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Evaluation of masseter muscle in different vertical skeletal patterns in growing patients

ABSTRACT

Aim *The present study aimed to evaluate the relationship between masseter size, maxillary intermolar width and craniofacial vertical skeletal pattern.*
Materials and methods *Study design: The study followed a prospective longitudinal design and enrolled 61 subjects (30 males and 31 females), 9-14 years of age (mean age 11.5) at 2-3 CVM stage. The participants were divided into three groups based on their vertical skeletal pattern which was estimated using the Frankfurt-mandibular plane angle: low-angle group (L-A), normal-angle group (N-A), high–angle group (H-A). An additional gender-based distinction was made. Maxillary intermolar width was measured on the maxillary cast of each patient by means of an electronic caliper; masseter volume was estimated by using magnetic resonance (MR) and masseter thickness was measured by means of ultrasonography (US). The US registrations were performed during the relaxation state (RS) and the maximum voluntary contraction (MVC) of the muscle. The indipendent samples T- test was used for sex comparisons; the analysis of variance test (ANOVA) was used to evaluate the differences between the three groups in males and females, and the Pearson r correlation coefficient was employed to assess the correlation between maxillary intermolar width and masseter volume.*

Conclusion *Maxillary intermolar width, masseter volume and thickness showed significant gender differences; all the tested variables decreased significantly according to the facial vertical pattern, with greater values in females, especially in low- and normal-angle subjects; maxillary intermolar width and masseter volume showed significant correlations, higher in females.*

Keywords *Gender; Magnetic resonance imaging; Masseter muscle ultrasonography; Masticatory muscles; Maxilla.*

Introduction

Craniofacial morphology is the result of a complex interaction between genetic and environmental factors. The role of all these factors was widely evaluated and several authors studied the influence of function on craniofacial form and showed a correlation between the morphology of craniofacial skeleton and the function and morphology of masticatory muscles [Van Spronsen et al., 1991; Raadsheer et al., 1996]. This relationship was investigated in both experimental animal and human clinical studies [Tuxen et al., 1999; Farella et al., 2003; Kwon et al., 2007; Tsai et al., 2010; Uchida et al., 2011]. Many factors such as size, fiber content, metabolism and biomechanics of masticatory muscles seem involved in the development of the craniofacial complex [Al-Farra et al., 2001; Sciote et al., 2012; Gregor et al., 2013] but their role is not completely clarified.

Among the masticatory muscles, the masseter was mainly investigated exploring the relationship between size and the vertical and transversal skeletal pattern. Masseter size measurements were performed using several techniques, including computed tomography (CT) [Chan et al., 2008; Becht et al., 2014], magnetic resonance (MR) [Boom et al., 2008] and ultrasonography (US) [Kiliaridis and Kalebo, 1991; Rohila et al., 2012; Tircoveluri et al., 2013].

In most studies masseter size was found to be significantly related to the vertical and transversal skeletal dimensions [Raadsheer et al., 1996; Rohila et al., 2012]. The association between masseter size and face height seemed to be negative, while the relationship between size and facial width seemed to be positive. Scientific evidence showed that masseter muscle thickness might be considered as one of the factors influencing the maxillary dental arch width [Kiliaridis et al., 2003; Tircoveluri et al., 2013].

Starting from the literature data, the present study aimed to evaluate the relationship between masseter size, maxillary intermolar width and craniofacial vertical skeletal pattern, using US and MR examinations.

Matherials and methods

Population and study design

The present study followed a prospective longitudinal design and enrolled subjects among the patients who underwent an orthodontic evaluation at the Division of Orthodontics, DSCM, University of Insubria (Varese, Italy). As a routine procedure, a signed informed consent for the release of diagnostic records for scientific purposes was obtained from the parents of the patients prior to entry in the study. The study protocol followed the World Medical Organization Declaration of Helsinki.

The initial study sample consisted of 125 subjects (60 males and 65 females) aged 8 to 15 years (mean age 12.2 yrs.), at the 2-4 cervical vertebral maturation (CVM) stage [Litsas and Ari-Demirkaya, 2010a; Litsas and Ari-Demirkaya, 2010b; Perinetti et al., 2014].

Primary and secondary exclusion criteria were applied to the initial sample (Table 1).

A total of 61 subjects (30 males and 31 females), 9-14 years of age (mean age 11.5 yrs.) at the 2-3 CVM stage were enrolled as the final sample of the study.

The participants were divided into three groups based on their vertical skeletal pattern, which was estimated using the Frankfurt-mandibular plane angle (FMA) according to the Tweed cephalometric analysis performed on standardised lateral cephalograms. The low-angle group (L-A) consisted of subjects with a FMA angle lower than 20°, the normal-angle group (N-A) included subjects with a FMA angle ranged from 21° to 28°, and the high–angle group (i.e. H-A) comprised subjects with a FMA angle greater than 29°. According to the selection criteria the following groups were created: 20 subjects for L-A group, 22 subjects for N-A group, 19 subjects for H-A group.

An additional gender-based distinction was made.

Alginate impressions were taken from each subject. The maxillary intermolar width was measured on the maxillary cast of each patient by means of an electronic caliper (CD 1105T, MJ Cnc Automation, Turin, Italy). The distance recorded was the shortest one between the lingual surfaces of the first permanent molars as suggested by the orthodontic literature [Kiliardis et al., 2003].

Magnetic resonance (Toshiba Vantage 1.5 Tesla, Toshiba Medical System SRL, Rome, Italy) and ultrasonography (7.5 Mega-Hertz linear transducer. Toshiba Power-Vision 6000 Ultrasound System, Toshiba Medical System SRL, Rome, Italy) examinations of masseter muscles were performed. MR was performed with the patients in a supine position. Axial and coronal scan series were taken bilaterally with an interslice gap of 2 cm, using T2 weighted sequences and fat suppression in order to better evaluate the intensity of the signal and the volume of the muscle. The US registrations were performed with the subjects seated on a chair with headrest. Firstly, they were requested to maintain slight interocclusal contacts, then to clench maximally in the intercuspal position in order to assess masseter thickness during the relaxation state (RS) and the maximum voluntary contraction (MVC) of the muscle. Both left and right masseter muscles were scanned. The measurement site was better identified by tracing two straight lines on the skin of the patient (Fig. 1). The first line was drawn parallel to and 2 cm distant from the mandibular base, which is approximately the bulkiest part of the superficial masseter; the registration was performed at a point on this line keeping the probe 2 cm from the posterior edge of the mandibular ramus, where the second line was drawn. Masseter thickness was measured directly on the screen of the scanner. The registrations were repeated three times per side;

Initial sample	125
Primary exclusion criteria	
Previous orthodontic treatment	4
Bad habits	9
Oro-facial disorders	6
Skeletal malocclusion (Class II o III)	10
Dental malocclusion (Class II o III)	14
Maxillo-facial asymmetries	7
Transversal discrepancies	12
Secondary exclusion criteria	
General contraindication to instrumental examinations	2
Final sample	61

TABLE 1 Primary and secondary exclusion criteria. Primary and secondary exclusion criteria were applied to the initial sample and a total of 61 subjects were enrolled as the final sample of the study.

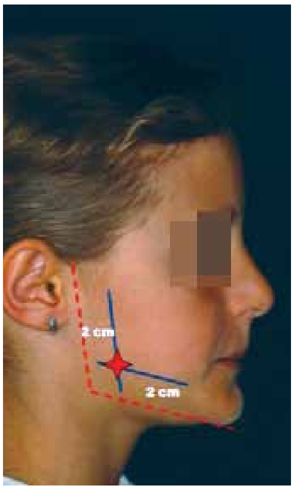

FIG. 1 Lines drawn on the skin of the patient to perform ultrasonography. The US measurement site was better identified by tracing two straight lines on the skin of the patient. The first was drawn parallel to and 2 cm distant from the mandibular base; the registration was performed at a point on this line keeping the probe 2 cm from the posterior edge of the mandibular ramus, where the second line was drawn.

Variables
Maxillary intermolar width (mm)
Masseter volume (cm^3)
Masseter thickness – RS (mm)
Masseter thickness – MVC (mm)

TABLE 2 Variables tested in the sample. Four measurements were selected as variables to study in L-A, N-A and H-A group, in both males and females.

the muscular thickness was calculated as the mean of those measurements and the muscular thickness of the patient was calculated as the mean of the two final values of the right and left muscles.

Finally, four measurements were selected as variables for each group (Table 2).

Statistical analysis

Means and standard deviations (SD) were calculated for the four variables in each group. All statistical analyses were performed using software MedCalc Version 11.5.1.0 (MedCalc Software, Mariakerke, Belgium). Parametrical methods were used after having tested the existence of the assumptions through the Shapiro-Wilk test for the normality of the distributions.

After the demonstration of statistically significant differences in the evaluated variables between males and females by means of Student t-test, the statistical analysis was performed considering the components of each group divided according to the gender.

The analysis of variance test (ANOVA) was used to determine if there were statistically significant differences in the evaluated variables between groups (L-A, N-A, H-A). Bonferroni correction was applied for statistically significant differences after post-hoc analysis. Pearson's r correlation coefficient was then used to assess the correlation between maxillary intermolar width and masseter volume.

Results

The gender-based analysis revealed statistically significant differences between males and females (Table 3). Maxillary intermolar width was found to be significantly greater in males than females with the same vertical skeletal pattern in all the groups, except H-A. Males exhibited significantly higher values of masseter volume than females with the same vertical skeletal pattern in all the groups, except H-A. Males had a significantly greater masseter thickness during the relaxation state in L-A and N-A groups, while females had a significantly greater masseter thickness during the maximum voluntary contraction in L-A and N-A groups. No statistically significant differences in masseter thickness were found between males and females in H-A group during both relaxation state and maximum voluntary contraction.

The ANOVA test showed that maxillary intermolar width, masseter volume and masseter thickness progressively decreased in L-A, N-A and H-A groups; differences between groups were statistically significant for each variable and these results were confirmed when the variables were separately analyzed in males

Variable		Males	Females	
		Mean±SD	Mean ±SD	p
Maxillary intermolar width (mm)	L-A	34.27±2.14	33.40±1.98	*
	N-A	32.40±1.63	31.05±1.90	*
	H-A	30.15±0.81	29.95±0.92	NS
Masseter volume (cm^3)	L-A	28.50±0.90	27.30±1.90	*
	N-A	26.20±1.89	24.35±1.74	*
	H-A	23.70±2.01	22.95±2.30	NS
Masseter thickness RS (mm)	L-A	8.52±0.78	7.80±1.19	*
	N-A	7.58±0.78	6.80±0.77	*
	H-A	6.70±0.36	6.00±0.52	NS
Masseter thickness MVC (mm)	L-A	10.50±0.69	12.60±0.80	*
	N-A	9.75±1.13	10.71±1.21	*
	H-A	8.96±0.74	9.05±0.90	NS

TABLE 3 Independent samples T- test results for sex comparisons. Data are shown as mean±SD. Intragroup comparison showing the sexual dimorphism of maxillary intermolar width, masseter volume, masseter thickness RS and masseter thickness MVC in L-A, N-A and H-A group, using independent samples T- test at significance level *$p<0.05$. NS: not statistically significant.

Variable	L-A	N-A	H-A
	Mean±SD	Mean±SD	Mean±SD
Maxillary intermolar width (mm)	34.27 ±2.14†‡	32.40±1.63*‡	30.15±0.81*†
Masseter volume (cm^3)	28.50±0.90†‡	26.20±1.89*‡	23.70±2.01*†
Masseter thickness RS (mm)	8.52±0.78†‡	7.58±0.78*‡	6.70±0.36*†
Masseter thickness MVC (mm)	10.50±0.69†‡	9.75±1.13*‡	8.96±0.74*†

* Statistically significant differences compared to L-A group; significance level $p<0.05$
† Statistically significant differences compared to N-A group; significance level $p<0.05$
‡ Statistically significant differences compared to H-A group; significance level $p<0.05$

TABLE 4 ANOVA results for inter-group comparisons in males. Data are shown as mean±SD. ANOVA results of maxillary intermolar width, masseter volume, masseter thickness RS and masseter thickness MVC relative to the vertical skeletal pattern in males.

Variable	L-A	N-A	H-A
	Mean±SD	Mean±SD	Mean±SD
Maxillary intermolar width (mm)	33.40±1.98†‡	31.05±1.90*‡	29.95±0.92*†
Masseter volume (cm^3)	27.30±1.09†‡	24.35±1.74*‡	22.95±2.30*†
Masseter thickness RS (mm)	7.80±1.19†‡	6.80±0.77*‡	6.00±0.52*†
Masseter thickness MVC (mm)	12.60±0.80†‡	10.71±1.21*‡	9.05±0.90*†

* Statistically significant differences compared to L-A group; significance level p<0.05
† Statistically significant differences compared to N-A group; significance level p<0.05
‡ Statistically significant differences compared to H-A group; significance level p<0.05

TABLE 5 ANOVA results for inter-group comparisons in females. Data are shown as mean±SD. ANOVA results of maxillary intermolar width, masseter volume, masseter thickness RS and masseter thickness MVC relative to the vertical skeletal pattern in females.

and females (Table 4, 5). By excluding the comparison between L-A and H-A groups, the greater differences were found in females between L-A and N-A groups.

According to the correlation analysis, maxillary intermolar width showed a significant positive correlation with masseter volume; in females this correlation was stronger and more statistically significant (Table 6).

Discussion

The present work aimed to evaluate the relationship among masseter size, maxillary intermolar width and vertical skeletal pattern. In literature it is generally accepted that representative indices of masseter size are thickness, maximum cross-sectional area (CSA) and volume. According to Boom et al. [2008] vertical craniofacial dimensions are more strongly related to muscular volume than to CSA; on the basis of this assumption masseter volume was selected as an index of masseter size together with thickness. In order to reduce radiographic exposure, masseter volume was estimated using MR rather than CT. Masseter thickness was measured using US, which reported a good rate of safety and reliability [Bakke et al., 1992].

The gender-based analysis revealed statistically significant differences between males and females. Maxillary intermolar width, masseter volume and masseter thickness during relaxation were found to be significantly greater in males than females with the same vertical skeletal pattern in L-A and N-A groups. No statistically significant differences were found between males and females in H-A group.

	Males	Females
r	0.57	0.74
p	0.03	0.01

TABLE 6 Correlation coefficient analysis. Correlation matrix between masseter volume and maxillary intermolar width using Pearson's correlation test in males and females.

The higher values recorded in males appear to be consistent with the literature [Raadsheer et al., 1996; Zhao et al. 2001; Palinkas et al., 2010; Rohila et al., 2012; Tircoveluri et al., 2013]; however the results of several previous investigations are not comparable to those of the present study due to the characteristics of the sample, such as older age [Rohila et al., 2012; Tircoveluri et al., 2013], size [Raadsheer et al., 1996] and non-homogeneity in terms of craniofacial pattern [Zhao et al., 2001; Palinkas et al., 2010].

Unusual findings of the gender-based analysis derived from the evaluation of masseter thickness during maximum voluntary contraction: in the present study this parameter was found to be greater in females than in males, except for H-A subjects. However masseter thickness measured with the patient clenching maximally in the intercuspal position might not be indicative of the true contraction potential of the muscle [Raadsheer at al., 1996].

The lack of statistically significant differences between males and females for all the variables in H-A group confirms the results reported by Rohila et al. [2012]. This might be related to the vertical pattern of these subjects who might have a decreased muscular size irrespective of the gender.

Maxillary intermolar width, masseter volume and masseter thickness, during both relaxation and maximum voluntary contraction, were significantly different in the three groups classified according to the vertical skeletal pattern. All the variables showed progressively decreasing values in L-A, N-A and H-A groups.

The negative relationship between facial height and masseter size is consistent with the literature [Raadsheer et al., 1996; Satiroğlu et al., 2005; Rohila et al., 2012; Lione et al., 2013]. Van Spronsen et al. [1991] quantified the reduction of the jaw muscles thickness in high-angle subjects compared to normo-angle subjects as a percentage and they reported values up to 33%. The authors explained the decreased dimensional values of jaw muscles in long-faced subjects, as well as their reduced intrinsic strength, as the result of a disuse hypotrophy that occurs during the development of craniofacial morphology in long-faced subjects.

According to this interpretation, the reduced size of jaw muscles could be the effect of an excessive vertical growth pattern rather than the effect of the causal factor of the vertical facial type.

Another possible explanation might involve the forces produced by muscular stretching, which might affect the skeletal growth pattern and dental eruption. In long-faced subjects the weaker forces would result in a major eruption of upper molars leading to the vertical growth; similarly, the greater contraction of masticatory muscles in short-faced individuals would generate a mechanical load that could influence bone growth producing a more acute gonion angle [Rohila et al., 2012].

As of now, whether the masticatory muscles control the facial vertical development or *vice versa* is still not completely clarified.

Boom et al. [2008] underlined that posterior facial height seems to be more strongly related to the jaw muscular volume compared with anterior facial height, probably because the muscle-bone interaction is more marked in the posterior area, where vertical growth is mainly determined by the development of the gonial region and its muscles.

Even if most studies [Bakke et al., 1992, Rani and Ravi, 2010; Uchida et al., 2011] found a correlation between facial height and masseter size, some discordant data exist in literature. Prabhu and Munshi [1994] measured masseter and temporalis muscular thickness using ultrasonography and reported no changes of these parameters in different facial morphologies. This literature inconsistency could be ascribed to the lack of an adequate sample size and homogeneity, as well as to the use of different methods.

The purpose of this study was also to evaluate the correlation between maxillary intermolar width and muscular size. Scientific evidence showed that these parameters are significantly related [Tircoveluri et al., 2013]. This could be explained considering that the growth of the midpalatal suture would be influenced by the functional demand. Maxillary bone apposition at midpalatal suture has been shown to be smaller in rats with decreased functional demands resulting in a narrower maxillary arch [Katsaros et al., 2002].

In this study a statistically significant correlation between maxillary intermolar width and masseter volume was found in both males and females, but females showed a higher and more significant correlation than males. Kiliaridis et al. [2003] found no correlation between maxillary intermolar width and masseter thickness in males but a significant positive correlation in females, supposedly due to the greater capacity of female masticatory muscular system to influence maxillary growth. This could explain the stronger and more significant correlation between maxillary intermolar width and masseter volume found in the female sample of this study and also the higher differences found in females comparing L-A and N-A groups.

If an intimate relationship between craniofacial morphology and muscular system is generally believed to exist, it remains unclear whether the morpho-structural modifications are linked only to the muscular size or if an essential role is played by other factors, such as neuromuscular mechanisms, metabolism, fiber distribution and muscular bio-mechanics.

The findings of the present investigation reveal the existence of close connections among masseter size, maxillary intermolar width, and vertical skeletal pattern. A stronger influence of muscular structures on craniofacial development has been hypothesized to exist in females rather than males. However, on the basis of the current knowledge it is not possible to deeply understand the pathophysiological mechanisms involved.

Further investigations are required to clarify the muscular factors involved in the craniofacial development. It would be desirable to extend the sample size and analyse neuromuscular and biomechanical mechanisms, as well as the fiber profile of masseter muscles, in order to explain how masticatory muscles could actually affect craniofacial morphology.

Conclusions

According to the present results the following conclusions might be drawn.

- Maxillary intermolar width, masseter volume and thickness showed significant differences between sexes.
- All the tested variables significantly decreased according to the facial vertical pattern, with more significant values in females, especially in low- and normal-angle subjects;
- Maxillary intermolar width and masseter volume showed significant correlations, higher in females.

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