Original Article

Angular and positional changes of the maxillary third molars after orthodontic treatment with different premolar extraction patterns

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ABSTRACT

Objectives: To evaluate the angular and positional changes of the maxillary second (M2) and third molars (M3) after orthodontic premolar extraction treatment according to patient skeletal classification and growth pattern.

Materials and Methods: Panoramic radiographs of patients treated with extraction of the first or second premolars (n = 116) and patients treated without extraction (n = 92), taken before orthodontic treatment (T0) and after completion of multibracket appliance therapy (T1) were analyzed. Angle classification, growth pattern, crowding, and incisor inclination were recorded. The palatal (PP) and interorbital planes (IOP) were used as reference lines. Changes in the M3 angulation relative to PP and IOP (T0–T1) within the same group were evaluated with paired *t*-tests. One-way analysis of variance (ANOVA) and Kruskal-Wallis tests were used for comparisons between the groups. Accordingly, pairwise comparisons were performed with Mann-Whitney *U*-tests or independent *t*-tests ($P < .05$).

Results: The M3 angulation related to the PP and the IOP did not differ significantly between the extraction and nonextraction groups. The M2 angulation improved in the premolar extraction group between T0 and T1 (M2/PP, $P < .001$). According to Archer's classification, the change in the vertical position of M3 differed significantly between the extraction and nonextraction groups ($P < .001$).

Conclusions: The angulation of M3 improved over time regardless of the extraction decision. The vertical eruption pattern of M3 was positively influenced only in the extraction group. M2 became significantly more upright in the orthodontic extraction treatment groups. (*Angle Orthod.* 2023;93:135–143.)

KEY WORDS: Third molars; Maxillary premolar extraction; Orthodontic treatment; Molar angulation; Extraction therapy

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INTRODUCTION

The impaction rate for the third molar (M3) is higher than any other tooth, approaching 24% worldwide.[1] M3 impaction is more frequently observed in the mandible than in the maxilla.[2] The leading causes of maxillary impaction are the poor compensatory bone apposition at the posterior periosteal maxillary tuberosity[3] and the limited space available in the retromolar region.[4]

M3s show significant variability in size, shape, mineralization time, position, and eruption pathway.[5] Commonly cited complications of unerupted M3s are pericoronitis, root resorption, periodontitis, infections, and cysts.[6] Nevertheless, M3 prophylactic extraction is still controversial. Asymptomatic M3s are not necessarily disease free. Therefore, many authors support prophylactic extraction.[7] In contrast, other investigators consider prophylactic extraction an unnecessary surgical risk.[8]

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The impaction incidence of M3s may be lower in patients with orthodontic premolar extraction due to mesial movement of the posterior teeth. However, a recent systematic review showed an overall low evidence level, with only moderate evidence for M3 angulation after maxillary extraction treatment of Class II patients.[9] Consequently, it remains unclear whether extraction therapy promotes M3 eruption into a more functional position.

The location of the extracted tooth relative to the M3 has been proposed as a prognostic factor for eruption.[10] Nevertheless, no reliable evidence addresses differences in M3 angulation after first or second premolar extraction in the maxilla. Other factors such as maxillary growth potential, crowding, incisor inclination, and anchorage mechanics may define the absolute effect of extraction therapy on M3s.[11]

Therefore, this study aimed to investigate the effect of orthodontic first and second premolar extraction therapy on the position and angulation of maxillary second (M2s) and third (M3s) molars and evaluate whether the response varies based on patient skeletal classification and growth pattern.

MATERIALS AND METHODS

This multicenter retrospective study was carried out by evaluating standardized pre- and posttreatment panoramic radiographs (OPGs) of 208 growing patients undergoing extraction or nonextraction orthodontic treatment with multibracket fixed appliances. OPGs, taken before the fixed appliance therapy (T0) and at the end of orthodontic treatment (T1), allowed evaluation of M3 developmental stages according to Demirjian's classification[12] system. M3s included were at least at developmental stage 4.

At least one maxillary premolar extraction was performed in 116 patients (45 males, 71 females, mean age: 13.64 $\pm$ 2.64 years at T0). Patients were allocated according to the extraction of the first (group PM1; $n = 87$) or second (group PM2; $n = 29$) premolar. In case of a single premolar extraction ($n = 2$), the other side was discarded and not evaluated. Thirty-two patients had extractions only in the maxilla, and 87 had extractions in both jaws. Ninety-two orthodontic patients (42 males, 50 females) with a mean age of 13.20 $\pm$ 1.74 years at T0 had nonextraction orthodontic treatment and served as controls (Table 1). The data were obtained from the Department of Orthodontics and Dentofacial Orthopedics, Charité – Universitätsmedizin Berlin, Athens Naval and Veterans Hospital, and a specialized orthodontic private practice in Berlin. Ethics committee approval was obtained from the Charité – Universitätsmedizin Berlin (EA2/231/18). Patients were excluded if the M3s at T0 had already erupted or if there were dental abnormalities (hypo-/hyperdontia, impaction, dental transposition, microdontia, or cysts), distalization mechanics, congenital malformations, or syndromes.

The panoramic radiographs were digitized using a scanner (Epson Expression 1680 Pro, SEIKO Epson Corporation, Suwa, Nagano, Japan) and traced with Sidexis XG software (version 2.63, Sirona Dental Systems, Bensheim, Germany) for the angular assessments. The interorbital plane (IOP) was constructed between the most inferior points of the right and left orbital rims. The palatal plane (PP) was defined as a line tangent to the cranial contour of the hard palate. The long axes of the M2s and M3s were the bisecting lines of the maximum mesiodistal width of each molar following the pulp chamber course (Figure 1). The following angles were recorded:

- M3/IOP: formed by the M3 long axis and the IOP
- M3/PP: M3 long axis and PP
- M2/IOP: M2 long axis and IOP
- M2/PP: M2 long axis and PP

A decrease of these angles from T0 to T1 indicated an uprighting of the molars. In addition, the angulation and vertical position of the M3s were recorded using a modification of Archer's classification[13] (Figures 2 and 3). Archer's classification stages with a similar eruption prognosis were merged to facilitate the comparison among the groups:

1. Archer's inclination stages 1 and 2 (questionable), stage 3 (good), and stages 4, 5, 6, and 7 (poor)
2. Archer's vertical classification stages 1 and 2 (good), 3 and 4 (questionable), and 5 (poor).

Statistical Analysis

Statistical analyses were performed using SPSS (version 27, IBM, Armonk, NY). Sample size determination performed a priori for detecting differences in M3 inclination (power = 80%, $\alpha = .05$) required 27 patients in each group (G*Power, Heinrich-Heine-Universität Düsseldorf, Germany).

Thirty randomly selected radiographs were assessed twice by two authors at an interval of 1 month. Intra- and interclass or Cohen's Kappa correlation coefficients were calculated, as applicable. Descriptive statistics, including frequencies and percentages, were calculated for the categorical variables. All continuous variables were tested for normal distribution (Kolmogorov-Smirnov test).

Differences between T0 and T1 within the same group were evaluated using paired *t*-tests. Comparisons among the groups were assessed by one-way analysis of variance (ANOVA) and Kruskal-Wallis H. Indepen-

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Table 1. Descriptive Statistics of the Sample[a]

	Nonextraction Group (n = 92) (%)	Extraction Group (n = 116; PM1 = 87, PM2 = 29) (%)	*P* Value	
Skeletal classification			.117	NS
Class I	38 (41.8)	31 (27.9)		
Class II	41 (45.1)	63 (56.8)		
Class III	12 (13.2)	17 (15.3)		
Angle's classification			.324	NS
Class I	38 (41.3)	41 (33.7)		
Class II	47 (51.1)	59 (50.9)		
Class III	7 (7.61)	16 (13.8)		
Growth pattern			.002*	S
Neutral	49 (54.4)	33 (33.7)		
Vertical	19 (21.1)	44 (44.9)		
Horizontal	22 (24.4)	21 (21.4)		
Dental crowding			< .001*	S
No crowding	36 (42.4)	10 (11.2)		
Light crowding (<3 mm)	31 (36.5)	27 (30.3)		
Medium crowding (3–5 mm)	8 (9.41)	22 (24.7)		
Severe crowding (>6 mm)	10 (11.8)	30 (33.7)		
Overjet			.463	NS
Reduced or negative (≤1 mm)	5 (5.43)	9 (8.33)		
Normal (2–3 mm)	33 (35.9)	31 (28.7)		
Increased (>3 mm)	54 (58.7)	68 (63.0)		
Overbite			.032*	S
Reduced (≤1 mm)	12 (13.0)	19 (17.4)		
Normal (2–3 mm)	25 (27.2)	45 (41.3)		
Increased (>3 mm)	55 (59.8)	45 (41.3)		
Upper incisor inclination (Is–NL)			.404	NS
Proclined	16 (17.6)	11 (11.7)		
Orthoaxial	58 (63.7)	60 (63.8)		
Reclined	17 (18.7)	23 (24.5)		

[a] PM1 indicates first premolar extraction; PM2, second premolar extraction; NS, not significant; S, significant. Chi-square test was used (if criteria were not met, Fisher's exact test was used).

* *P* values <.05 are considered significant.

dent *t*-tests or Mann-Whitney *U*-tests were used for pairwise comparisons and adjusted using Hochberg's step-up Bonferroni method. The chi-square test was used to calculate the differences among groups for the categorical variables. In addition, univariate regression analysis was performed to detect those variables that could positively affect the posttreatment M3 vertical position. Only the variables with $P < .20$ were tested in a multivariate regression model. The threshold for the statistical significance for all tests was set at $P < .05$.

RESULTS

Reproducibility measures achieved an intraclass correlation coefficient of greater than 0.93. The interclass correlation coefficient ranged between 0.87 and 1.00 for all measurements, showing high reproducibility. Cohen's Kappa tests were used for categorical variables and showed a value of 0.87 for the Demirjian classification ($P < .001$). It ranged between 0.92 and 1.00 for both of Archer's classifications ($P < .001$).

There was a statistically significant improvement in the median M2 angulation in the PM1 and PM2 extraction groups compared with the nonextraction group (M2/PP angle, $P < .05$; Table 2).

The M3 angulation to the PP and the IOP did not differ significantly between the extraction and nonextraction groups.

The groups showed no statistically significant changes in the M3 Archer's inclination classification at the defined time points. However, the M3 vertical position, according to Archer's classification,[13] showed statistically significant changes in the extraction group compared with the nonextraction group ($P < .001$; Figure 4). At T0, no M3 occlusal surface was more erupted than the cementoenamel junction of the M2. At T1, the M3 was erupted beyond the cementoenamel junction of the M2 in 14.80%, 33.90%, and 39.60% of the nonextraction, PM1, and PM2 groups, respectively.

None of the studied variables showed a statistically significant difference between the two extraction groups.

In a univariate regression analysis, the variables tested for significance of M3 vertical position according to Archer's classification[13] at T1 were premolar

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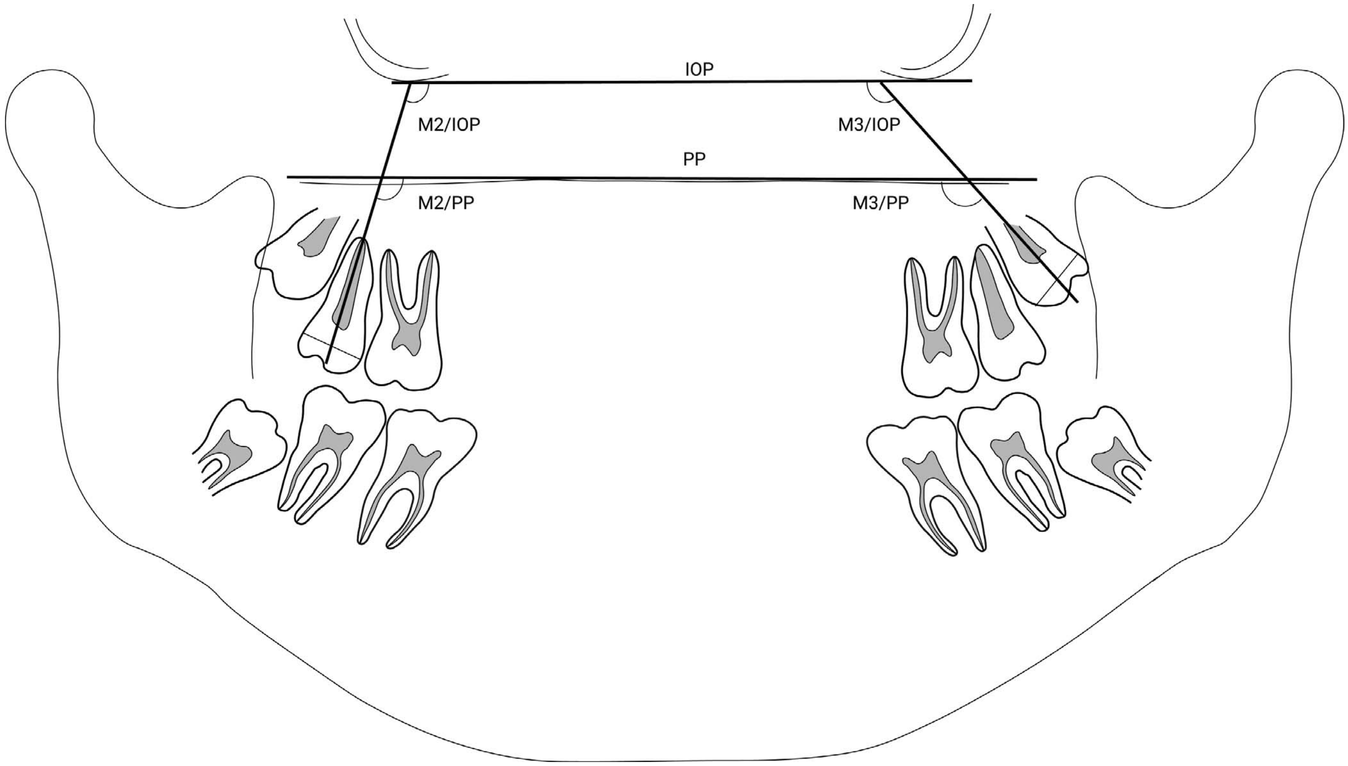

Figure 1. Reference lines and angles used in this study.

extraction, Angle classification, skeletal classification, growth pattern, crowding, overjet, overbite, and maxillary incisor inclination. Only premolar extraction and the Angle Class showed $P < .20$ and entered the multivariate regression model (Table 3). Patients with PM1 or PM2 extraction had a significantly higher probability of a good vertical prognosis according to Archer (stage 1 or 2) at T1 compared with the nonextraction group (odds ratio [OR] = 2.776; 95% confidence interval [CI]: 1.63 to 4.79, $P < .001$). Angle Class III patients had a lower probability of a favorable score (Archer's vertical stages 1 or 2) compared with Class I patients (OR = 0.0318, 95% CI: 0.109 to 0.924, $P = .035$; Table 3).

DISCUSSION

This study aimed to investigate whether premolar extraction therapy positively influenced the angulation and vertical position of maxillary M3s. In addition, M2 angular changes in these patients were assessed. Apart from the M3 morphology and mesiodistal width, the available retromolar space[14] and the eruption pathway[11] are crucial for M3 eruption.

The current study showed that premolar extraction treatment did not improve maxillary M3 angulation during the observation period. These results were in agreement with previous findings,[15] in which nonsignificant M3 angulation changes over time were observed between extraction and nonextraction groups. In a comparative study, although mandibular M3 angulation significantly improved with premolar extraction therapy, maxillary M3 showed no significant difference between extraction and nonextraction therapy.[5]

There was a statistically significant difference in growth pattern ($P = .002$), dental crowding ($P < .001$), and overbite ($P = .03$) between the extraction and nonextraction groups (Table 1), justifying the extraction treatment approach. Previous studies have used the occlusal[5,16–21] or the mandibular[11,21] planes to evaluate angulation changes. It has been argued that these planes are susceptible to developmental and orthodontic modifications. The PP is also subject to remodeling processes,[22] but it is considered more stable during growth and orthodontic treatment.[23] Therefore, this study used PP and IOP as reference lines. The selected planes are not exactly parallel to each other (eg, due to slight asymmetry in the infraorbital points), which explains the observed differences in the results. Because the IOP is easier to locate, not subjected to growth changes, and is widely accepted, it is suggested that IOP be used in future studies.

Linear measurements have been considered unreliable on conventional radiographs, since they are influenced by projection and magnification.[24] Because

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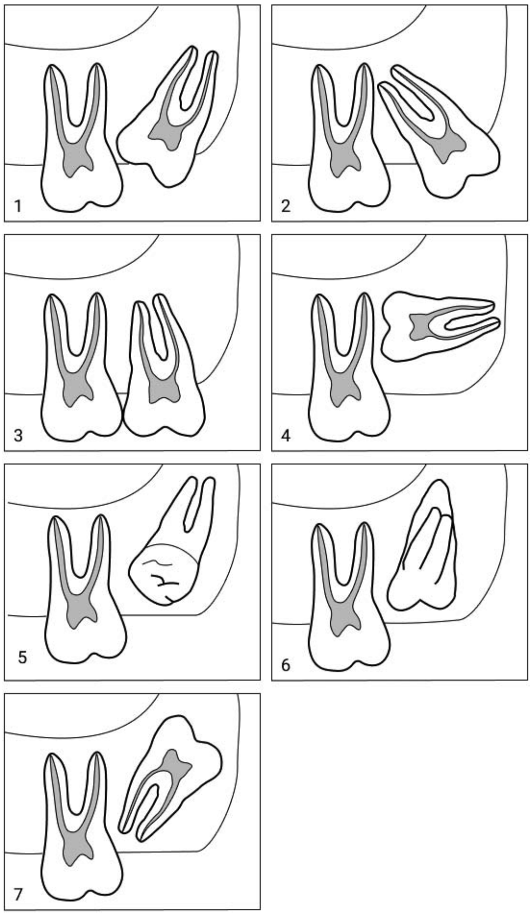

Figure 2. Archer's qualitative classification of maxillary M3 inclination relative to the long axis of M2: (1) mesioangular, (2) distoangular, (3) vertical, (4) horizontal, (5) buccoangular, (6) linguoangular, (7) inverted.

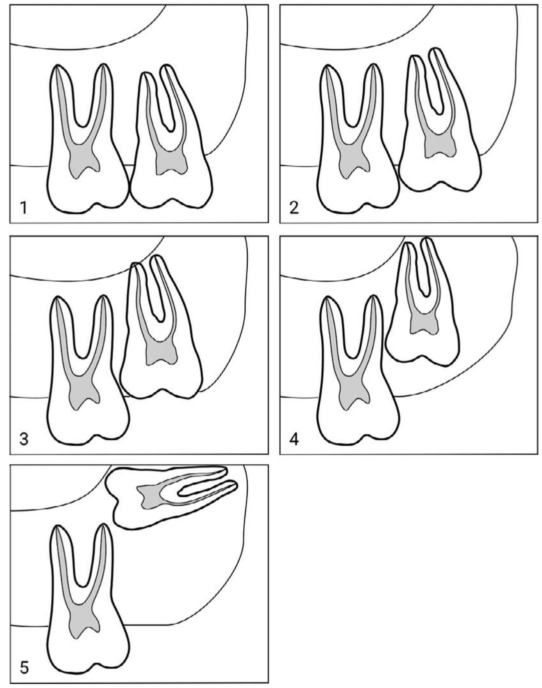

Figure 3. Archer's qualitative classification of maxillary M3 vertical position compared with the M2s. (1) M3 and M2 occlusal surfaces at the same level. (2) Occlusal surface erupted past the cementoenamel junction of the M2. (3) Occlusal surface at the level of the cementoenamel junction. (4) Occlusal surface not erupted to the cementoenamel junction. (5) Occlusal surface above the apex of the M2.

radiographs taken at different time points (T0, T1) and orthodontic practices were analyzed in this study, only angular variables on panoramic radiographs were considered.[25,26] In contrast to other investigators,[27] no linear measurements were carried out. Vertical position changes of the M3 were assessed using Archer's classification (Figure 3).[13]

There are concerns about the accuracy of lateral cephalograms for assessing angular variables related to asymmetrically superimposed teeth, the contralateral M3s. In addition, lateral cephalograms are not considered a normal part of standard final records for orthodontic patients according to the radiation guidelines in Germany for radiation exposure protection. Yet, cephalograms were assessed at T0 to determine the skeletal pattern, facial configuration, and initial inclination of the maxillary incisors.

A recent study used CBCT images to analyze the spatial position of maxillary M3s and found that angulation changes after premolar extraction were not significant.[28] Three-dimensional (3D) images allow for the evaluation of the rotational changes of the M3s, showing significant outward rotation of the M3 crowns in the extraction group.[28] However, 3D images for increased diagnostic accuracy are not yet performed routinely because of their higher x-ray exposure and cost.

Appliances or mechanics that hold or tip back the molars have been considered to increase the risk of M3 impaction.[29] Therefore, patients who had been treated with maxillary distalization mechanics (eg, headgear, fixed intermaxillary Class II appliances, or mini-screws) were excluded from the present study. However, recent studies indicated that the impact of these appliances on the retromolar space available and M3 eruption might be overestimated.[27,30] A study by Kang et al.[27] suggested that M3s erupted spontaneously over the long term despite their position after maxillary molar distalization with modified C-palatal plates.

A general shortcoming of the published studies on this topic is the small patient sample sizes and the limited information available on the craniofacial and dental characteristics of the groups involved. Despite the retrospective character of the current study, the patients included were well documented and matched in their initial characteristics, thus limiting potential

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Table 2. Comparison of the Mean and Median of the Pairwise Comparisons for All Mentioned Classifications[a]

						Pairwise Comparison		
Variable	Measurement	NE	PM1	PM2	*P* Value	*P* Value NE vs PM1	*P* Value NE vs PM2	*P* Value PM1 vs PM2
3M/IOP	T0							
	Mean (SD)	131° (13.4)	126° (13.4)	130° (15.8)	.015*[b]	.013*[c]	.930[c]	.249[c]
	Median [range]	131° [121; 142]	128° [117; 135]	132° [122; 142]	.002*[d]	.002*[e]	.986[e]	.128[e]
	T1							
	Mean (SD)	118° (14.0)	113° (12.7)	114° (14.8)	.013*[b]	.016*[c]	.150[c]	.998[c]
	Median [range]	120° [107; 127]	112° [104; 124]	112° [102; 126]	.013*[d]	.015*[e]	.125[e]	.933[e]
	T0–T1							
	Mean (SD)	13.1° (12.2)	12.9° (15.0)	16.7° (13.0)	.263[b]	.992[c]	.290[c]	.262[c]
	Median [range]	13.7° [5.77; 20.9]	12.9° [4.08; 21.0]	16.1° [10.6; 27.3]	.196[d]	.895[e]	.164[e]	.164[e]
3M/PP	T0							
	Mean (SD)	131° (13.4)	127° (13.8)	129° (15.5)	.004*[b]	.003*[c]	.808[c]	.518[c]
	Median [range]	130° [121; 140]	129° [118; 135]	130 [123; 139]	.067[d]	.065[e]	.730[e]	.352[e]
	T1							
	Mean (SD)	118° (13.2)	114° (13.1)	113° (13.8)	.007*[b]	.002*[c]	.003*[c]	.739[c]
	Median [range]	120° [109; 126]	114° [105; 123]	112° [101; 120]	.005*[d]	.017*[e]	.020*[e]	.481[e]
	T0-T1							
	Mean (SD)	12.7° (12.2)	12.6° (14.5)	16.6° (12.2)	.130[b]	1.000[c]	.139[c]	.140[c]
	Median [range]	12.6° [5.92; 21.3]	12.7° [4.42; 20.9]	16.6° [10.1; 26.1]	.063[d]	.961[e]	.057[e]	.059[e]
2M/IOP	T0							
	Mean (SD)	116° (8.82)	115° (8.87)	116° (8.45)	.208[b]	.237[c]	.987[c]	.418[c]
	Median [range]	116° [110; 123]	115° [109; 121]	116° [112; 121]	.300[d]	.406[e]	.918[e]	.406[e]
	T1							
	Mean (SD)	110° (7.80)	106° (8.28)	105° (7.81)	<.001*[b]	<.001*[c]	.001*[c]	.587[c]
	Median [range]	109° [105; 114]	106° [101; 112]	105° [99.6; 110]	<.001*[d]	.001*[e]	.001*[e]	.302[e]
	T0–T1							
	Mean (SD)	6.15° (9.29)	8.06° (10.60)	11.5° (8.04)	.007[b]	.242[c]	.005*[c]	.118[c]
	Median [range]	7.15° [1.80; 11.5]	9.20° [0.63; 15.6]	11.8° [6.12; 17.8]	.005[d]	.136[e]	.003*[e]	.071[e]
2M/PP	T0							
	Mean (SD)	116° (8.44)	115° (12.2)	117° (8.70)	.321[b]	.517[c]	.808[c]	.362[c]
	Median [range]	117° [110; 122]	116° [109; 123]	118° [111; 120]	.707[d]	.739[e]	.739[e]	.739[e]
	T1							
	Mean	111° (8.09)	112° (78.7)	105° (8.02)	.632[b]	.945[c]	.734[c]	.603[c]
	Median [range]	110° [105; 115]	106° [99.9; 112]	105° [98.9; 110]	<.001*[d]	<.001*[e]	<.001*[e]	.221[e]
	T0–T1							
	Mean (SD)	5.77° (9.30)	2.74° (80.0)	12.7° (8.98)	.473[b]	.851[c]	.666[c]	.443[c]
	Median [range]	6.50° [0.00; 11.9]	9.40° [1.93; 17.6]	12.7° [7.30; 19.6]	<.001*[d]	.005*[e]	<.001*[e]	.005[e]
Archer (inclination), %	T0							
	Stage 1 and 2	43.4	40.2	49.1				
	Stage 3	55.5	59.8	49.1				
	Stage 4, 5, 6, and 7	1.10	0.00	1.89				
	T1							
	Stage 1 and 2	44.0	31.5	28.3				
	Stage 3	55.5	67.9	71.7				
	Stage 4, 5, 6, and 7	0.55	0.61	0.00				
	T0–T1 change (to stage 3)				.140[f]	.215[f]	0.940[f]	.503[f]
Archer (vertical), %	T0							
	Stage 1 and 2	0	0	0				
	Stage 3 and 4	87.9	82.3	77.4				
	Stage 5	12.1	17.7	22.6				
	T1							
	Stage 1 and 2	14.8	33.9	39.6				
	Stage 3 and 4	84.6	65.5	58.5				
	Stage 5	0.55	0.61	1.89				
	T0–T1 (to stage 1 and 2)				<.001*[f]	<.001*[f]	<.001*[f]	.557[f]
Demirjian, %	T0				.013*[f]	.017*[f]	.180[f]	.880[f]
	Stage 1, 2, 3, and 4	85.7	73.2	75.5				
	Stage 4, 5, 6, 7, and 8	14.3	26.8	24.5				
	T1			3.77	.694[f]	.985[f]	.985[f]	1.000[f]
	Stage 1, 2, 3, and 4	2.75	4.24					
	Stage 4, 5, 6, 7, and 8	97.3	95.8	96.2				

[a] NE indicates nonextraction; PM1, first premolar extraction; PM2, second premolar extraction; T0, before orthodontic treatment; T1, after completion of orthodontic treatment; SD, standard deviation.

[b] One-way analysis of variance test.

[c] Independent *t*-test, with Bonferroni correction.

[d] Kruskal-Wallis H test.

[e] Mann-Whitney *U*-test, with Bonferroni correction.

[f] Chi-square test (if criteria are not met, Fisher's exact test).

* Significant when $P < .05$.

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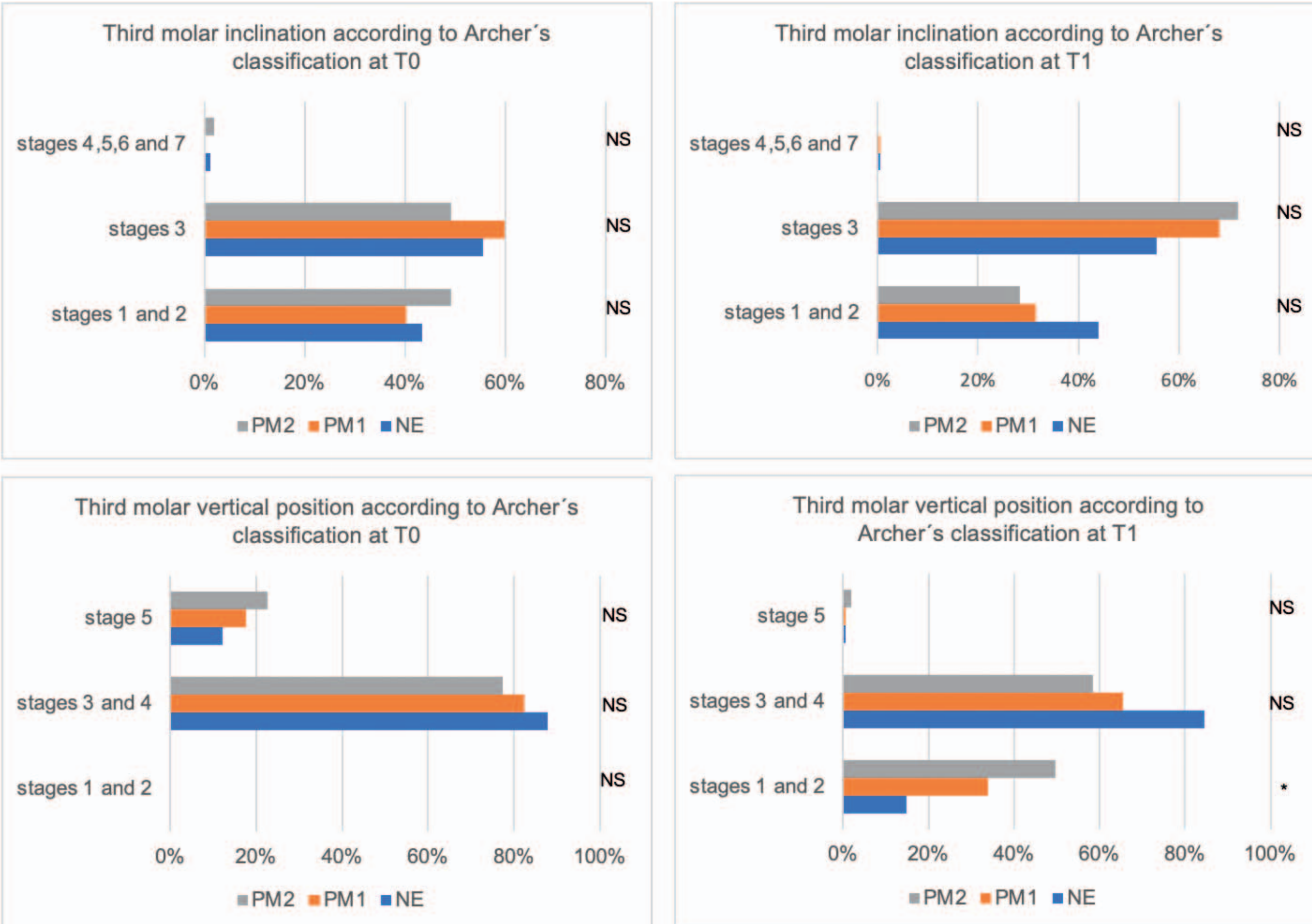

Figure 4. Third molar inclination and vertical position according to Archer's classification in the extraction and nonextraction group before (T0) and after orthodontic treatment (T1). *Archer's vertical position classification stages 1 and 2 were statistically significantly different at T1 between the nonextraction (NE) and the extraction groups (PM1, first premolar; PM2, second premolar extraction; $P < .001$).

confounding variables. The severity of crowding, a decisive factor in extraction therapy, and the anchorage control may determine the space available for molar mesial drifting and the final position of M3s. In addition, due to the ALARA (as low as reasonably achievable) regulations for radiation protection, there was no information on the incisors' final position, which could also be decisive for M3 space available.

In this study, the first and second premolar extraction groups were unevenly distributed, emphasizing the lower frequency of PM2 extractions.

This study aimed to assess the influence of premolar extraction therapy on M3 position in different skeletal facial types. Horizontal facial growth patterns were previously associated with increased retromolar space in the mandible[31,32] but not in the maxilla.[32] In the current study, no association between craniofacial configuration and M3 impaction was found.

The duration of treatment was longer in the extraction groups ($P < .001$), potentially influencing the vertical position of the M3s. However, Demirjian's M3 classification after treatment (T1; Table 2) was not significantly different between the groups ($P = .694$). Finally, it could not be confirmed whether, in some patients, the M3s did not erupt because of the patient's age at T1. Kim et al.[3] found that M3s may erupt at the final root developmental stages even when they appeared impacted. Therefore, further studies with extended follow-up are required to clarify the M3 eruption pathway after orthodontic extraction treatment.

The findings could help clarify the M3 changes caused by orthodontic premolar extraction. M3 angulation changes related to orthodontic extraction treatment might be smaller than previously suggested.

CONCLUSIONS

- A statistically significant improvement could be identified in the vertical position of the M3 after orthodontic premolar extraction treatment compared with the nonextraction group.

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Table 3. Multivariate Regression Analysis for the Prediction of Archer's 3M Vertical Classification at T1 (Dependent Variable: Stages $1 + 2$ According to Archer's Vertical Classification)[a]

Variable	*P*	OR	95 CI
Group			
Nonextraction group	NS		
Premolar extraction group	$<.001^*$	2.776	1.630 to 4.729
Angle's classification			
Class I	.003*		
Class II	.054	1.762	0.911 to 3.132
Class III	.035*	0.318	0.109 to 0.924
Skeletal classification			
Class I	NS		
Class II	NS		
Class III	NS		
Growth pattern			
Neutral	NS		
Vertical	NS		
Horizontal			
Maxillary dental crowding			
No crowding	NS		
<3 mm crowding	NS		
3–5 mm crowding	NS		
≥ 6 mm crowding	NS		
Overjet			
≤ 1 mm	NS		
2–3 mm	NS		
>3 mm	NS		
Overbite			
≤ 1 mm	NS		
2–3 mm	NS		
>3 mm	NS		
Inclination Is at T0	NS		
Constant	.311	0.579	

[a] T0, before orthodontic treatment; T1, after completion of orthodontic treatment; Is, upper incisor; OR, odds ratio; CI, 95% confidence interval; NS, not significant.

* Significant when $P < .05$.

- There was an improvement in the angulation of the M3 regardless of the orthodontic therapy.
- No statistically significant differences were found between the PM1 and PM2 extraction groups.

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