



Comparative cone-beam computed tomography evaluation of the osseous morphology of the temporomandibular joint in temporomandibular dysfunction patients and asymptomatic individuals

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Received: 4 October 2016 / Accepted: 21 February 2017 / Published online: 16 March 2017
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Abstract

Objective We examined the bone components of the temporomandibular joint (TMJ) in asymptomatic individuals and patients with temporomandibular dysfunction (TMD) using cone-beam computed tomography (CBCT).

Methods Two hundred asymptomatic individuals and 200 patients with TMD were included in this study. Condyle position, eminence height, eminence inclination, condyle shape, and fossa shape were assessed on CBCT images of the 800 temporomandibular joints.

Results The eminence inclination ($P < 0.05$), eminence height ($P < 0.0001$), mediolateral width of condyle ($P < 0.0001$), and anterior joint space ($P < 0.0001$) were significantly greater in male subjects compared with female subjects in both the asymptomatic group and TMD group. Comparisons of the asymptomatic group and TMD group revealed significant differences in the anterior joint space ($P < 0.0001$), ratio of anterior joint space to posterior joint space ($P < 0.001$), posterior joint space ($P < 0.05$), eminence inclination ($P < 0.05$), eminence height ($P < 0.05$), condyle shape ($P < 0.0001$), and fossa shape ($P < 0.05$).

Conclusions The present analyses suggest that a steeper articular eminence inclination may be risk factor for TMD. The presence of TMD was associated with the condyle position in the TMJ.

Keywords Cone-beam computed tomography · Articular eminence · Temporomandibular dysfunction · Temporomandibular joint morphology

Introduction

Between the temporal bone and the mandible is a sophisticated articular system known as the temporomandibular joint (TMJ). The inferior portion of the bone is known as the mandibular condylar process, while the superior portion of the bone is called the glenoid fossa. The articular eminence is a part of the temporal bone on which the mandibular condyle slides during mandibular movements [1].

The clinical significance of condyle–fossa relationships in the TMJ remains controversial [2]. A better understanding of TMJ morphology may help clinicians to define the normal range of variation within the asymptomatic population and pathological anomalies that require regular treatment [3]. Previous studies have evaluated the morphology of the TMJ and investigated the correlations between specific clinical parameters and temporomandibular dysfunction (TMD) [4–7]. The widely studied variables include the articular eminence inclination, condyle position, condyle shape, and fossa shape [5, 6, 8, 9].

Many methods have been used to examine the TMJ morphology. Standard two-dimensional projections of the TMJ, taken for example from the transcranial view, are of limited clinical utility. For example, superimposition of overlying structures can limit the ability to visualize pathological changes in the TMJ [10, 11]. Cone-beam computed tomography (CBCT) is often recommended as a dose-sparing technique for maxillofacial imaging [12]. Previous reports have suggested that CBCT can provide accurate and

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reliable linear measurements for reconstruction and imaging of the TMJ bones [7, 13, 14].

In the present study, we simultaneously evaluated multiple TMJ parameters in a large population of patients and asymptomatic individuals. The aim of the study was to evaluate the bone components of the TMJ in asymptomatic individuals and TMD patients using CBCT.

Methods

The study received approval from the Ethics Committee in Research of Ataturk University (Protocol No. 334/2013), and an informed consent form was signed by those who agreed to participate, as well as their legal guardian in the case of minors aged <18 years. The TMD group consisted of 200 patients (152 females with mean age of 31.13 ± 11.09 years and 48 males with mean age of 30.56 ± 10.16 years) who showed signs and symptoms consistent with TMD, including joint click/crepitation, joint pain, muscle pain, mouth-opening limitation, and non-harmonic movements of the joint. All patients were referred to the Department of Oral and Maxillofacial Radiology for treatment of TMD and required CBCT.

The asymptomatic group consisted of 200 individuals (138 females with mean age 25.09 ± 6.23 years and 62 males with mean age of 25.35 ± 5.64 years) who underwent CBCT examination for any indication other than TMD. The criteria for exclusion from the asymptomatic group were: occlusal interference resulting in functional mandibular deviation, history of orthodontics, signs and symptoms consistent with TMD or history of TMD treatment, visible facial asymmetry or teeth craniofacial abnormalities

including cleft palate, history of facial trauma, or general conditions potentially affecting the TMJ.

After the subjects were enrolled according to the selection criteria, all measurements were carried out in a blinded manner regarding whether or not the subjects had TMD. A single examiner performed all of the measurements. The intraobserver error rate was evaluated by the intraclass correlation coefficient, and the intraclass correlation coefficients were between 0.82 and 0.95.

Imaging procedures

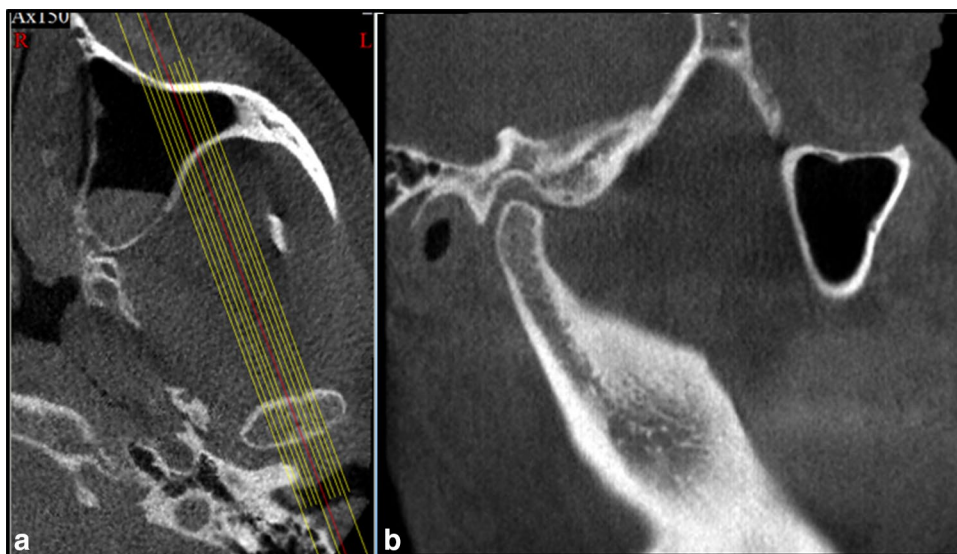
CBCT imaging was conducted using a NewTom 3G device (Quantitative Radiology, Verona, Italy). The maximum output of the scanner was 110 kV and 15 mAs, with 0.16-mm voxel size and 30-cm field of view. For imaging, the subject was positioned in a seated posture with the head held upright, the eyes focused on a point straight ahead, and the teeth in centric occlusion (maximum intercuspation). The X-ray tube-detector system performed a 360° rotation around the head of the patient and the scanning time was 36 s.

Measurements

The axial view presenting the maximum mediolateral dimension of the condyle using 0.5-mm thickness axial slices was selected for measurements. Next, 1-mm sagittal slices created in each slice from the medial condyle to the orbitale were selected (Fig. 1). Using the obtained reconstructed sagittal images, the eminence inclination and joint spaces were examined and measured (Fig. 2).

The eminence inclination was measured using two complementary methods. The best-fit line method

Fig. 1 **a** Axial view showing the longest mediolateral length of the condyle. The *red line* shows the slice selected as the reference view for joint space and eminence measurements. **b** Sagittal cross-sectional image corresponding to the *red line* shown in **(a)**



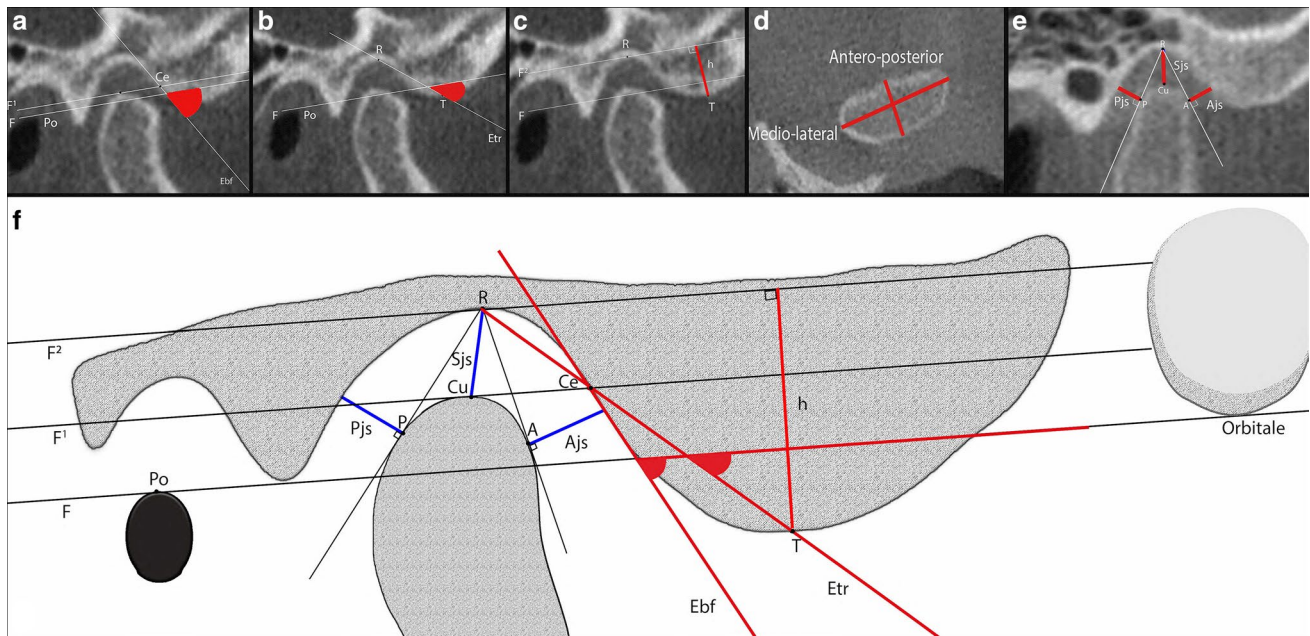


Fig. 2 Eminence measurements. **a** Best-fit line method. **b** Top-roof line method. **c** Eminence height. **d** Axial condylar morphology. **e** Joint spaces. **f** Points and planes used in this study. *Ce* point at which line F1 cuts through eminence posterior surface, *Cu* maximum point of condylar process, *R* maximum point of fossa, *T* minimum point of articular eminence, *Po* portion (highest point of auditory meatus), *A*

most prominent point on anterior aspect of condyle, *P* most prominent point on posterior aspect of condyle, *Ebf* best-fitting plane of articular eminence inclination connecting to *Ce*, *Etr* plane passing through points *Ce* and *R*, *F* (Frankfort horizontal), plane passing through point *Po* and orbitale, *F1* plane passing through point *C* and parallel to *F*, *F2* plane passing through point *R* and parallel to *F*

measures the angle between *Ebf* and *F*, while the top-roof line method measures the angle between *Etr* and *F*. The eminence height (*h*) was determined by the perpendicular distance from the minimum point of the articular eminence to the maximum point of the fossa (Fig. 2).

The superior joint space (*Sjs*), posterior joint space (*Pjs*), and anterior joint space (*Ajs*) were measured on the above-described sagittal images to determine the position of the condyle. Initially, a horizontal line was drawn from *R* to *Cu*. The *R* point was sequentially connected to *A* and *P*. The *Ajs* and *Pjs* were defined as the perpendicular distance from the *A* and *P* tangent points to the glenoid fossa, respectively. As a result, measurement of the *S* point and the superior prominent point of the condylar head reflected the *Sjs* (Fig. 2). If the *Ajs/Pjs* ratio was >1 , the condyle was noted as being in a posterior position. If the same ratio was <1 , the condyle was assessed as being in an anterior position.

The axial condylar morphology was evaluated by measuring the maximum mediolateral width and maximum anteroposterior width using a view containing the maximum mediolateral view of the condyle (Fig. 2). The condyle shape was estimated from the central coronal slice, and the fossa shape was determined from the central sagittal slice. Condyle shapes were recorded as convex, round, flattened, angled, or other. Fossa shapes were

classified as oval, triangular, angled, trapezoidal, or other (Fig. 3).

Statistical analysis

All statistical analyses were performed using SPSS for Windows (IBM Corporation, Armonk, NY, USA). Kolmogorov–Smirnov statistics was used for the normality test. Differences in eminence inclination, eminence height, *Ajs*, *Pjs*, *Sjs*, *Ajs/Pjs* ratio, anteroposterior width of condyle, and mediolateral width of condyle were compared between sexes and groups by Student's *t* test. Differences in eminence height and eminence inclination between the groups were evaluated by one-way ANOVA. Differences in condyle distribution and fossa shape between the groups were evaluated by the Chi-square test. A value of $P < 0.05$ was established as the threshold of statistical significance.

Results

The eminence height and eminence inclination findings in the asymptomatic and TMD groups are shown in Table 1. The eminence inclination ($P < 0.05$) and eminence height ($P < 0.0001$) were both significantly greater in males than in females.

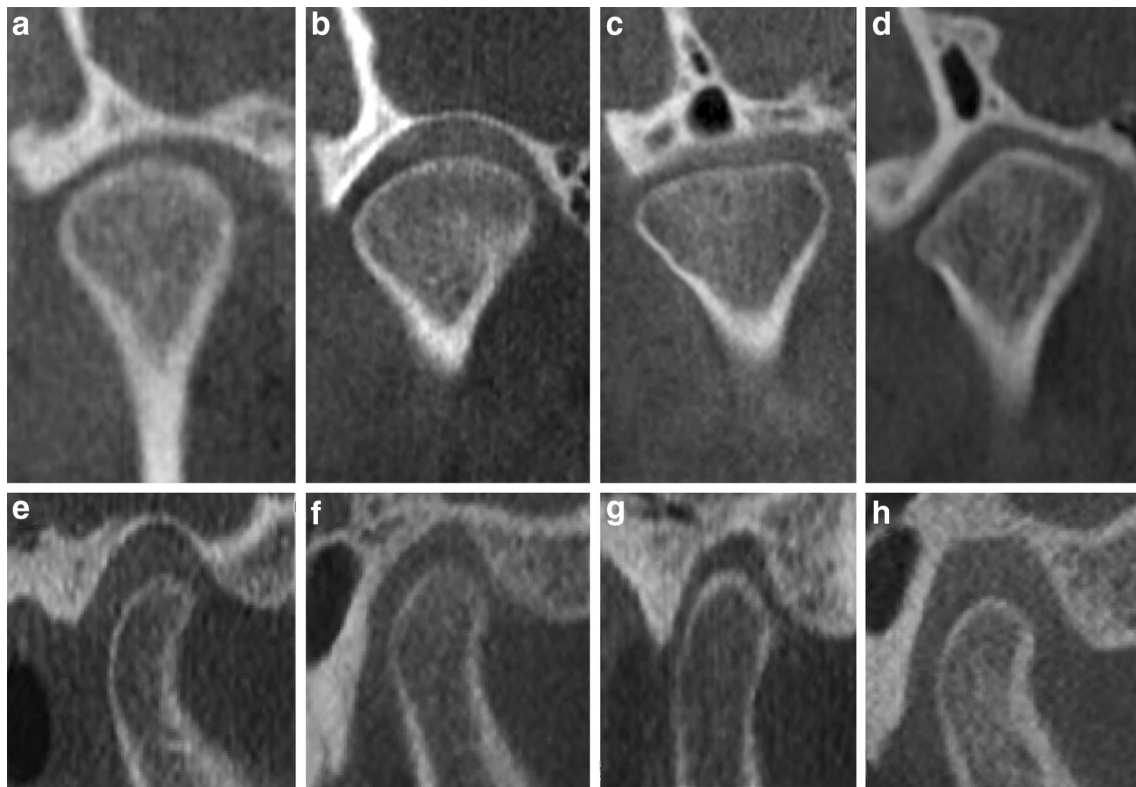


Fig. 3 Condyle and fossa shapes. **a–d** Condyle shapes: **a** convex, **b** round, **c** flattened, **d** angled. **e–h** Fossa shapes: **e** oval, **f** triangular, **g** angled, **h** trapezoidal

As shown in Table 2, both the eminence inclination and eminence height differed significantly between the TMD group and the asymptomatic group ($P < 0.05$). The eminence inclination was lower in the TMD group compared with the asymptomatic group using either the best-fit line method or the top-roof line method.

Significant differences in the Ajs ($P < 0.0001$), Pjs ($P < 0.05$), Ajs/Pjs ($P < 0.0001$), and condyle dimensions were observed between the asymptomatic group and the TMD group (Table 2). A posterior condylar position was noted more frequently in the TMD group. Although there were significant differences for all measurements of condyle dimensions ($P < 0.05$) and joint spaces ($P < 0.0001$) between the sexes in the asymptomatic group, only Sjs ($P < 0.0001$) and mediolateral width of condyle ($P < 0.0001$) differed significantly between males and females in the TMD group (Table 1).

The study group was stratified according to the age of the subjects. The eminence inclination ($P < 0.0001$), mediolateral width ($P < 0.05$), eminence height ($P < 0.05$), and anteroposterior width of condyle ($P < 0.0001$) differed significantly with age in the asymptomatic group (Table 1). Both the eminence measurements and condyle dimensions were lowest among patients aged 15–20 years and highest among patients aged 21–30 years in the asymptomatic

group. Among the patients in the TMD group, the eminence height, Sjs, and anteroposterior width of condyle increased with age.

The proportional distributions of the fossa and condyle shapes in the asymptomatic and TMD groups are shown in Table 2. The types of condyle and fossa shapes differed significantly between the asymptomatic group and the TMD group ($P < 0.05$ for condyle shape; $P < 0.0001$ for fossa shape). An oval-shaped condyle and fossa were the most common variant in both the asymptomatic group and the TMD group.

Discussion

All components of the TMJ are part of a functional whole. Imaging of the complex anatomical structures of the TMJ requires the use of specialized techniques. Clear imaging of the mandibular condyle, glenoid fossa, and articular eminence is essential for identifying the source of any dysfunction. The head of the condyle in the joint and the angle of the long axis of the eminence are not parallel to the mid-sagittal plane, and this angle can vary among individuals. Thus, the angle of the X-ray should be adjusted along the condyle axis for radiographic examination and visualized

Table 1 All TMJ measurements in the TMD and asymptomatic groups according to sex and age

	Male	Female	<i>P</i> ^a	15–20 years	21–30 years	31–40 years	>40 years	<i>P</i> ^b
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
	(<i>n</i> = 124)	(<i>n</i> = 276)		(<i>n</i> = 102)	(<i>n</i> = 204)	(<i>n</i> = 56)	(<i>n</i> = 38)	
Asymptomatic group								
Best-fit line	63.44 (13.33)	59.58 (12.63)	0.006*	57.91 (12.78) ^a	62.88 (12.84) ^b	62.85 (13.08) ^b	54.15 (10.40) ^a	0.0001**
Top-roof line	40.43 (6.89)	38.14 (6.68)	0.002*	36.96 (6.66) ^a	40.13 (6.91) ^b	39.68 (5.93) ^b	35.83 (6.07) ^a	0.0001**
Eminence height (mm)	8.12 (1.33)	7.52 (1.33)	0.0001**	7.45 (1.45)	7.87 (1.24)	7.75 (1.31)	7.44 (1.63)	0.039*
Anterior joint space (mm)	2.56 (0.86)	2.27 (0.68)	0.0001**	2.36 (0.82)	2.32 (0.75)	2.46 (0.65)	2.41 (0.76)	0.634
Superior joint space (mm)	4.02 (0.84)	3.30 (0.95)	0.0001**	3.34 (0.90)	3.59 (0.98)	3.70 (0.94)	3.38 (1.11)	0.065
Posterior joint space (mm)	2.44 (0.67)	2.26 (0.69)	0.014*	2.33 (0.58)	2.33 (0.74)	2.26 (0.60)	2.30 (0.82)	0.906
Mediolateral width of condyle (mm)	20.43 (2.61)	18.30 (2.45)	0.0001**	18.65 (2.4) ^a	18.98 (2.61) ^{ab}	19.84 (3.41) ^b	18.38 (2.33) ^a	0.026*
Anteroposterior width of condyle (mm)	8.19 (1.70)	7.82 (1.36)	0.022*	7.57 (0.92) ^a	8.18 (1.73) ^b	8.15 (1.16) ^b	7.28 (1.30) ^a	0.0001**
	Male	Female	<i>P</i> ^a	15–20 years	21–30 years	31–40 years	>40 years	<i>P</i> ^b
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
	(<i>n</i> = 96)	(<i>n</i> = 304)		(<i>n</i> = 102)	(<i>n</i> = 204)	(<i>n</i> = 56)	(<i>n</i> = 38)	
TMD group								
Best-fit line	62.27 (11.83)	57.97 (12.88)	0.003*	59.27 (12.88)	58.11 (12.34)	57.79 (11.96)	61.88 (13.96)	0.117
Top-roof line	40.35 (6.60)	37.11 (6.29)	0.0001**	38.03 (5.64)	37.52 (6.42)	37.22 (6.75)	39.42 (6.84)	0.128
Eminence height (mm)	8.11 (1.39)	7.31 (1.19)	0.0001**	7.82 (1.10) ^a	7.61 (1.28) ^a	7.16 (1.26) ^b	7.46 (1.37) ^{ab}	0.010*
Anterior joint space (mm)	2.84 (1.05)	2.68 (0.89)	0.133	2.78 (0.97)	2.82 (0.99)	2.52 (0.77)	2.70 (0.92)	0.820
Superior joint space (mm)	3.94 (0.97)	3.23 (0.96)	0.0001**	3.14 (0.96) ^a	3.31 (0.92) ^a	3.39 (0.98) ^a	3.78 (1.17) ^b	0.001*
Posterior joint space (mm)	2.24 (0.79)	2.12 (0.91)	0.224	2.25 (0.76)	2.14 (0.72)	2.10 (1.03)	2.16 (1.05)	0.795
Mediolateral width of condyle (mm)	19.64 (2.16)	17.88 (2.20)	0.0001**	17.66 (1.80)	18.15 (2.35)	18.62 (2.45)	18.68 (2.29)	0.300
Anteroposterior width of condyle (mm)	7.64 (1.11)	7.65 (1.16)	0.917	7.15 (1) ^a	7.59 (1.02) ^b	7.79 (1.26) ^{bc}	7.96 (1.24) ^c	0.0001**

TMJ temporomandibular joint, TMD temporomandibular disorder, *n* number of joints, *SD* standard deviation

Superscript letters in the data indicate values that differ significantly at the 0.05% probability level (Duncan test)

P^a*P* values obtained by Student’s *t* test, *P*^b*P* values obtained by one-way ANOVA, * *P* < 0.05, ** *P* < 0.0001

in the direction in which translational movement of the condyle-disk complex is realized in the eminence sagittal plane [10, 11]. As a result, conventional methods are not sufficient. Magnetic resonance imaging (MRI) is superior for imaging of soft tissues [15, 16], while computed tomography (CT) and CBCT are more reliable for evaluating bone structures [13, 17]. However, CT involves high cost and high radiation exposure. CBCT is an ideal method for evaluating TMJ bone structures [7, 12, 18]. In this study, we utilized CBCT to image the bones of the TMJ in three planes and reconstruct the condyle head along the longitudinal axis. This approach allowed us to evaluate the structures without angular or dimensional distortion or

superposition, and involved lower doses of radiation compared with conventional tomography and CT.

The eminence inclination is the angle between the horizontal planes (F, occlusal plane, or palatal plane) and the posterior slope of the articular eminence. The most commonly used plane is F [6, 9, 19–21]. In our study, F was used as the reference plane. Some investigators have evaluated the slope of the eminence by measuring the angle between a line connecting the rock-bottom eminence point and the deepest projection of the fossa and the F plane [6, 8, 9]. Other investigators have evaluated the articular eminence inclination by measuring the angle between a line connecting the posterior aspects of the articular eminence

Table 2 The difference in all measurement of TMJ and distributions of the condyle and fossa shapes in the TMJ dysfunction and asymptomatic groups

	Asymptomatic group Mean (SD) (<i>n</i> = 400)	TMJ dysfunction group Mean (SD) (<i>n</i> = 400)	<i>P</i> ^a
Best-fit line (°)	60.78 (12.96)	58.94 (12.70)	0.044*
Top-roof line (°)	38.85 (6.82)	37.89 (6.51)	0.042*
Eminence height (mm)	7.70 (1.35)	7.50 (1.29)	0.031*
Anterior joint space (mm)	2.36 (0.76)	2.72 (0.93)	0.0001**
Superior joint space (mm)	3.52 (0.97)	3.40 (1.01)	0.081
Posterior joint space (mm)	2.32 (0.69)	2.15 (0.88)	0.003*
Anterior joint space/posterior joint space	1.11 (0.52)	1.47 (0.81)	0.0001**
Medio-lateral width of condyle (mm)	18.96 (2.69)	18.30 (2.31)	0.0001**
Antero-posterior width of condyle (mm)	7.93 (1.48)	7.65 (1.15)	0.003*
	Asymptomatic group Mean (SD) <i>n</i> (%)	TMJ dysfunction group Mean (SD) <i>n</i> (%)	<i>P</i> ^c
Condyle shape			
Oval	250 (62.5)	185 (46.3)	
Round	54 (13.5)	70 (17.5)	
Flattened	54 (13.5)	60 (15)	0.0001**
Angled	22 (5.5)	26 (6.5)	
Other	20 (5)	59 (14.8)	
Fossa shape			
Oval	307 (76.8)	307 (76.8)	
Triangular	57 (14.2)	60 (15)	
Angled	25 (6.3)	12 (3)	0.019*
Trapezoidal	9 (2.3)	9 (2.3)	
Other	2 (0.5)	12 (3)	

n number of joints, *SD* standard deviation

P^a Results of Student *t* test, *P*^b χ^2 test; * *P* < 0.05, ** *P* < 0.0001

and the F plane [22, 23]. In our study, two different methods were used to evaluate the articular eminence inclination, with each reflecting different characteristics of the eminence. The best-fit line method (measurement made by drawing a tangent on the posterior surface of the eminence) involves measurement of the articular eminence posterior surface to reveal the movement path of the condyle. The top-roof line method (measurement of a line connecting the top of the eminence and the roof of the fossa) measures the connection between the top of the eminence and the top of the fossa. Eminence height development can have a major effect on top-roof line measurements. In addition, the top-roof line method reveals the articular eminence morphology.

Sülün et al. [21] evaluated the slope of the eminence on central, medial, and lateral MRI sections where the head of the condyle and glenoid fossa are visible. The central section of the eminence is the steepest portion, and thus the

most appropriate section for evaluating the eminence. This section is one of the best images for achieving accurate measurements and has been used in several studies [6, 24]. In our study, measurements of the eminence were made on the section equivalent to a reference line passing from the sagittal oblique direction in sections of 1-mm thickness and taken at 1-mm intervals on the axial section where the head of the condyle is widest, at the middle part of the condyle and the orbitale.

The height of the articular eminence may be an important factor in TMD. Sülün et al. [21] proposed that a steep slope of the eminence forms a basis for the development of reducing disc displacement, following a study involving the use of MRI to evaluate 52 symptomatic patients and 25 asymptomatic patients. In a study of 34 asymptomatic individuals and 71 patients, Ren et al. [23] described that a steep slope of the eminence is more apparent in asymptomatic individuals than in patients with TMD. The reduced

eminence height in patients may arise through degenerative changes in the bone or remodeling. Likewise, Keller and Carano [20] reported that the angle between the occlusal plane and the eminence ridge is lower in patients with TMD than in asymptomatic patients, and therefore a low eminence angle can be a predisposing factor for TMD. In the present study, both the height and inclination of the eminence were lower in the TMD group than in the asymptomatic group. However, other studies have reported contrary results [25, 26]. The reason for the discrepancy may be that the measurements in the previous studies were made on conventional radiography.

Morphological differences in craniofacial structures between men and women become evident during adolescence as a result of the influence of sex hormones [3]. In addition, the intensity of the functional forces carried over the TMJ during functional activities such as chewing may differ between men and women and may cause morphological differences between the sexes [27]. Previous studies reported a lower eminence height in women compared with men [6, 9, 24, 28]. Our results are consistent with these earlier studies.

Previous studies have indicated that morphological changes occur as the eminence flattens with advanced aging [28, 29]. Our observations are consistent with these studies. In the asymptomatic group, the eminence inclination was lowest in individuals aged 16–20 years, peaked in individuals aged 21–30 years, and was relatively lower in patients aged >30 years. However, there was no association between age and eminence anatomy among the patients in the TMD group.

In a study that utilized MRI to evaluate 47 individuals, Major et al. [30] demonstrated a relationship between disk displacement and variation in condyle position. Paknahad et al. [8] argued that the Ajs has lower values than the Sjs and Pjs in normal joints. The posterior position of the condyle in the mandibular fossa varies widely among patients with TMD. Likewise, in our study, the condyle was positioned more toward the posterior in the TMD group. However, the Ajs and Pjs were similar in the asymptomatic group. Contrary to this, some studies have concluded that dysfunction is not related to the position of the condyle and that the condyle may be positioned toward the posterior in asymptomatic individuals [5, 31].

In a study of condyle size using autopsy specimens, Solberg et al. [32] found a significant difference in condyle width between female and male specimens. Likewise, in a study using CBCT, Al-Koshab et al. [33] found that the condyle width was greater in males than in females on coronal sections. In sectional radiological examinations, the axial section is the most appropriate for evaluating condyle width and thickness. In our measurements made on axial images, the condyle width

was greater in males than in females in both groups. The anteroposterior width of the condyle was significantly different in asymptomatic males and females, but did not differ meaningfully in patients with TMD. A limited number of studies have evaluated anteroposterior condyle width and mediolateral width in patients with TMD relative to healthy subjects. Okur et al. [34] measured condyle width in both the anteroposterior direction and mediolateral direction using CT, and reported significant differences between patients and control subjects. In our study, condyle width was greater in the asymptomatic group.

In the present study, the joint spaces varied significantly within the asymptomatic group. In the TMD group, we observed significant sex differences in the Sjs only. While these results are consistent with those of Kinniburgh et al. [35], Ikeda and Kawamura [36] did not report differences in joint spaces related to sex.

In a study of condyle shapes in skulls, Yale et al. [37] reported that convex and oval shapes are much more common. Likewise, Matsumoto et al. [38] classified condyle shapes as convex, angled, flat, and other, with the convex type being the most frequently observed condyle shape in both normal joints and joints with disorders. Farias et al. [39] evaluated coronal condyle morphology on MRI, and also reported that the convex shape is the most common. In the present study, an oval-shaped condyle was the most common.

Matsumoto et al. [38] reported that the most common fossa shape was concave in a study classifying fossa shapes as concave, angled, flat, and other. Using lateral tomograms of 47 individuals with Class II Division 2 malocclusion, Katsavrias [40] evaluated sections passing through the middle line of the fossa to evaluate the shape of fossa. The fossa shapes were classified as oval, triangular, trapezoidal, and angled. The most commonly observed fossa shape was oval in that study. Different classification systems have been used in different studies. Thus, convex shapes in one study might be classified as round or oval in other studies. Differences in the images used for the analyses may also have affected the interpretation. Therefore, small differences between studies may reflect differences in methodology rather than anatomic variation. Other reasons for variation among published studies may include differences in study populations, numbers of individuals evaluated, horizontal reference planes, and measurement and visualization techniques. These considerations should be taken into account when summarizing the cumulative results of numerous studies on the subject of TMJ anatomy.

In conclusion, the articular eminence inclination was steeper in the TMD group than in the asymptomatic group in the present study. The presence of TMD was associated with the condyle position in the TMJ. Our data could

help toward a better understanding of anatomic variation between asymptomatic individuals and patients with TMD. Compliance with Ethical Standards

Conflict of interest Yasin Yasa and Hayati Murat Akgül declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all patients for being included in the study.

Research involving human participants and/or animals All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

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