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ORIGINAL RESEARCH ARTICLE

The Influences of Incisor Positional Changes Due to Fixed Appliance Therapy on Tongue Position

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Abstract

Purpose: The positional changes of the tongue due to fixed appliance therapy, the relationship between the positional changes of lower incisor, the tongue and the attribution of this relationship was investigated.

Materials and Methods: In this retrospective study, lateral cephalograms of pre and posttreatment records were evaluated. According to the mathematical differences of 1-NB parameters, subgroups namely; retrusion and protrusion groups were formed. Pretreatment measurements were performed from horizontal and vertical reference planes defined, posttreatment measurements were evaluated with the transportation of these reference lines using the total structural superimpositional method. The positional and linear dimensional changes of the tongue were evaluated using Rakosi tongue analysis with a modification of the reference line. Cephalometric tongue images were digitized and the tongue area were calculated.

Results: The positional changes of the tip of the tongue were concordant with the positional changes of the lower incisor, elevation of the dorsum of the tongue and stoopness of the base of the tongue occurred in the retrusion group. Tongue area increased in both groups.

Conclusions: The tip of the tongue followed the movement of the lower incisor altered with fixed appliance therapy in sagittal direction. In cases where upper incisor retraction is planned, tongue tip should be expected to adapt by rising vertically.

Keywords: Cephalometric tongue position; Fixed appliance therapy; Incisors; Rakosi tongue analysis; Tongue area

Introduction

In orthodontics, skeletal and dental structures have much been focused on, but the roles of surrounding muscle structures have often been ignored.¹⁻³ Dental and skeletal morphology affects physiological functions whereas functional compensations occur due to physiological needs. However, there are limits both for morphological and functional adaptations.⁴ It has not yet been fully clarified whether the tongue determines the form of the dental arch or whether it functions according to dentoalveolar morphology.⁵ Orthodontic fixed appliance therapy has functional and positional effects on dentoalveolar structures as well as on orofacial soft tissues. The functional and resting position of the tongue, as well as the dimensional influence of the tongue were emphasized, but there is still little to no information whether there exists a relationship between fixed appliance therapy and changes in tongue position or lower incisor position.⁶ The aimed to investigate whether there exists a relationship. The study hypothesizes that the tongue tip adjusts to the lower incisors' positional changes altered with the fixed appliance therapy.

Material and Methods

A retrospective study was conducted on 34 patients who had undergone fixed edgewise therapy with Angle Class 1 and 2 anomalies on permanent or late mixed dentition stage from the archive of Ankara University Faculty of Dentistry Department of Orthodontics. The lateral cephalograms taken at pre and posttreatment on which the tongue can be seen clearly, and the patient did not swallow during exposure were selected. Patients with Angle Class 3 anomalies, crossbites and missing teeth were excluded. Total of 34 patients (total group), two subgroups were formed according to the linear measurement of position of the lower incisor to line NB. Individuals exhibiting lower incisor movement between 0-1 mm were used only in the total group. The values <0 mm was grouped as retrusion group ($\bar{x} = -2,21$ mm) (n=10), the values >1mm were grouped as protrusion group (\bar{x} =2,36mm) (n=10). Chronological mean ages were 13.69 ±0.42 for total group, 13.40 ±0.52 for retrusion group and 14.32 ±1.17 for protrusion group. The landmarks and measurements used in the study are shown on Table 1, Figure 3. The borders of the cephalometric tongue image are limited







Figure 1. Superimpositon of tongue areal changes of a retrusion case

with tongue tip (TT) anteriorly, the dorsum above and posteriorly and the visible tongue image up to the epiglottis (E). In order to determine the tongue base, the E and Hy points are used for identifying a line which was extended to the innermost and lowest point on the lingual side of the symphysis. The lingual borders of the symphysis are drawn up to the TT and completed. Tongue images were digitized on an AccuTab GTCO table (GTCO CalComp 14557 N, 82nd St. Scotsdale, AZ 85260, US). The tongue area was calculated with the MicroStation 95 Programme (Bentley Systems, Incorporated, 685, Stockton Drive Exton, PA, 19341, US). Pre and posttreatment cephalometric radiographs were superimposed due to Björk and Skieller's local mandibular structural superimposition technique.⁷ Digitized and superimposed as seen on Figure 1,2. The dimensional and linear changes of the tongue were evaluated by Rakosi tongue analysis.⁸ Referring to Rakosi tongue analysis a reference line was conducted among the most prominent lower incisors incisal edge passing through distocervical enamel cement junction of lower first molar and this line is limited by the lowest point or the projection of that point of soft palate (Figure 4). The midpoint is marked as point O. Six angles of 30° were made through point O. The cut off points of these linear extensions on tongue were named as 'tg' points and on hard and soft palate were 'ltg' points starting from anterior to posterior ranking from 1 to 7 (Figure 5). In the study, the modification was made in the reference plane. The lower permanent first molar was used instead of terminal molar as described in Rakosi tongue analysis. Linear and angular measurements were done on cephalometric radiographs. For total measurements, a vertical reference plane (VR) was conducted with a line passing through the point Sella perpendicular to the occlusal plane (OD) and a horizontal reference plane (HR) was conducted with a line parallel to OD. For maxillary measurements; the ANS-PNS plane is used as the maxillary horizontal reference plane (max.HR), and the maxillary vertical reference plane (max.VR) was formed with the line drawn perpendicular to this plane from the point ANS. For mandibular measurements, Menton-Gonion was used as the mandibular horizontal reference plane (mand.HR), and the perpendicular descending from Pogonion to this plane was used as the vertical reference plane (mand.VR). Descriptive statistics of pre and posttreatment characteristics were done. Intragroup and intergroup differences were tested with student t-test and paired t-test (Table 2,3). Pearson correlation analysis was applied in order to evaluate the relationship between the parameters and tongue (Table 4).



Figure 2. Superimpositon of tongue areal changes of a protrusion case



Figure 3. Tongue length (TGL) and tongue height (TGH) measurement



Figure 4. Rakosi reference line

Results

Descriptive statistics and comparison of the parameters were done (Table 2). Rakosi parameters which are O-tg1 and O-ltg1, were

Table 1. Definition	n of landmarks and measurements used in the study				
Landmark/ Measurement	Definition				
OD	(occlusal plane) the line passing through the midpoint of the occlusal closure points of first upper and lower permanent molars and midpoint of the occlusal closure points of most prominent upper and lower incisors				
VR	(vertical reference plane) a 90° vertical line from the point Sella to the occlusal plane				
HR	(horizontal reference plane) a parallel line to the occlusal plane passing through point Sella				
TT	(tip of the tongue) the most prominent point of the tongue in the sagittal plane				
TGL	(length of the tongue) the linear distance between point TT and point E				
u	The shortest linear distance between the ANS-PNS plane and the dorsum of the tongue				
U1	the most prominent incisal point of the upper incisor in the sagittal plane				
L1	the most prominent incisal point of the lower incisor				
E-Shor	linear distance of the epiglottis point to the horizontal reference plane				

Table 2. Descriptive statistics of parameters of subgroups at pretreatment and posttreatment and the comparison (student t-test) according to the subgroups

	t	0		t1			
	Retrusion	Protrusion	tost	Retrusion	Protrusion	tost	
	X ±Sx	X ±Sx	lest	X ±Sx	X ±Sx	lest	
1/NB	29.47 ±2.66	26.43 ±1.66		21.51 ±2.63	33.80 ±2.94	**	
1-NB	6.85 ±0.84	5.75 ±0.52		4.64 ±0.85	8.11 ±0.71	**	
L1/mand.HR	96.55 ±2.75	93.43 ±2.08		89.09 ±2.66	99.62 ±3.73	*	
APog-1	3.29 ±0.59	2.86 ±0.38		0.98 ±0.62	5.20 ±0.42	***	
U1-max.HR	28.64 ±0.86	31.92 ±0.80	*	29.07 ±0.74	33.67 ±1.20	**	
0-tg1	29.81 ±0.92	31.71 ±0.66		30.75 ±0.94	33.36 ±0.49	*	
0-tg2	23.91 ±1.03	25.49 ±1.33		26.52 ±0.93	26.85 ±0.76		
0-tg3	20.74 ±1.28	22.82 ±1.56		24.02 ±1.12	23.72 ±1.09		
O-tg4	20.60 ±1.30	22.26 ±1.47		23.78 ±1.03	23.71 ±1.21		
0-tg5	22.51 ±1.00	24.02 ±1.25		24.75 ±0.94	24.98 ±1.14		
O-tg6	26.70 ±0.82	27.54 ±0.98		27.59 ±1.08	27.92 ±0.83		
O-tg7	34.00 ±1.22	34.62 ±0.83		34.46 ±1.49	35.28 ±0.72		
O-ltg1	35.25 ±0.92	35.15 ±0.39		33.89 ±1.02	36.39 ±0.31	*	
0-ltg2	28.83 ±0.71	30.21 ±0.79		30.44 ±0.83	30.59 ±0.42		
0-ltg3	28.63 ±0.32	29.86 ±0.75		30.92 ±0.53	31.90 ±0.73		
0-ltg4	27.76 ±0.55	28.53 ±1.08		29.81 ±0.51	30.24 ±0.81		
0-ltg5	26.36 ±0.54	27.02 ±1.02		27.31 ±0.85	27.88 ±0.84		
0-ltg6	28.42 ±0.71	29.06 ±1.02		29.52 ±1.07	29.35±0.75		
0-ltg7	34.99 ±0.94	35.12 ±0.34		33.65 ±1.27	36.18 ±0.93		
TGL	71.96 ±1.42	73.35 ±1.82		74.52 ±1.50	75.93 ±1.17		
TGH	33.15 ±1.56	36.37 ±1.45		36.13 ±1.13	38.85 ±1.63		

X: mean value, Sx: standart deviation of mean value, to: pretreatment, t1: posttreatment. *p<0.05, **p<0.01 ***p<0.001.

	Retrusion Group		Protrusion Group		
	D±Sd	test	D±Sd	test	test
Hy-Svert	1.98 ±2.17		5.60 ±2.26	*	
Hy-Shor	7.39 ±2.18	**	5.43 ±2.44		
Pd	0.62 ±0.19	*	0.77 ±0.22	**	
U1-maxVR	-2.72 ±1.19	*	-0.54 ±0.79		
L1/mand.HR	-7.47 ±1.70	**	6.19 ±2.89		**
1/NB°	-7.96 ±1.70	**	7.37 ±2.77	*	***
1-NB	-2.21 ±0.50	**	2.36 ±.0.36	***	***
APog-1	-2.32 ±0.59	**	2.34 ±0.44	***	***
L1-mand.VR	3.60 ±0.67	***	-1.14 ±0.78		***
0-tg1	0.94 ±0.74		1.65 ±0.61		*
O-tg3	3.28 ±1.22	*	0.90 ±1.02		
O-tg4	3.18 ±1.13	*	1.45 ±0.86		
0-ltg1	-1.36 ±0.62		1.24 ±0.41	*	**
0-ltg2	1.60 ±0.34	**	0.39 ±0.57		
0-ltg3	2.29 ±0.42	***	2.04 ±0.54	**	
O-ltg4	2.05 ±0.59	**	1.70 ±0.69	*	
0-ltg7	-1.34 ±0.84		1.06 ±0.69		*
TGH	2.97 ±0.95	*	2.48 ±0.90	*	
TT-mand.HR	2.26 ±0.88	*	1.70 ±0.95		
TT-mand.VR	1.79 ±0.64	*	-0.89 ±0.79		*
TT-Shor	4.17 ±0.59	***	2.77 ±1.34		
TT-Svert	0.20 ±1.01		2.10 ±0.80	*	
E-Shor	7.63 ±2.08	**	4.25 ±2.47		
Tongue Area	3.84 ±0.98	**	2.08 ±0.66	*	
u	-0.84 ±1.00		0.56 ±0.88		
TGL	2.56 ±1.73		2.57 ±2.19		

Table 3. Descriptive statistics of subgroup parameters $(D\pm Sd)$ achieved with the treatment, comparison of the differences (paired t-test) and comparison of head differences a(student t-test)

D: mean difference, Sd: standard error of mean difference, * p<0.05, ** p<0.01, *** p<0.001



Figure 5. Cephalometric landmarks of Rakosi tongue analysis

found to be similar at pretreatment but were statistically differed at posttreatment. Other Rakosi parameters did not show any significant difference. TGL and TGH parameters were found to be similar. Paired t-test was done for pre and posttreatment evaluation of values for each subgroup (Table 3). Tongue area was significantly increased in both subgroups. TT-mand.HR and TT-mand.VR were significant in retrusion group. Intergroup differences were compared with student t-test (Table 3). Hy-Svert increased in protrusion group, Hy-Shor increased in retrusion group, O-ltg1 increased in protrusion group and was significantly different between groups. TT-mand.VR increased in retrusion group and significantly different between groups. According to Pearson correlation analysis for the total group TT-mand.VR was found to be correlated with 1-NB, 1/NB, A-Pog1, L1-mand.VR and L1-mand.HR (Table 4). Correlation analysis results for retrusion group showed that TTmand.VR and L1-mand.VR, 1-NA and TT-Shor were significantly correlated while for protrusion group TT-mand.VR was negatively

correlated with APog-1 and 1-NB. TT-mand.HR was correlated with L1-mand.HR and TGH was negatively correlated with U1-max.HR and L1/mand.HR.

Discussion

The positional changes of the tongue due to fixed appliance therapy, the relationship between the positional changes of lower incisor, the tongue and the attribution of this relationship was investigated. Our study showed that TT followed the movement of the lower incisor in sagittal direction. The tongue is positioned on the floor of the mouth, while TT taking part behind the incisal edges of lower incisors, the dorsum of the tongue over the teeth levels and spreading all over the oral cavity.⁹ At rest, the TT is positioned on the lingual surface of the lower incisors exerting a certain amount of resting pressure and has a strong relationship with the mandibular arch.^{10,11} Changes in the size of the oral cavity due to different treatment protocols might affect tongue position.¹² Soft tissues, affect hard tissues and orthodontic treatment results, therefore, soft tissue limitations should be assessed more precisely.¹³ An alteration of incisors and soft tissue position could potentially affect tongue position.^{14,15} These studies have shown that; there exists a close relationship between the TT and the lower incisors. The lower incisors are an important factor in providing ideal overbite and overjet, in determining the profile esthetics, and have been heavily involved in almost all of the cephalometric analyses.^{16,17} Considering that the fixed orthodontic treatment plans are made by targeting the lower dentition; this relationship needs to be taken into account. In this context, in the study; two subgroups were formed as the retrusion and the protrusion group according to 1-NB.

Rakosi tongue analysis enables the evaluation of positional changes of the tongue in horizontal and vertical dimensions.⁸ In original Rakosi tongue analysis the mandibular terminal molar

TOTAL GROUP	TGL	TT-Svert	E-Svert	TT-mand.VR	TT-Shor	E-Shor	TT-mand.HR	tongue area
1/NA		0.365 *						
U1/max.HR	0.398 *	0.382 *						
U1-max.HR					0.361 *			
1/NB				-0.706 ***		-0.396 *		
L1/mand.HR				-0.733 ***		-0.373 *		
1-NB				-0.662 ***				
APog-1				-0.626 ***		-0.368 *		
L1-mand.VR				0.803 ***		0.414 *		0.374 *
L1-mand.HR	0.426*		-0.572 ***	0.371 *		0.415 *	0.578 ***	
PROTRUSION GROUP	TGH	TT-OD	E-Svert	TT-mand.VR	TT-Shor	TT-mand.HR	u	
1/NA			-0.743 *					
U1/max.HR			-0.739 *					
1/NB	-0.680 *						-0.647 *	
L1/mand.HR				-0.708 *				
1-NB	-0.724 *	-0.642 *					-0.900 ***	
APog-1				-0.784 **				
L1-mand.VR				-0.788 **				
Pg-NB				0.888 **				
L1-mand.HR					-0.639 *			
RETRUSION GROUP			-0.755 *	0.717 *		0.867 **		
1-NA	0.648 *							
U1-max.HR			0.680 *					
L1-mand.VR		0.735 *						
Pg-NB				0.678 *				

 Table 4. Pearson correlation analysis results for total group, protrusion group and retrusion group

*p<0.05, **p<0.01, ***p<0.001.

is used as an identification point of the reference plane. We used mandibular first molar instead because permanent first molars are the identification landmarks of occlusion and are widely used in cephalometric analysis.¹⁸

This study revealed that tongue-related parameters are heavily correlated with the mandibular dentoalveolar parameters. The significant negative correlation between parameters 1-NB and TTmandVR, 1/NB and TT-mandVR, L1/mand.HR and APog-1 and TTmand.VR shows that TT moves backwards in cases of advanced retrusion, that is, the TT tries to adapt to the form by following the linear and angular changes of the lower incisor (Table 4). The significant positive correlation between L1-mandVR and TT-mandVR supports the fact that the TT moves in the same direction as the lower incisor (Table 4). As contraray to our finding the relationship of TT with the lower incisors was told to be preserved at the end of the treatment, regardless of the lower incisor position.³ However; our results revealed that the tongue adapts to the current morphological state of the oral cavity rather than forming the dental arches.¹⁹ Besides, TGL is also found to be correlated with L1-mand.HR (Table 4). So; we can confirm the opinion that the tongue adapts to the changing occlusion and environment and that altering incisor and soft tissue position and arch dimension could significantly reduce TGL, as a result, affect the tongue position.²⁰ Additionally, the correlations between the parameters 1/NB and TT-mand.VR, L1-mand.HR and TGL can be explained with the adaptation of the tongue to the altered incisal inclinations, unlike the authors who claimed that incisor inclinations were not due to muscle action (Table 4).²¹ The correlation between 1/NA and TT-Svert, U1/max.VR and TT-Svert indicates that forward or backward movement of the upper incisor was followed by the similar movement of TT on sagittal dimension (Table 4). The positive correlation between L1-mand.VR and TT-mand.VR also supports the fact that TT moves in the same direction as the incisor (Table 4). So the tongue adapts to the surrounding structures when the occlusion is changed. 14,15,22 Altered incisor and soft tissue position and arch dimension could significantly reduce TGL and as a result affect the tongue position.²⁰

In vertical dimension we found that vertical position of the upper incisor relative to its base and the vertical position of the TT are statistically corraleted (Table 4). However, it is not clear whether this correlation causes lowering of TT as a result of the altered vertical position of the upper incisor or whether it is due to dentoalveolar development. It is also well accepted that the vertical position of the TT showed a highly positive correlation with the mandible, lower incisors and mandibular dentition rather than the upper incisor.²

Tongue area were measured digitally with planimeter devices.^{23,24} According to our results; tongue area decreased in lower incisor protrusion cases and increased in retrusion cases. It is known that the TT moves forward to the incisors and lips to provide anterior oral seal, and the tongue spreads to the floor of the mouth when the length of the oral cavity increases.²⁵ But at this point the transversal dimension should be taken into consideration. In cases with protrusion, while TT is similarly positioned forward, the increase in the dorsum region is less in the retrusion group. It can be assumed that the tongue has undergone a positional change by spreading itself in the transversal dimension. Also, the backward movement of the lower incisors in the sagittal direction was accompanied by an increase in the vertical direction. In fixed appliance therapy, the incisal edge was positioned higher in the vertical direction due to the retrusion of the lower incisors.²⁶ So this may be due to retrusion and/or dentoalveolar development. In mandibular retrognathic Class 2 cases, the tongue is told to be positioned higher than normal occlusion cases, which is attributed to the soft tissues adaptation to environment in order to meet physiological needs as evident with our findings.² TT-Shor and E-Shor measurements revealed that the dimensional increase of the tongue in the vertical dimension is not only due to the rise in the dorsum region, but also to the lowering of the tongue base (Table 4). This was also re-

flected in the tongue area as a significant increase in the retrusion group (Table 3). According to this study, when retrusion occurs in the upper incisor, TT rises (Table 4). In cases where upper incisor retraction is planned, TT should be expected to adapt by rising vertically. In lower incisor protrusion cases, the tongue accompanied in a similar way with the lower incisor. The TT was positioned significantly ahead of the vertical reference plane (Table 4), increased significantly in size (O-ltg1) (Table 3), and showed non-important forward movement relative to mandibular vertical plane (Table 3). In the vertical direction, the TT did not show a significant lowering relative to the reference plane, no significant elevation was detected relative to the mandibular base (Table 3). The insignificant forward movement of E in the protrusion group can be considered as an attempt to compensate for the significant forward movement of TT (Table 3). With this possible compensation mechanism, it can be thought that TGL may have been tried to be preserved. Dimensional increase in tongue dimensions in both genders at different times was reported.²³ In the vertical direction; the elevation of the lower incisor relative to the mandibular base is also observed at TT. As in line with parameters u and TGH, the distance between the dorsum of the tongue and the maxillary base may decrease in relation to the positional and angular changes in the upper and lower incisors due to protrusion (Table 4). The shape of the tongue and dimensions are as important as incisor position and inclination during diagnosis.¹⁹

The hyoid bone and tongue descend in line with age, profile flattens, crowding occurs in the mandibular anterior region, and this is attributed to the lowering of the tongue. When retraction was performed, the tongue was told to be positioned below.²⁷ In this study the significant lowering of TT, Hy and E gives information about the vertical positions of the TT (Table 3). In the protrusion group, there is a non-significant decrease. So, when incisor retraction is planned in the early period, it should be taken into account that the advanced Hy and tongue will descend and the profile will tend to flatten. A positive relationship is reported between Hy and mandibular position.²⁸ The most protruding part of the dorsum of the tongue facing the pharyngeal cavity is located further back in Class 2 cases, independent of the tongue root and Hy position. This was explained as an adaptation in the genioglossus muscle which allows the tongue to protrude. In Class 3 cases; Hy was told to be positioned further due to increased genioglossus activity. In our study O-ltg7 showed significant difference between groups, and can be interpreted as tongue dorsum and Hy adaptation to the changing environment (Table 3). Besides postextraction tongue position is reported to influence the Hy.²⁹ But tongue size was stated as independent of the sizes of bone structures and gender differences.³⁰ Also tongue length was reported as not to differ in gender in Class 1 and 2, but it was found to be shorter in Class 3. 31 In our study, E and TT moved similarly in both groups in sagittal and vertical dimensions which suggests that TGL will not show a significant change (Table 3). Our findings confirm that the tongue does not show a dimensionally relationship with the dimensions of the skeletodental structures. However, TGL was significantly correlated with the distance of the upper and lower incisors to their bases (Table 3). TGL may be related to the vertical position of the incisors in the sagittal direction rather than the movement. In an opposite conclusion, Al Maaitah et al reported a decrease in TGL in bimaxillary extraction cases.²⁰ In our study, O-tg3 and O-tg4 parameters, increased significantly in the retrusion group, whereas no significant change was found in protrusion group (Table 3). However, it should be considered that, the movement of the lower incisors may cause a change in the Rakosi reference plane and should be evaluated together with other parameters. It may be more accurate to evaluate the dorsum of the tongue not only with Rakosi parameters, but also with the directly measured TGH and u parameters. In the retrusion group 'u' decreased at an insignificant level, indicating that the dorsum approached the maxillary base, in the protrusion group it decreased at an insignificant level, indicating that it moved away. However, these changes were not statistically significant either within the

group or between the groups (Table 3). It is seen that there is a less increase in the dorsum region (O-tg2, O-tg3, O-tg4, O-tg5, O-tg6) in protrusion cases compared to the retrusion cases. However, it was determined that the forward movement was more pronounced in the anterior region (O-ltg1, TT-Svert) in the sagittal direction. Also, TGH increases in size, but it does not show a significant approximation to the maxillary base. The insignificant elevation of the dorsum of the tongue towards the hard palate is a finding contrary to what other studies have reported.²³ Besides dorsal tongue height was reported as higher in children compared to adults.²⁵ Besides changing tooth positions is told to affect tongue posture and functions, and these changes may also be reflected in palatal form and volume.^{10,32–34} The reader should take into account that our study was conducted on lateral cephalometric films so were 2D and the transversal dimension were ignored.

Conclusion

The TT followed the movement of the lower incisor in sagittal direction. The tongue tried to adapt to the new form altered by the fixed therapy in the vertical direction as if following the mandibular dentition. In cases where upper incisor retraction is planned, TT should be expected to adapt by rising vertically. TGH increases in size, but it does not show a significant approximation to the maxillary base spatially in the oral cavity. In cases where incisor retraction is planned in the early period, it should be taken into account that the advanced Hy and tongue will descend, and the profile will tend to flatten. The readers should evaluate the findings as treatment results. Long-term results should be considered.

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Author Contributions

Conception and Design : All Authors Data Collection : A.A. Data Analysis and interpretation : A.A. Draft of the manuscript : A.A. Critical revision of manuscript : All Authors Final approval of manuscript : All Authors

Conflict of Interest

Authors declare that they have no conflict of interest.

Ethics Approval

Due to the "Ankara University Faculty of Dentistry, Clinical Research Ethics Committee" decision about this study; ethical approval was not deemed necessary at the specified dates (2006). Decision No: 36290600/57/2024

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