference (mm)		The result of the paired t-test	
	Mean	Standard deviation	P-value	t
Right transverse (rest and contraction)	-0.247	0.150	<0.001	-6.569
Right longitudinal (rest and contraction)	-0.213	0.137	<0.001	-6.216
Left transverse (rest and contraction)	-0.267	0.123	<0.001	-8.675
Left longitudinal (rest and contraction)	-0.217	0.116	<0.001	-7.462

Table 2. Comparison of the left and right masseter muscle thickness in the rest and contraction states in the malocclusion II group

Group	Difference (mm)		The result of the paired t-test	
	Mean	Standard deviation	P-value	t
Right transverse (rest and contraction)	-0.232	0.107	<0.001	-8.655
Right longitudinal (rest and contraction)	-0.215	0.122	<0.001	-7.050
Left transverse (rest and contraction)	-0.203	0.109	<0.001	-7.464
Left longitudinal (rest and contraction)	-0.171	0.097	<0.001	-7.007

Table 3. Comparison of the left and right masseter muscle thickness in the rest and contraction states in the malocclusion III group

Group	Difference (mm)		The result of the paired t-test	
	Mean	Standard deviation	P-value	t
Right transverse (rest and contraction)	-0.269	0.114	<0.001	-9.410
Right longitudinal (rest and contraction)	-0.234	0.136	<0.001	-6.879
Left transverse (rest and contraction)	-0.190	0.077	<0.001	-9.889
Left longitudinal (rest and contraction)	-0.274	0.184	<0.001	-5.936

Table 4. Pearson correlation coefficient between BMI and muscle thickness

Muscle condition	Total	CL I	CL II	CL III
Right transverse (rest)	$r=0.552$ $P<0.001$	$r=0.632$ $P<0.001$	$r=0.548$ $P=0.028$	$r=0.678$ $P<0.001$
Right transverse (contraction)	$r=0.543$ $P<0.001$	$r=0.772$ $P<0.001$	$r=0.458$ $P=0.074$	$r=0.653$ $P<0.001$
Right longitudinal (rest)	$r=0.495$ $P<0.001$	$r=0.572$ $P<0.001$	$r=0.501$ $P=0.048$	$r=0.618$ $P=0.011$
Right longitudinal (contraction)	$r=0.502$ $P<0.001$	$r=0.657$ $P<0.001$	$r=0.443$ $P=0.086$	$r=0.635$ $P<0.001$
Left transverse (rest)	$r=0.582$ $P<0.001$	$r=0.670$ $P<0.001$	$r=0.583$ $P=0.018$	$r=0.783$ $P<0.001$
Left transverse (contraction)	$r=0.503$ $P<0.001$	$r=0.664$ $P<0.001$	$r=0.463$ $P=0.071$	$r=0.793$ $P<0.001$
Left longitudinal (rest)	$r=0.420$ $P<0.001$	$r=0.495$ $P=0.051$	$r=0.350$ $P=0.184$	$r=0.770$ $P<0.001$
Left longitudinal (contraction)	$r=0.535$ $P<0.001$	$r=0.584$ $P=0.018$	$r=0.495$ $P=0.051$	$r=0.697$ $P<0.001$

\*Pearson correlation coefficient



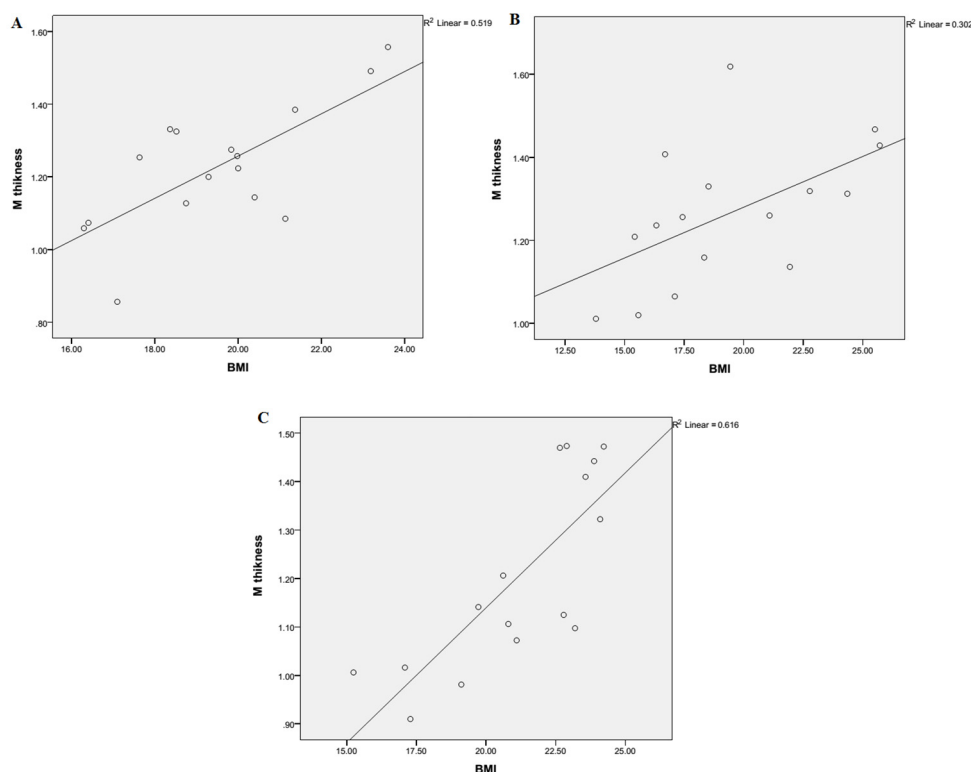


Figure 2. Scatter plot of BMI and the average masseter muscle thickness in malocclusion class I (A), II (B) and III (C)

Table 5. The average masseter muscle thickness varies across different situations based on the chewing side within each of the three malocclusion groups

Chewing side	Right side	Left side	Both sides	P-value*
Muscle condition (mm)	n = 2 Mean ± SD	n = 9 Mean ± SD	n = 37 Mean ± SD	
Right transverse (rest)	1.48±0.09	0.91±0.21	1.09±0.19	0.001
Right transverse (contraction)	1.73±0.08	1.16±0.17	1.34±0.21	0.002
Right longitudinal (rest)	1.51±0.01	1.04±0.26	1.17±0.20	0.020
Right longitudinal (contraction)	1.64±0.10	1.29±0.22	1.39±0.20	0.081
Left transverse (rest)	1.14±1.11	1.06±0.17	1.09±0.18	0.825
Left transverse (contraction)	1.32±0.11	1.31±0.20	1.30±0.22	0.986
Left longitudinal (rest)	1.22±0.28	1.16±0.14	1.15±0.19	0.904
Left longitudinal (contraction)	1.63±0.23	1.36±0.19	1.37±0.17	0.120

\* ANOVA

the highest frequency was also related to pattern type I (81.3%), while in the malocclusion III group, it was related to pattern type II (68.8%) (Figure 3 A).

When examining the right masseter muscle pattern in the longitudinal plane, we found that in the malocclusion I group, the highest frequency was related to pattern type I (56.3%). In the malocclusion II group, the highest frequency was related to pattern type II (50.0%), while in the malocclusion III group, it was related to pattern type II (81.3%) (Figure 3 B).

When examining the left masseter muscle pattern in the transverse plane, within the malocclusion I group, the highest frequency is associated with pattern type II (56.3%), while in the malocclusion II group, it is linked to pattern type I (68.8%); for the III malocclusion group, the highest frequency corresponds to pattern type II (62.5%) (Figure 4 A)

In the examination of the left masseter muscle pattern in the longitudinal plane, among the malocclusion I group, the maximum occurrence relates to pattern type I (50.0%), as does the malocclusion II group (50.0%); however, in the III malocclusion group, the highest frequency pertains to pattern II (75.0%) (Figure 4 B).

#### Relationship between the masseter muscle pattern with age, gender, and BMI of patients with class I, II, III malocclusion

Our findings revealed that as average age increased across all three malocclusion classes, the frequency of type II patterns surpassed type I but remained lower than type III; however, these differences were not statistically significant. In addition, our study results showed that there was no substantial relationship between the masseter muscle pattern and either patient sex or BMI under various

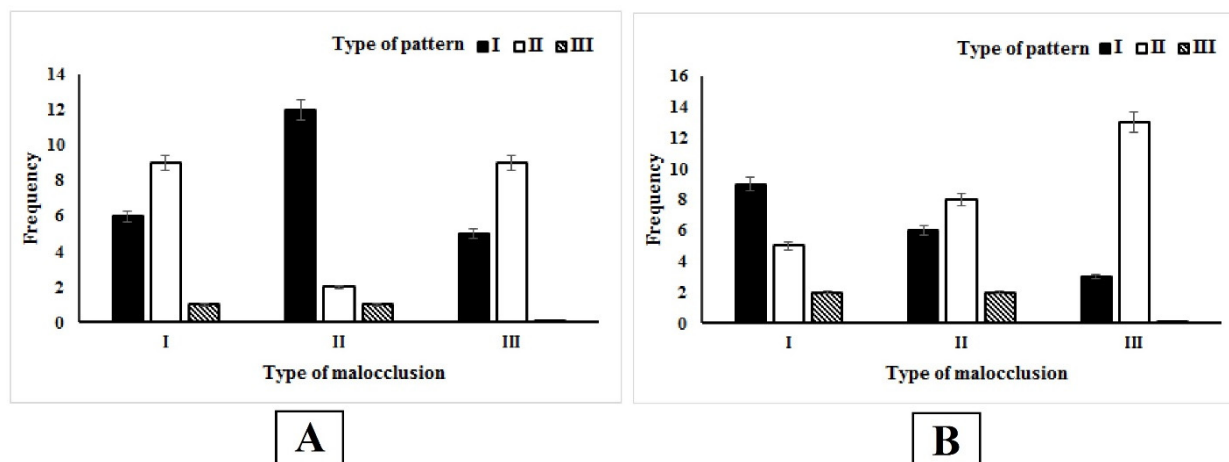


Figure 3. A) The frequency of pattern types in the right masseter muscle for each of the three groups of malocclusion in the transverse plane. B) The frequency of pattern types in the right masseter muscle for each of the three groups of malocclusion in the longitudinal plane.

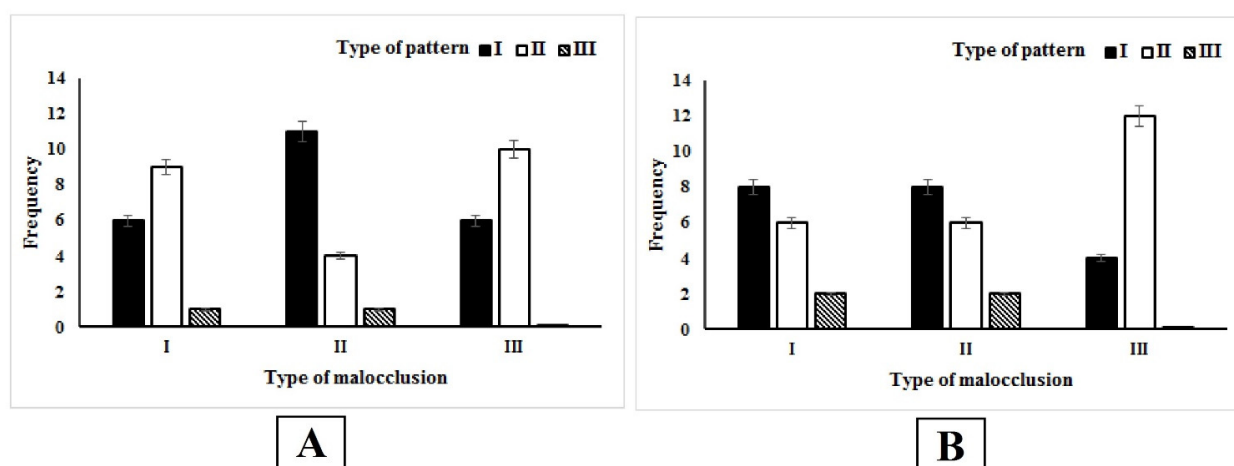


Figure 4. A) The frequency of pattern types in the left masseter muscle for each of the three groups of malocclusion in the transverse plane. B) The frequency of pattern types in the left masseter muscle for each of the three groups of malocclusion in the longitudinal plane.

conditions ( $P > 0.05$ ).

## Discussion

Studies have shown that ultrasound can be used as an alternative method to reveal the morphology of the masseter and other muscles located on the bone surface (7). In the present study, ultrasound findings indicated no significant difference between the thickness of masseter muscles (left and right) in both resting and contraction states in all three groups, which is consistent with the results of Uchia, Park, and Leone et al. (9, 11, 12). Additionally, Raadsheer et al.'s study, which compared masseter muscle thickness using ultrasound and MRI, also did not reveal a significant difference in the thickness of the right and left masseter muscles (13). Our study revealed a notable alteration in masseter muscle thickness between the resting and contraction states in all three groups, consistent with the findings of Kiliardis (14), Bakke et al. (15), and Rani et al. (16). Uchida (11) also reported an increase in masseter muscle thickness in the contraction state compared to the resting state. Olabimpe (17) reported a similar increase in muscle thickness during contraction, but the difference

from the resting state was not significant. In our study, the average right masseter muscle thickness was 1.07 mm at rest and 1.32 mm at contraction. In this regard, Satioglu (18), Kubota et al. (19), and Olabimpe et al. (17) reported a higher mean masseter muscle thickness than our study in Turkey, Japan, and Nigeria, respectively, while the values reported in the Benington study (20) in the British population were lower than in our study. This variation in masseter muscle thickness among different populations may be due to racial diversity, environmental conditions, genetics, and diet. Therefore, genetic effects and environmental variations on muscle development cannot be ignored (17).

In the present study, a significant correlation was found between masseter muscle thickness and BMI of the subjects in all three groups, so a higher relationship was observed in the class I and III malocclusion than in class II malocclusion. In line with our study, a positive relationship has been reported between masseter muscle thickness and BMI in the studies of Satioglu et al. (18), Raadsheer (13), and Rani et al. (16).

Considering the completion of anterior-posterior growth at the age of 15 years, the age range of the patients in our

research was chosen as 15-20 years. It should be noted that in older age, the probability of TMD increases, and because the patients of this study were in the same age range, no significant difference was observed between muscle thickness and age, which was in line with the study of Kiliardis et al. (14) and Raadsheer et al. (13). As the average age increased in all three malocclusion groups, we observed a higher frequency of pattern type II than type I, and a higher frequency of type III than type II.

Park et al. (9) and Soyoye et al. (21) reported that the masseter muscle thickness has an inverse correlation with the mandible angle, so that as the angle of the mandible decreases, the thickness of the muscle increases and also as the angle increases, the thickness of the muscle decreases. However, in our study, all patients were within the Frankfort mandibular plane angle (FMA) normal range and no significant relationship was found between the masseter muscle thickness and facial morphology in terms of the horizontal relationship of skeletal class I, II, and III malocclusions. In the context of masseter muscle thickness, contradictory findings have been reported in studies involving both men and women. Kiliardis (14), Raadsheer (13), Park (9), and Rani et al. (16) found that men have greater masseter muscle thickness than women, while Biondi and colleagues (22) reported the opposite. Our study, however, observed no significant difference in masseter muscle thickness between the two sexes, aligning with the findings of Soyoye et al. (21). Furthermore, our study revealed notable alteration in thickness of the right transverse masseter muscle (in rest and contraction state) and the right longitudinal masseter muscle (in rest state) based on the chewing side in all three groups. This contrasts with the findings of Park et al. (9), who found no significant difference between the right and left sides used for chewing.

In terms of internal pattern frequency in malocclusion groups, we observed that the highest frequency of type II patterns was related to malocclusion I and III groups, while malocclusion II showed the highest frequency of type I patterns. These changes are likely associated with muscle inflammation, as indicated by studies such as Imanimoghaddam (10), and Arijji (5), which highlighted structural indicators of masseter muscle inflammation. It was also observed in Nishi's study (23) that, in terms of electromyography in class II subjects, the masseter muscle activity increased with the increase of overjet. Additionally, our study found that in class II malocclusion patients with increased overjet, the most frequent internal muscle pattern was type I. This was attributed to the increased use of the masseter muscle due to the ability to use the lower jaw in chewing and biting. This finding is consistent with the report by Soyoye et al. (21) regarding individuals with a long face. In comparison, no significant relationship was found between masseter muscle pattern and gender or BMI in our study.

This study has both strengths and limitations. The key strength of our research is its data collection for the related factors of skeletal malocclusions class I, II, and III (e.g., size and pattern of the masseter muscle) with sonographic images for the first time. These findings show

that sonographic images can provide useful information in patients with class I, II, and III malocclusions, which can be useful in choosing their treatment model before and during the orthodontics stage. Indeed, sufficient information about the pattern and thickness of the masseter muscles can effectively stabilize orthodontics treatment and it may be possible to solve some dentoalveolar problems before starting orthodontic treatment.

However, the study has some limitations, too. For instance, in our study, there was no significant relationship between BMI and masseter muscle pattern. However, the most likely confounding factor in this study is BMI. To properly assess the effect of skeletal malocclusion on masseter muscle characteristics, the researchers should have controlled for BMI by either matching or adjusting for it in the analysis.

Additionally, the descriptive design of this study does not permit drawing any causal inferences regarding the temporal sequence between the related factors. So, further research is recommended to determine the other variables related to skeletal malocclusions class I, II, and III and efficient ways to reduce possible biases.

## Conclusion

Based on the findings of the present study, we discovered that skeletal malocclusions in the anterior-posterior dimension can affect the pattern and internal structure of the masseter muscle. The presence of specific structural changes in the internal patterns of the masseter muscle in all types of skeletal malocclusions can indicate the relationship between facial morphology and the internal pattern of muscles. We also observed that BMI can be an effective factor in muscle thickness in patients with various types of skeletal malocclusions in both sexes. According to our study, the ultrasound evaluation of the masseter muscle was effective in screening patients for muscle pattern changes before starting and during orthodontic and surgical treatments and differential diagnosis of muscle hypertrophy or lesions in this area.

## Authors' Contributions

M.I.: Development of the project, analysis of the findings, and editing of the manuscript; Mt.Sh.: Analysis of the data; F. S.: Assisted in data collection and writing; F.F.: Contributed to results interpretation and manuscript editing; Z.J.P.: Contributed to the work designing and analysis, writing the manuscript, and editing the revised manuscript.

All authors reviewed and approved both the submitted and modified versions of the manuscript. Furthermore, each author acknowledged their personal responsibility for their contributions and committed to addressing any inquiries regarding the accuracy or integrity of any aspect of the work, ensuring that such issues are thoroughly investigated and resolved.

## Ethical Considerations

Written informed consent was obtained from all patients to disseminate the data for educational and research pur-

poses. The Mashhad University of Medical Sciences Ethical Committee approved the study (Ethics No. IR.MUMS.DENTISTRY.REC.1398.122). All procedures adhered to the ethical standards set forth by the appropriate committees on human experimentation, both institutional and national, as well as the principles outlined in the Helsinki Declaration of 1975, revised in 2008.

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### Conflict of Interests

The authors declare that they have no competing interests.

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