## **ORIGINAL RESEARCH**



# New regression equation for predicting the size of unerupted canines and premolars in a Turkish sample

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#### Abstract

**Background**: One of the methods used for predicting the size of unerupted canines and premolars is regression equaiton. This retrospective study aimed to evaluate the reliability of regression equations developed for predicting the size of unerupted canines and premolars and to develop a new regression equation. Methods: Mesiodistal diameters of all permanent teeth except second molars were measured on orthodontic plaster models of 265 patients (133 females, mean age 15.09 years; 132 males, mean age 15.25 years). Actual values measured with digital caliper on orthodontic plaster models were compared with the predicted values of 3 regression equations developed for the Turkish population. For data analysis, Linear Regression Analysis was used to make measurement predictions. Intraclass correlation was used to evaluate intraobserver reliability. Based on the parameters of this study, a new regression equation was developed. **Results**: Three regression equations developed previously for the Turkish population underestimated the mesiodistal dimension of permanent canines and premolars. Mesiodistal diameters of teeth were significantly different between genders in both the maxillary and mandibular arches. A new regression equation was developed using the data of this study. Conclusions: Based on our population, the new regression equation would provide the closest prediction value for the sum of the mesiodistal widths of unerupted permanent canines and premolars. Therefore, it enables more realistic orthodontic treatment planning to be conducted.

#### **Keywords**

Regression equation; Mixed dentition; Tooth size

## 1. Introduction

During the period of mixed dentition, space analysis is an important part of the diagnostic and treatment procedure in determining whether the treatment plan includes serial extraction, eruption guidance, space maintenance, space regaining or only periodic observation of the patient [1]. Space analysis measurements should therefore be taken carefully [2]. One of the following four methods is usually used: mean values of the mesiodistal dimensions of the permanent canines and premolars, correlation or regression methods, combination of correlation and radiographic methods and radiographic methods [3].

Tooth size may vary between different ethnic groups, gender, genetic and environmental factors [4, 5]. Males' mesiodistal crown widths are consistently greater than females' [5– 9]. Prediction tables and regression equations developed for North Americans may not provide reliable results for other populations, causing treatment planning errors [10]. Turkish populations come from a variety of regions, thus creating a wide variety of gene pools [11]. For the mixed dentition period, the methods developed are only valid within the community where they were developed due to ethnic differences [12]. Clinically, estimation methods developed for different populations should not be used without modifications [13].

Published studies on the estimation of the size of unerupted permanent canines and premolars in the Turkish population show that the reliability of Tanaka and Johnston equations and Moyers probability charts were low [10, 11, 13, 14]. Arslan *et al.* [15] also reported that mesiodistal widths of permanent canines and premolars were overestimated using the Tanaka Johnston equation and were not suitable for the Turkish population. Based on the width of the four permanent incisors in the mandible, they developed a new regression equation [15]. Uysal *et al.* [11] demonstrated a new regression equation with dental models obtained from Turkish patients. Sağlam Aydınatay *et al.* [16] calculated a new regression equation using the sum of permanent first molar, mandibular central and lateral tooth size and gender variable.

It is not clear which of the different regression equations developed for the Turkish population is more reliable. Therefore, this study aimed to evaluate the reliability of the regression equations developed by Arslan *et al.* [15], Uysal *et al.* [11] and Sağlam Aydınatay *et al.* [16] for the estimation of unerupted permanent tooth size in the Turkish population and to develop a new regression equation with the measurements we made in our patients in case of incompatibility and to provide orthodontists with a more realistic tooth size analysis opportunity in treatment planning.

### 2. Materials and methods

This retrospective study was approved by Health Sciences Ethics Committee of Ankara Yıldırım Beyazıt University (06 October 2022-14). Informed consent to participate was obtained from all of the subjects and/or their legal guardian(s). The research material consists of pretreatment orthodontic plaster models of patients undergoing orthodontic treatment between November 2022 and November 2023.

The G-Power 3 programme (G\*Power; version 3.1.9.213, Franz Faul, Universitet Kiel, Dusseldorf, NRW, Germany) was used to calculate the sample size. Using an error margin of  $\alpha = 0.05$ , an effect size of 0.2, and a power of 0.90, 265 orthodontic models were determined. Orthodontic plaster models of 265 Turkish patients (133 females, mean age 15.09; 132 males, mean age 15.25) were included based on the following criteria:

- Turkish parents, living in Ankara.

- Full eruption of all permanent teeth except the third molars.

- Angle Class I molar and canine occlusion.

- Teeth without caries, restorations, fractures, attrition, abrasions or hypoplasia affecting the mesiodistal dimension.

- Absence of dental anomalies such as tooth deficiency, microdontia and macrodontia.

- Orthodontic plaster models with good clarity and quality.

- Abrasion, fractures, air bubbles and excesses that would affect measurements were not present on models.

Exclusion citeria were as follows:

- Systemic or dentofacial deformities.

- Orthodontic or orthognathic treatment history.

- Syndromes or cleft lip and/or palate.

- Presence of cysts or other craniofacial pathology.

A standard impression procedure in the clinic was used to obtain plaster models for the study. Orthodontic plaster models were obtained by taking impressions with disposable plastic impression spoons and orthodontic fast-setting alginate-based hydrocolloid impression material, applying standard procedures during impression disinfection, and casting type 3 dental hard plaster. In this study, the mesiodistal dimensions of all teeth except the permanent second molars were measured. The mesiodistal crown widths of the relevant teeth were measured using a digital caliper (Karl Hammacher GmbH HSL 246-15, Solingen, NRW, Germany) with an accuracy of 0.01 mm. The digital caliper was calibrated before measurements. The digital caliper was held at right angles to the long axis of the teeth and the maximum mesiodistal distance between the buccal and aproximal contact points was measured (Fig. 1). Between models, the eyes rested for 5 minutes to reduce eye fatigue and minimise error. To ensure measurement reliability, only 10 models were measured by a single researcher (RY) per day.

Statistical Method: Data analysis was performed using SPSS 21 package programme (SPSS Inc., Chicago, IL, USA; version 15.0 for Windows). *t*-test was used to evaluate differences between genders and between symmetrical teeth dimensions. Measurement predictions were made using linear regression analysis.

Method error: To assess observer reliability, the same researcher repeated tooth size measurements of randomly selected 30 cases after 1 month. The observer reliability was evaluated by ICC (IntraClass Correlation) method.

#### 3. Results

To evaluate observer reliability, ICC was used to compare the first and second measurements in 30 cases. For all tooth size measurements, the ICC value ranged from 0.870 to 0.984. Both measurements were in very high agreement (Table 1).

The mesiodistal width measurements of the left and right symmetrical teeth of the dental arch did not differ significantly. Therefore, the mesiodistal widths of the symmetrical teeth were summed and averaged (Table 2).

*t*-tests were used to compare mesiodistal width measurements in orthodontic plaster models of male and female patients. In all teeth, the mesiodistal tooth sizes of males and females were statistically significant (p < 0.05). Male's tooth dimensions were discovered to be significantly larger than female's (Table 3).



FIGURE 1. Mesiodistal crown diameter measurements of incisor, premolar and molar teeth using digital caliper.

	IADLE I, I	intraobserver correlation table betwee	en mist and second measur	ements.
Tooth	ICC	95% Confidence	р	
		Lower border	Upper border	
11	0.951	0.898	0.977	< 0.001
12	0.984	0.966	0.992	< 0.001
13	0.870	0.752	0.938	< 0.001
14	0.904	0.873	0.971	< 0.001
15	0.966	0.929	0.984	< 0.001
16	0.947	0.890	0.975	< 0.001
21	0.981	0.960	0.991	< 0.001
22	0.982	0.962	0.991	< 0.001
23	0.947	0.889	0.975	< 0.001
24	0.945	0.885	0.974	< 0.001
25	0.896	0.782	0.951	< 0.001
26	0.904	0.797	0.954	< 0.001
31	0.974	0.946	0.988	< 0.001
32	0.972	0.941	0.987	< 0.001
33	0.931	0.854	0.967	< 0.001
34	0.951	0.897	0.977	< 0.001
35	0.967	0.930	0.984	< 0.001
36	0.971	0.939	0.986	< 0.001
41	0.953	0.902	0.978	< 0.001
42	0.964	0.924	0.983	< 0.001
43	0.952	0.899	0.977	< 0.001
44	0.947	0.888	0.975	< 0.001
45	0.928	0.849	0.966	< 0.001
46	0.959	0.914	0.981	< 0.001

TABLE 1. Intraobserver correlation table between first and second measurements.

ICC: IntraClass Correlation.

## TABLE 2. Comparison of mesiodistal dimensions of the symmetrical teeth on the right and left sides in the upper and

	lower jaws.													
Teeth	eeth Right/Left											<i>t</i> -te	t-test	
				Right					Left					
	n	Mean	Median	Minimum	Maximum	sd	Mean	Median	Minimum	Maximum	sd	t	р	
11–21	265	8.92	8.90	7.47	10.50	0.56	8.90	8.90	7.39	10.40	0.56	0.312	0.755	
12-22	265	7.036	7.050	5.760	8.420	0.548	7.018	7.030	5.760	8.740	0.536	0.387	0.699	
13–23	265	8.09	8.10	6.86	9.30	0.43	8.06	8.07	6.99	9.29	0.44	0.926	0.355	
14–24	265	7.40	7.39	6.13	8.73	0.44	7.41	7.42	6.12	8.50	0.42	-0.393	0.694	
15–25	265	7.13	7.15	6.06	8.27	0.43	7.15	7.19	5.91	8.39	0.45	-0.533	0.594	
16–26	265	10.44	10.40	9.13	12.38	0.51	10.48	10.41	9.15	12.31	0.54	-0.903	0.367	
31-41	265	5.72	5.70	4.63	6.75	0.36	5.71	5.71	4.65	6.82	0.36	0.403	0.687	
32–42	265	6.24	6.21	5.23	7.55	0.39	6.24	6.22	5.25	7.27	0.39	-0.044	0.965	
33–43	265	7.00	6.95	5.75	8.06	0.43	7.00	6.99	5.94	8.07	0.41	-0.102	0.918	
34-44	265	7.39	7.38	6.16	8.54	0.44	7.39	7.37	6.15	8.67	0.45	0.046	0.963	
35–45	265	7.55	7.56	6.22	8.70	0.46	7.53	7.52	6.13	8.86	0.47	0.378	0.706	
36–46	265	11.30	11.31	9.74	12.93	0.62	11.29	11.35	9.79	12.95	0.62	0.151	0.880	

sd: standard deviation.

Tooth						Ger	nder						i	t-test
				Female						Male				
	n	Mean	Median	Minimum	Maximum	sd	n	Mean	Median	Minimum	Maximum	sd	t	р
11	133	8.80	8.81	7.47	9.83	0.48	132	9.03	9.06	7.72	10.50	0.60	3.368	0.001
21	133	8.78	8.83	7.39	10.00	0.49	132	9.02	9.06	7.51	10.40	0.60	3.523	0.001
12	133	6.935	7.010	5.760	8.040	0.519	132	7.138	7.125	5.810	8.420	0.560	3.055	0.002
22	133	6.94	7.02	5.76	8.04	0.52	132	7.10	7.05	5.82	8.74	0.54	2.496	0.013
13	133	7.94	7.99	7.01	9.10	0.42	132	8.25	8.24	6.86	9.07	0.39	6.050	< 0.001
23	133	7.89	7.91	6.99	9.10	0.40	132	8.23	8.24	7.07	9.29	0.41	6.799	< 0.001
14	133	7.31	7.32	6.13	8.51	0.39	132	7.48	7.50	6.36	8.73	0.47	3.248	0.001
24	133	7.35	7.33	6.12	8.50	0.41	132	7.48	7.53	6.63	8.49	0.43	2.475	0.014
15	133	7.06	7.05	6.06	8.19	0.43	132	7.19	7.17	6.20	8.27	0.42	2.479	0.014
25	133	7.08	7.12	5.91	8.39	0.46	132	7.21	7.26	5.96	8.36	0.43	2.466	0.014
16	133	10.27	10.29	9.30	11.70	0.48	132	10.60	10.55	9.13	12.38	0.48	5.699	< 0.001
26	133	10.34	10.29	9.35	11.95	0.53	132	10.62	10.54	9.15	12.31	0.51	4.322	< 0.001
31	133	5.67	5.64	4.87	6.68	0.34	132	5.78	5.76	4.63	6.75	0.38	2.351	0.019
41	133	5.67	5.66	4.68	6.42	0.32	132	5.76	5.78	4.65	6.82	0.38	2.078	0.039
32	133	6.16	6.15	5.37	6.97	0.35	132	6.31	6.27	5.23	7.55	0.42	3.128	0.002
42	133	6.15	6.16	5.25	7.27	0.36	132	6.33	6.33	5.43	7.26	0.39	3.800	< 0.001
33	133	6.78	6.77	5.75	7.80	0.34	132	7.22	7.25	6.17	8.06	0.40	9.580	< 0.001
43	133	6.80	6.80	5.94	7.70	0.35	132	7.21	7.24	6.31	8.07	0.36	9.420	< 0.001
34	133	7.34	7.35	6.25	8.13	0.40	132	7.45	7.44	6.16	8.54	0.46	2.142	0.033
44	133	7.31	7.29	6.28	8.23	0.42	132	7.47	7.48	6.15	8.67	0.46	2.905	0.004
35	133	7.45	7.49	6.22	8.61	0.46	132	7.64	7.64	6.54	8.70	0.44	3.469	0.001
45	133	7.42	7.43	6.13	8.57	0.45	132	7.64	7.60	6.59	8.86	0.47	3.799	< 0.001
36	133	11.11	11.08	9.79	12.52	0.57	132	11.50	11.51	9.74	12.93	0.61	5.313	< 0.001
46	133	11.10	11.08	9.80	12.26	0.59	132	11.49	11.50	9.79	12.95	0.59	5.307	< 0.001

TABLE 3. Comparison of mesiodistal tooth dimensions in the maxilla and mandible according to gender.

sd: standard deviation.

There was a significant difference (p < 0.05) between the actual dimensions measured on plaster models and the predicted values of the regression equation developed by Arslan *et al.* [15], Uysal *et al.* [11] and Sağlam Aydınatay *et al.* [16] (Table 4, Ref. [11, 15]; Table 5, Ref. [16]). The regression equations of Arslan *et al.* [15], Uysal *et al.* [11] and Sağlam Aydınatay *et al.* [16] had significantly lower predictions than the actual tooth dimensions (Tables 4 and 5).

A new regression equation was developed to predict the total mesiodistal dimension of unerupted permanent canines and premolars based on measurements (Tables 6 and 7).

For the new regression equation, the presence of teeth with an estimated mesiodistal width in the maxilla or mandible, as well as the individual's gender, were taken into account both as independent variables.

The dependent variable was significantly explained by three independent variables. Based on the regression equation developed in our study, it was observed that three independent variables explained 53.5% of the dependent variable according to the model predicting the dependent variable using independent variables (Table 6).

The regression equation developed was as follows:

Y = 9.316 + 0.525X + 0.684 (Maxilla/Mandible) - 0.411 (Female/Male)

Y indicates the sum of the mesiodistal width of the unerupted canines and premolars.

Jaw variable should be set to 1 for the mandible and 2 for the maxilla. Gender variable should be set to 1 for male and 2 for female. X indicates the sum of the mesiodistal dimensions of the lower four incisors.

## 4. Discussion

Nowadays, regression equations are the most commonly used methods to estimate unerupted permanent tooth size due to their advantages such as ease of application, not taking much time and not requiring additional equipment such as X-rays. Moyers probability tables and Tanaka Johnston equations developed for European Americans are the most preferred ones [11, 15, 17–20]. The usability of Moyers probability tables and Tanaka Johnston equation have been evaluated with many ethnic groups such as Asian American [19], Syrian [21], Saudi Arabian [17], Jordanian [6], Indian [22], Senegalese [23], Nepalese [24], Iraqi [25] populations. It was observed that unerupted canines and premolars over- or under-predicted mesiodistal crown size. A population-based reorganization of reference values was made to prevent possible clinical errors [17–19, 25].

Many factors affect the reliability of the study findings. A key factor is the size of the material. The accuracy of measurements increases with the number of measurements made by the investigator [26]. This study was conducted on a larger sample size than similar studies in the literature. By using the ICC method, we evaluated intraobserver reliability. As intraobserver reliability increases, the ICC value increases and approaches 1.0 [27]. Our study found that the ICC value ranged from 0.870 to 0.984 for all tooth size measurements. High repeatability and intraobserver reliability were observed for all teeth.

Attention was paid to standardization in obtaining orthodontic plaster models. To obtain dental models in our study, alginate impression material and dental hard plaster were used in many studies in the literature [6, 15, 16, 25, 28–36]. Statistically significant differences were found when the same measurements were performed by different investigators [26,

**TABLE 4.** Agreement of the actual mesiodistal size of unerupted teeth with the equation developed by Arslan *et al.* [15] and Uysal *et al.* [11] (y = mesiodistal width of unerupted maxillary canine and premolar teeth, x = sum of the mesiodistal width of the four permanent incisors).

width of the four permanent incisors).									
		Arslan et al. [15] equations	Uysal et al. [11] equations						
		Female	Female						
		maxilla $y = 9.775 + 0.50x$	maxilla $y = 5.32 + 0.71x$						
		mandible $y = 9.145 + 0.50x$	mandible $y = 4.51 + 0.71x$						
		Male	Male						
		maxilla $y = 9.98 + 0.50x$	maxilla y = $3.82 + 0.78x$						
		mandible $y = 9.54 + 0.50x$	mandible $y = 4.17 + 0.73x$						
Maxilla									
Male	Ζ	-8.782	-3.565						
Wide	р	< 0.001	<0.001						
Female	Ζ	-7.674	-3.013						
1 childle	р	< 0.001	0.003						
Mandible									
Male	Ζ	-8.410	-6.305						
Ividic	р	<0.001	<0.001						
Female	Ζ	-7.154	-3.524						
remate	р	< 0.001	< 0.001						

TABLE 5. Agreement of the actual mesiodistal size of unerupted teeth with the equation developed by Sağlam
Aydinatay et al. [16] (X0 indicates gender, the value 2 is used for women and 1 for men. X1 refers to the sum of the tooth
size widths of the permanent upper first molar, lower central and lateral incisors).

	1 11 /	,
	Sağlam Aydinatay et al. [16] equations	
	maxilla Y = 5.243 - 0.249(X0) + 0.386(X1)	
	mandible $Y = 5.008 - 0.227(X0) + 0.378(X1)$	
Maxilla		
Ζ	-8.915	
р	< 0.001	
Mandible		
Ζ	-6.671	
р	<0.001	

#### TABLE 6. New regression equation for determining mesiodistal crown diameters of unerupted canines and premolars.

	R	R Square	Adjusted R Square	Durbin-Watson	And	ova
					F	р
1	0.733	0.538	0.535	2.103	203.776	0.0001

a. Independent Variables: (Constant value), Female/Male, Maxilla/Mandible, X

b. Dependent variables: Y

IADLE 7. Coefficients of the newly developed regression equal	ΤA	ſ.	A	B	L	Е	7.	<b>Coefficients</b>	of	the	newly	develo	ped	regression	eq	uatio	n	۱.
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Variable	Coefficient	р
(Constant value)	9.316	< 0.001
Х	0.525	< 0.001
Maxilla/Mandible (2 Maxilla/1 Mandible)	0.684	< 0.001
Female/Male (2 Female/1 Male)	-0.411	< 0.001

37]. Based on these data, all measurements used in our study were performed by the same researcher (RY).

It was reported that plaster models measured 0.1 mm larger on average [26]. Intraoral measurements are harder than plaster models. Since precisely obtained dental plaster models are more reliable and precise in the measurement of tooth dimensions, plaster models were preferred in our study [25, 26, 32].

Tooth sizes may vary by genetic factors, society and region. In previous studies, patients were considered suitable if they had at least one generation of ancestors who belonged to that community [11, 12, 16, 18, 19, 23, 24, 28]. In our study, we paid attention to the Turkish patients and parents.

Orthodontic callipers [14], Boley gauge [18, 19, 21, 28, 35, 36] or digital callipers [6, 10–13, 15, 17, 23–25, 31–34, 38–41] are commonly used to measure mesiodistal tooth size. With a caliper, Hunter and Priest found it was more accurate and easier to measure tooth dimensions than with a compass [26].

Researchers have reported significant differences between the mesiodistal dimensions of the teeth on the right and left sides of the dental arch, but many report these differences insignificant. In our study, mesiodistal crown size measurements of symmetrical teeth showed no statistically significant differences (p > 0.05). In general, studies used the average of the right and left sides measurements for tooth size analysis [11, 15, 19, 25], while some used the measurement values from either size [16, 39]. According to many studies, we used the average of right and left side symmetrical tooth size measurements [11, 15, 19].

Based on gender-related tooth sizes, male and female tooth sizes differed statistically significantly (Table 3) (p < 0.05). Males have larger mesiodistal tooth sizes than females. The maximum difference in mesiodistal crown size between male and female was observed in maxillary and mandibular canines, maxillary and mandibular permanent first molars and mandibular lateral teeth. The least difference was observed in mandibular right first premolars.

The difference between the regression equation of Arslan *et al.* [15] and the actual tooth size was statistically significant (Table 4) (p < 0.05). The regression equation of Arslan *et al.* [15] gave a smaller prediction than the actual tooth size. Sağlam Aydınatay *et al.* [16] tested the reliability of the regression equation developed by Arslan *et al.* [15] and reported that it underestimated tooth size in accordance with our findings. Possibly, the reason for this difference is that Arslan *et al.*'s [15] sample group was made up of individuals from southeastern Anatolia region of Turkey, while our sample group was made up of individuals from central Anatolia region of Turkey.

The difference between Uysal *et al.*'s [11] regression equation and the actual tooth size was statistically significant (Table 4) (p < 0.05). Uysal *et al.*'s [11] regression equation pre-

dicted smaller dimensions than the actual dimensions. However, results closer to the actual Y value were obtained according to Arslan *et al.*'s [15] regression equation. Uysal *et al.*'s [11] regression equation developed for the maxilla in females gave the closest value to the actual Y value. Sağlam Aydınatay *et al.* [16] also tested the reliability of Uysal *et al.*'s [11] regression equation and reported that Uysal *et al.*'s [11] regression equation overestimated mesiodistal widths of maxillary canine and premolar teeth and underestimated mesiodistal widths of mandibular canine and premolar teeth.

The difference between Sağlam Aydınatay *et al.*'s [16] regression equation and the actual tooth size was statistically significant (Table 5) (p < 0.05). Sağlam Aydınatay *et al.*'s [16] regression equation underestimated the actual tooth size.

Ankara is thought to better reflect Turkish society since it is the capital, has more education and job opportunities, is centrally located, and receives a lot of migration from other provinces. Our sample size is larger than previously reported studies, which increases the reliability of our findings. Additionally, the homogeneous gender distribution of the sample provides more accurate regression equation results. A continuous change in tooth sizes occurs in populations over time, which indicates that the current mixed dentition period space analyses need to be modified periodically [8, 34, 42, 43]. For these reasons, our study is important for being conducted on a larger sample than previous studies and being up-to-date.

We found that the regression equations developed for the Turkish population were not reliable when tested on our sample population. A new and updated regression equation was calculated using our sample group data (Tables 6 and 7). The new correlation coefficient of the regression equation we developed using the mesiodistal widths of four mandibular incisors was 0.733. The *R* square value was 0.538. The Adjusted *R* Square value was 0.535 (Table 6). These values were quite high and acceptable.

Our multiple regression equation, developed by using gender, jaw variables and the sum of mesiodistal widths of the lower four incisors as independent variables, predicted that it could provide the closest prediction values for the sum of mesiodistal widths of the unerupted permanent canines and premolars for our population.

This study has some limitations. Mesiodistal diameters of all permanent teeth except second molars were measured. It will be more convenient to add the measurements of the second molar teeth. For future studies, it would be appropriate to evaluate the equation in more individuals, covering all geographical regions of Turkey, to assess its predictive value.

### 5. Conclusions

Three regression equations underestimated the mesiodistal widths of permanent canine and premolar teeth. For the Turkish population, this set of three equations had low reliability. An updated regression equation was developed to predict the mesiodistal tooth dimensions of unerupted permanent canines and premolars.

#### AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

#### **AUTHOR CONTRIBUTIONS**

RY and OUA—performed the research; wrote the manuscript. OUA—provided help and advice. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research protocol was reviewed and approved by the Health Sciences Ethics Committee of Ankara Yıldırım Beyazıt University (Date 06 October 2022–number 14), while the study was carried out in accordance with the Declaration of Helsinki. Informed consent to participate was obtained from all of the subjects and/or their legal guardian(s).

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#### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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