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Comparison of gingival and periodontal phenotype classification methods and phenotype-related clinical parameters: cross-sectional observational study

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Abstract

Background The aim of this study was to assess gingival and periodontal-phenotype by using Standard-Periodontal-Probing (SPP), Colored-Periodontal-Probing (CPP), and Cone-Beam-Computed-Tomography (CBCT) in comparison to gold standard transgingival-probing.

Methods Gingival-thickness of the maxillary anterior incisors and canines of 30 healthy individuals (6 teeth of each individual) was evaluated by transgingival-probing, SPP, CPP, and CBCT methods. The relationship between thin and thick phenotype and phenotypic parameters such as age, gender, Keratinized-Tissue-Width (KTW), and Buccal-Bone-Thickness (BBT) was tested with the Chi-square test, and the differences between the measurements were tested with the Mann-Whitney U test. Gingival-thickness, BBT, and related phenotypic parameters were measured from three buccal points (marginal-middle-apical) of each tooth, and Spearman-Rho Correlation Analysis was performed. Pearson chi-square and McNemar tests were used to assess the distribution of categorical data. Sensitivity, specificity and accuracy levels and kappa statistics were calculated for each method. Intra/interobserver agreement was assessed using the intraclass correlation coefficient. The significance level was set at $p < 0.05$.

Results There was no statistically significant difference for gingival-phenotype according to age and gender ($p > 0.05$). Higher KTW values were measured in areas with thick gingival-phenotype ($p:0.008$). The highest agreement in terms of detecting gingival-phenotype was found between transgingival-probing and CBCT ($p < 0.01$). All methods were found to be more accurate in the determination of thin phenotype ($p < 0.01$). Marginal gingival-thickness measurements were higher than those of middle and apical measurements ($p < 0.01$), and middle BBT measurements were higher than those of apical measurements ($p < 0.01$). Gingival-thickness measured by transgingival-probing and CBCT showed a significant correlation ($p < 0.01$). KTW was significantly correlated with BBT and marginal gingival-thickness ($p < 0.01$).

Conclusions Thin or thick phenotype is associated with different apical-coronal points and KTW. The CBCT method was found to be helpful in determining gingival and periodontal-phenotype.

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Clinical relevance Available CBCT images can be used to take precautions and assess prognosis before implant placement and orthodontic treatment.

Clinical trial number Not applicable.

Keywords Gingival phenotype, Periodontal phenotype, Gingival thickness, Transgingival probing, CBCT

Introduction

Accurate measurement of gingival dimensions is of paramount importance during periodontal, restorative, and orthodontic treatments and implant placement. Gingival thickness and keratinized tissue width (KTW) ensure the protection and maintenance of aesthetics, function, and tissue health surrounding teeth and dental implants [1, 2]. Most patients undergoing dental treatment desire functional integrity and aesthetic appearance, so assessment of periodontal phenotype is essential [3]. Gingival phenotype refers to the three-dimensional gingival volume, which includes gingival thickness and KTW. The gingival biotype term was used in most previous studies until the 2017 World Workshop, but then the use of the term “phenotype” was accepted instead of “biotype” [3, 4]. The term periodontal phenotype has been recommended to describe the combination of the gingival phenotype and the buccal bone thickness (BBT) [3].

Gingival shape and thickness vary depending on the individual patient and the location of the tooth in the dentition [5, 6]. Gingival phenotype should be considered before and after treatment, as it may affect the treatment plan and tissue healing process. In areas with thin gingiva, there is a tendency for less root coverage after periodontal surgery and more attachment loss after periodontal treatment [7, 8]. Thin gingiva is also susceptible to trauma, inflammation, and gingival recession, whereas thick gingiva is dense and fibrotic [1]. In addition, gingival thickness affects the success of regenerative procedures applied to bone and soft tissues [9]. Several previous studies reported a relationship between gingival thickness and BBT [10]. However, supportive evidence is limited due to the absence of a standard technique used to measure gingival thickness and BBT. Some of the non-invasive and reliable methods which were utilized to assess gingival thickness are visual examination, transgingival probing [11], ultrasound imaging, transparency of the periodontal probe through the gingival margin (TRAN) [12] with standard periodontal probe (SPP) or colored periodontal probe (CPP) set (Colorvue biotype probe, Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA) [13, 14] and cone-beam-computed tomography (CBCT) [15, 16].

The most frequently used subjective method is the TRAN, which includes the placement of a SPP into the gingival sulcus from the middle buccal region of the tooth [17, 18]. If the probe is visible through the gingival

sulcus, the gingiva is considered “thin,” but if it is invisible, then the gingiva is considered “thick” [12]. On the other hand, transgingival probing is a more objective and quantitative method, but it is invasive and compelling [19, 20]. On transgingival probing, the gingiva is considered “thin” when the gingival thickness measures < 1 mm and “thick” when it measures > 1 mm [12, 21].

Methods that are not as invasive as transgingival probing and more objective than the TRAN are ultrasound imaging [22], calipers [23], two-dimensional radiographic evaluation [18], and CBCT [13, 14]. TRAN method with CPP consists of three probes with white, green, and blue-colored tips. Depending on which of the three probes is visible from the gingival sulcus, gingival thickness can be classified into four categories as follows: “thin,” “medium,” “thick,” and “very thick.” Measurement with CBCT is objective and quantitative, like transgingival probing, but it has a significant disadvantage, such as exposure to radiation. Previous studies have compared different methods for measuring periodontal phenotypes and demonstrated that CBCT is a reliable technique for assessing gingival thickness [14, 24, 25]. However, available CBCT images taken for implant planning can be used for the assessment of periodontal phenotype without exposing the patient to extra radiation doses. There is no non-invasive technique other than CBCT that can provide images with sub-millimeter accuracy without distortion and magnification and objectively measure BBT quantitatively. This study aimed to comprehensively compare multiple qualitative and quantitative approaches for measuring gingival phenotype and also to evaluate the accuracy of proposed methods, and so far no studies have evaluated this new phenotype definition from this broad perspective.

In consideration of the importance of phenotype in periodontal treatment and potential differences among various methods, the present research aimed to compare SPP, CPP, and CBCT with gold standard transgingival probing to classify gingival and periodontal phenotype in the maxillary anterior incisor and canine teeth of individuals with healthy gingiva without periodontitis. We also assessed the relationship between periodontal phenotype at different reference points, including age, gender, KTW, and attached tissue width (ATW).

Materials and methods

Study design

This cross-sectional study was conducted by using clinical and radiographic data of individuals who had CBCT images taken for implant placement and who had maxillary incisors and canines in their mouth at Ankara Yıldırım Beyazıt University, Faculty of Dentistry. The STARD 2015 guidelines were used [26]. The study was approved by the Faculty of Medicine Clinical Research Ethics Committee (number:2637996/08, date:07.06.2023-07). Clinical procedures were carried out in conformity with the Declaration of Helsinki. Individuals who agreed to participate in the study signed informed consent forms and had intraoral examinations at the periodontology clinic. The individuals' age, gender, smoking status, and clinical periodontal parameters were recorded.

A power analysis was conducted prior to the study. The minimum number of measurements required with a type I error of 0.05 and 90% power for at least 0.70 agreement between measurements was determined as 87 for intra-class correlation coefficient (ICC) calculation [28], and 149 for kappa agreement [29]. However 180 measurements were taken for potential data loss or variability in clinical settings.

Inclusion and exclusion criteria

The study included 30 individuals over the age of 18, systemically healthy, defined as clinical gingival health without periodontitis [27], with maxillary incisors and canines in their mouth and probing-pocket-depth (PPD) ≤ 3 mm, bleeding-on-probing (BOP) (-), and no gingival inflammation with well-maintained oral hygiene. Individuals with intact periodontium without detectable interproximal clinical attachment loss (CAL) or radiographic bone loss were included. Inclusion criteria were assessed after a radiographic and clinical periodontal examination, including PPD, BOP, and CAL.

Patients with gingival recession, dental anomalies, endodontic lesions, caries, extensive restorations or fixed prostheses in the maxillary anterior region were excluded from the study. Patients using medication affecting the gingival condition or mucogingival complex, having clinical signs of gingivitis or having a history of surgical intervention in the relevant area, and having noticeable gingival pigmentation or gingival overgrowth were excluded from the study. Individuals undergoing active orthodontic treatment, pregnant or breastfeeding women were also excluded.

Obtaining clinical parameters

A calibrated researcher (D.İ.A.) performed all clinical measurements using a SPP (PCP-UNC-156, Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA). Prior to the study, the examiners were calibrated. Five volunteers were

assessed twice, leaving one hour between assessments. The second set was performed by blinding out the initial one. The reproducibility assessment resulted in 85% of sites for repeated measurements within ± 1 mm. A periodontology specialist student (D.İ.A.) was calibrated by a reference examiner (M.A.T) with more than 10 years of experience in periodontology. KTW measured the distance from the muco-gingival-junction (MGJ) to the free-gingival-margin in the middle buccal region. ATW was obtained by subtracting the PPD from KTW. Clinical periodontal parameters of the six maxillary anterior teeth were recorded in millimeters.

Gingival phenotype assessments

Gingival phenotype was determined using transgingival probing, SPP, CPP, and CBCT. Transgingival probing was accepted as the "gold standard" [21]. Three of these methods (SPP, CPP and transgingival probing) were performed in a single session when the participants came to the clinic. The sequence of the three clinical assessment methods was the same for all patients. Clinical measurements were also recorded on the day CBCT images were taken from individuals.

Assessment by transgingival probing

After applying a topical anesthetic spray, gingival thickness was measured from 3 points perpendicular to the long axis of each tooth in the middle buccal region using an endodontic spreader (ISO 20, Endo-Art, Türkiye) with a stopper until the alveolar bone was reached [17]. These points were determined as "1 mm apical to the free gingival margin", "1 mm coronal to the MGJ" and "the middle point of these two points". Points were named "marginal", "apical" and "middle" respectively. The gingival thickness was determined by measuring the spreader from the stopper to its tip and recorded using a digital caliper (Vernier, Altraco Inc., Sausalito, California, ABD) with a calibration of 0.01 mm (Fig. 1a). Thus, 540 measurements were conducted from these three reference points of six maxillary anterior and canine teeth.

Assessment by standard periodontal probing

If the SPP was visible or reflected through the free-gingival-margin in the middle buccal region, the gingiva was considered "thin", or vice versa it was considered "thick" [17] (Fig. 1b).

Assessment by colored periodontal probing

Gingival thickness was classified into four categories depending on which of the three probes in the CPP set (white-green-blue) reflected off the gingiva. Gingival thickness was determined as "thin" if the white tip was reflected, "moderate" if the white tip was not reflected but the green tip was reflected, "thick" if the green tip

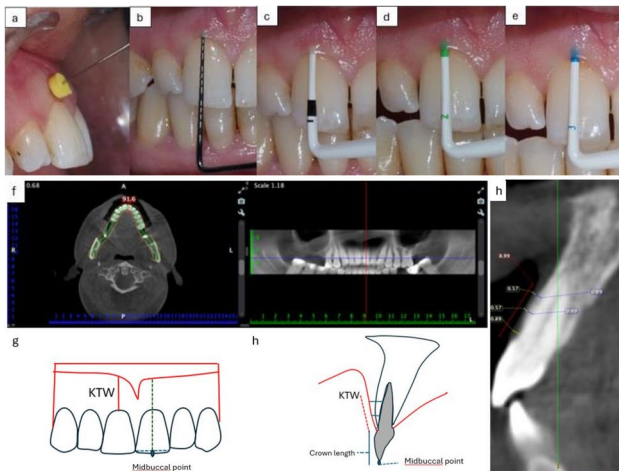


Fig. 1 Using an endodontic spreader for measurement with transgingival probing (a), Determining GT with a SPP (b), Determining gingival-thickness with a CPP set (c, d, e), Section passing through the horizontal intersection area perpendicular to the mid-buccal axis of a tooth in the CBCT image (f), KTW measurement (red), gingival-thickness measurement (yellow) and BBT measurement (blue) in the CBCT image (g-h)

was not reflected about the blue tip was reflected, and “very thick” if neither tip was reflected [15] (Fig. 1c-e).

Periodontal phenotype assessment by cone-beam computed tomography

Images were obtained by using a CBCT unit (Planmeca Promax 3D max, Helsinki, Finland; 90 kV, 8 mA, 18 s, field of view 200×62 mm, 0.2 mm voxel size). Before image analysis, a calibration session was conducted with a periodontology specialist student (D.İ.A) who performed clinical examinations accompanied by an Oral and Maxillofacial Radiologist (B.Ç) with 8 years of experience. In this session, six CBCT images that were not included in the study were used in pilot measurements. Measurements were performed using the built-in software (Planmeca Romexis 5.3 R) of the CBCT device. Before taking CBCT images, the lips and cheeks were lifted by placing a cotton roll with a retractor to visualize KTW in more detail. Cross-sectional images were created from the horizontal arch drawn from the maxilla anterior region (Fig. 1f). The middle buccal point of each tooth was determined as the exact middle of the mesio-distal dimension from the incisal edge of the tooth by the clinical points and cross-sectional images were created from the relevant point. In these sections, KTW was measured horizontally, starting from the gingival margin. For this purpose, the crown length of each tooth from the incisal edge to the free gingival margin was marked, the point where the gingival margin began was determined, and a longitudinal KTW was drawn. Gingival thickness, 1 mm below, 1 mm above, and at the exact middle point of this measured line, was measured horizontally between the alveolar bone and soft tissue. The BBT measurements

were also performed on the same CBCT slices used for gingival thickness assessment. These measurements were taken at the exact midpoint of each tooth’s crown, referencing the distance between the alveolar bone and the root surface. Since there was no buccal cortical bone at the marginal point, BBT was measured at the middle and apical points (Fig. 1g-h). For each tooth, measurements of buccal soft tissue thickness in mm were made twice on the CBCT images, and the average of these measurements was recorded. 30% of the CBCT measurements (for 54 teeth) were conducted twice separately between two-week intervals by two observers to determine intra- and inter-observer agreement.

Statistical analysis

Descriptive statistics for each variable were calculated. The numerical data were reported with mean ± standard deviation and/or median (minimum-maximum) values. For the categorical data, frequency (n) and percentages (%) were used. The normality distribution of the numerical data was checked using Shapiro Wilk test. Comparison of phenotypic variables according to thick and thin gingival-phenotype was assessed using Mann Whitney U test. A repeated measure ANOVA (for >2 reference points) and paired sample t test (for 2 reference points) was used to evaluate for comparing gingival and periodontal phenotypic variables at different reference points.

The Pearson chi-square test was utilized to assess the frequency distribution of independent categorical data, while the McNemar test was employed for dependent categorical data. Intraobserver and interobserver agreement were assessed using Intraclass correlation coefficient (ICC). Sensitivity, specificity and accuracy levels and kappa statistics for each gingival-phenotype determination methods were calculated with 95% confidence intervals. The relationship between variables was tested using Spearman-Rho Correlation Analysis. The statistical significance level was $p < 0.05$. Data were analyzed in SPSS 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Versiyon 22.0. Armonk, NY: USA).

Results

The frequency distributions between the thin or thick gingival thickness determined for 180 teeth with transgingival probing and the gender and smoking status of the individuals are shown in Table 1. No significant relationship was found between these parameters and gingival thickness ($p > 0.05$) (Table 1).

The mean age, KTW, and ATW measurements according to gingival thickness determined by transgingival probing are shown in Table 2. No significant difference was found between gingival thickness and individuals’ age ($p > 0.05$), but a significant difference was found

Table 1 Gender and smoking and of individuals according to thick and thin gingival-phenotype determined by transgingival-probing method

		Transgingival Probing (TP)				x ² and p value
			Thin	Thick	Total	
		n (%)	n (%)	n (%)	n (%)	
Gender	Female	21 (70%)	18 (14.3%)	108 (85.7%)	126 (100%)	x ² = 1.714, p = 0.19
	Male	9 (30%)	12 (22.2%)	42 (77.8%)	54 (100%)	
Smoking Status	No	19 (63.3%)	16 (14%)	98 (86%)	114 (100%)	x ² = 6.568, p = 0.087
	< 10 per day	2 (6.7%)	0 (0%)	12 (100%)	12 (100%)	
	10–20 per day	6 (20%)	10 (27.8%)	26 (72.2%)	36 (100%)	
	> 20 per day	3 (10%)	4 (22.2%)	14 (77.8%)	18 (100%)	
	Total	30 (100%) (patient)	30 (16.7%)	150 (83.3%)	180 (100%) (teeth)	

Table 2 Mean age and other phenotypic variables according to thick and thin gingival-phenotype determined by transgingival-probing method

Variable	Transgingival Probing (TP)	n	Mean ± SD	Median (Min.-Max.)	U [‡]	p
Age	Thin	30	41.9 ± 12.5	40.5 (20–59)	1995	0.327
	Thick	150	43.9 ± 9.6	43 (20–61)		
KTW (mm)	Thin	30	6.77 ± 1.91	7 (4–11)	1567.5	0.008*
	Thick	150	7.77 ± 1.64	8 (4–13)		
ATW (mm)	Thin	30	5.47 ± 1.68	5.5 (3–9)	1591	0.010*
	Thick	150	6.37 ± 1.66	6 (2–12)		

*p < 0.05 Statistically significant difference. [‡] Mann Whitney U test. KTW: Keratinized Tissue Width. ATW: Attached Tissue Width

Table 3 General distributions of thin and thick gingival-phenotype determination methods (SPP, CPP, transgingival-probing and CBCT)

		Transgingival Probing (TP)			Sensitivity % (95% CI)	Specificity % (95% CI)	Accuracy % (95% CI)	McNemar Test x ² (P value)	Kappa (95% CI)
		Thin n (%)	Thick n (%)	Total n (%)					
Standart Peri- odontal Probing (SPP)	Thin	26 (21.7%)	94 (78.3%)	120 (100%)	86.67	37.33	45.56	80.827	0.109
	Thick	4 (6.7%)	56 (93.3%)	60 (100%)	(69.28–96.24)	(29.58–45.6)	(38.13–53.13)	< 0.001	(0.035–0.184)
	Total	30 (16.7%)	150 (83.3%)	180 (100%)					
Colored Periodon- tal Probing (CPP)	Thin	27 (42.2%)	37 (57.8%)	64 (100%)	90	75.33	77.78	27.225	0.45
	Thick	3 (2.6%)	113 (97.4%)	116 (100%)	(73.47–97.89)	(67.64–82)	(70.99–83.62)	< 0.001	(0.318–0.581)
	Total	30 (16.7%)	150 (83.3%)	180 (100%)					
Cone Beam Com- puted Tomogra- phy (CBCT)	Thin	30 (60%)	20 (40%)	50 (100%)	100	86.67	88.89	18.05	0.684
	Thick	0 (0%)	130 (100%)	130 (100%)	(88.43–100)	(80.16–91.66)	(83.36–93.08)	< 0.001	(0.56–0.808)
	Total	30 (16.7%)	150 (83.3%)	180 (100%)					

CI: Confidence Interval

between KTW, ATW values, and thin or thick gingival thickness ($p < 0.05$). Significantly lower KTW ($p = 0.008$) and ATW ($p = 0.01$) were measured with thin gingival thickness (Table 2).

The relationship between the thin or thick gingival thickness assessment methods is shown in Table 3. All methods were significantly related to each other ($p < 0.01$). The thin/thick measurement numbers of 180 teeth are 30/150 with transgingival probing, 50/130 with CBCT, and 120/60 with SSP. With the CCP method, 64 thin, 98 medium, 12 thick and 6 very thick gingiva were measured. In other words, the percentages of thin/thick gingival thickness compared to the gold standard transgingival probing are 86.6/37.3 for SPP; 90/12 for CPP

and 100/86.6 for CBCT. The highest agreement was observed between transgingival probing and CBCT (kappa = 0.684), and the lowest agreement was between transgingival probing and SPP (kappa = 0.109). In addition, all methods are more consistent in determining the thin gingival thickness (CBCT = 100%, CPP = 90%, SPP = 86.6%). An excessive number of thin gingival thickness measurements have been made with SPP ($n = 120$), which is a routine, simple and practical method in the clinic (Table 3).

The correlation between gingival thickness and BBT measured from different points by transgingival probing and CBCT methods and other phenotypic parameters is shown in Table 4. A highly significant correlation was

Table 4 Correlation between periodontal phenotypic parameters measured according to different reference points (Spearman correlation test, Rho)

Phenotypic variables		TP (n = 180 teeth)			CBCT (n = 180 teeth)			
		Marginal	Middle	Apical	Marginal	Middle	Apical	Middle
		GT	GT	GT	GT	GT	GT	BBT
TP	Middle GT	0.519**	-					
	Apical GT	0.554**	0.477**	-				
	Marginal GT	0.886**	0.451**	0.479**	-			
	Middle GT	0.355**	0.777**	0.257**	0.428**	-		
	Apical GT	0.434**	0.371**	0.782**	0.451**	0.317**	-	
CBCT	Middle BBT	0.346**	0.019	0.226**	0.314**	-0.119	0.171*	-
	Apical BBT	0.317**	0.344**	0.116	0.356**	0.389**	0.096	0.442**
KTW		0.273**	0.067	0.091	0.298**	0.033	0.097	0.403**

* $p < 0.05$, ** $p < 0.01$ TP: Transgingival probing, CBCT: Cone Beam Computed Tomography, GT: Gingival thickness, BBT: Buccal bone thickness, KTW: Keratinized tissue width

Table 5 Comparison of gingival and periodontal phenotypic variables at different reference points

Phenotypic variables	Region	n	Mean \pm SD	Median (Min.- Max.)	Test statistics	P
TP - GT	Marginal	180	1.43 \pm 0.4 a	1.35 (0.51–2.54)	108,91	< 0.001*** ‡
	Middle	180	1.04 \pm 0.41 b	0.99 (0.27–2.43)		
	Apical	180	1.05 \pm 0.39 b	1.03 (0.24–2.67)		
CBCT-GT	Marginal	180	1.25 \pm 0.35 a	1.26 (0.4–2.33)	144,85	< 0.001*** ‡
	Middle	180	0.79 \pm 0.4 b	0.8 (0.4–1.79)		
	Apical	180	0.81 \pm 0.36 b	0.8 (0.4–2.15)		
CBCT- BBT	Middle	153	1.15 \pm 0.43	1.13 (0.4–2.53)	9,2	0.003** ◇
	Apical	175	1.03 \pm 0.44	0.89 (0.4–2.56)		

*** $p < 0.001$, ** $p < 0.01$, ‡ repeated measures ANOVA, ◇ paired sample t test. TP: Transgingival probing. CBCT: Cone Beam Computed Tomography. GT: Gingival thickness. BBT: Buccal bone thickness

a, b: Different letters in the same column represent statistically significant difference ($p < 0.05$)

found between transgingival probing and CBCT in terms of gingival thickness at all points (marginal-middle-apical, $Rho = 0.886, 0.777, 0.782$) ($p < 0.01$). Low to moderate correlations were found between gingival thickness and BBTs measured by CBCT and transgingival probing at the marginal point ($Rho = 0.346, 0.317$) ($p < 0.01$). No correlation was found between gingival thickness and BBT at the apical point ($Rho = 0.116$) and GT-BBT at the middle point ($Rho = 0.019$) measured on CBCT ($p > 0.05$). There was a low correlation between marginal gingival thickness and KTW in both transgingival probing ($Rho = 0.273$) and CBCT ($Rho = 0.298$) ($p < 0.01$), whereas KTW was found to have a positive correlation with middle ($Rho = 0.403$) and apical-BBT (0.225) ($p < 0.01$). In general, positive significant correlations were found at different levels between the quantitative measurements of the transgingival probing and CBCT methods (Table 4).

A comparison of gingival thickness and BBT measured by transgingival probing and CBCT according to different points is shown in Table 5. For both methods, marginal gingival thickness (1.43 mm and 1.25 mm) was significantly higher than gingival thickness measured from the middle and apical points (1.04 mm and 0.79 mm) ($p < 0.01$). No significant difference was found

between mid- and apical gingival thickness measurements ($p > 0.05$). A significant difference was found in CBCT for mid- and apical-BBT measurements ($p < 0.05$). Apical-BBT (1.03 mm) was found to be thinner than the middle BBT (1.15 mm) (Table 5) (Fig. 2).

Agreement between gingival thickness and BBT measurements was high in CBCT according to intra-observer ($ICC = 0.994 - 0.969$) and inter-observer ($ICC = 0.997 - 0.972$) reliability (Table 6).

Discussion

This study was performed on 180 upper anterior teeth of a limited Turkish population. This is the first study where all parameters of both gingival and periodontal phenotype were evaluated multidimensionally at different points along the keratinized gingiva. According to the most commonly used cutoff value of 1 mm to classify gingival phenotype as thin and thick [9, 12, 19, 30] 16.6% thin and 83.4% thick gingival phenotype was detected in this study. CBCT provided the best agreement with the gold standard transgingival probing [21] among the methods used. The methods were more accurate in detecting the thin phenotype than the thick phenotype. In addition, in areas where KTW was wider, gingival thickness was thicker. A previous study also supported

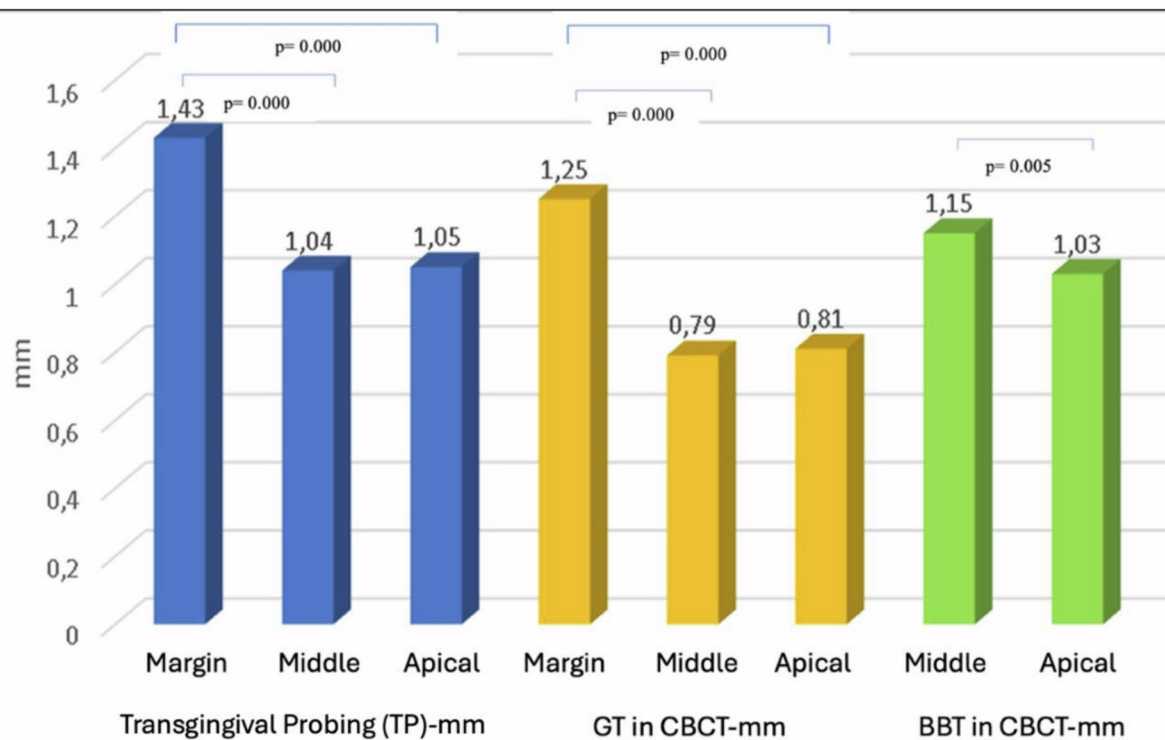


Fig. 2 Comparison of gingival and periodontal phenotypic variables at different reference points

Table 6 Intraobserver and interobserver agreement of phenotypic variables measured with CBCT

Observer (O)	Reading (R)	ICC (95% CI)	
		CBCT- GT	CBCT- BBT
Observer 1	R1 vs. R2	0.977 (0.966–0.988)	0.994 (0.989–0.99)
Observer 2	R1 vs. R2	0.969 (0.955–0.983)	0.981 (0.975–0.989)
Reading 1	O1 vs. O2	0.990 (0.987–0.993)	0.997 (0.995–0.998)
Reading 2	O1 vs. O2	0.972 (0.963–0.981)	0.982 (0.977–0.989)

ICC: Intraclass correlation coefficient, CI: Confidence Interval, O: Observer, R: Reading, CBCT: Cone Beam Computed Tomography, GT: Gingival thickness, BBT: Buccal bone thickness

that the middle and apical gingival phenotype at the vertical level on the alveolar bone decreased significantly according to the marginal point [18].

There is no accepted reliable or standard method to categorize gingival phenotype in the literature [31]. Different gingival points and cutoff values were considered to diagnose gingival phenotype as thin/thick by various methods, which made it difficult to compare the study results [3, 30, 32]. In addition, comparing the TRAN method performed with SPP and CPP with quantitative measurements (transgingival probing, CBCT) expressed different results in this study. In order to see the deficiency of binary classification, the CPP method was used and the medium-thick-very thick categories were classified as thick with the transgingival probing method. TRAN is a non-invasive and subjective method [3, 17, 33] but is not easily comparable [15] because the color,

design, thickness, and markings of the probes used in the studies may be different [10, 17, 18, 19, 34]. It may be affected by the light conditions, the gingival pigmentation, and the ethnic origine of the subjects. Patients with gingival pigmentation were not included in the study because pigmentation may limit the visibility of the probe and may cause errors in gingival thickness classification. Although the current study excluded subjects with significant pigmentation, we did not use a standard scale for pigmentation status. In addition, breastfeeding mothers were excluded because the rapid post-partum hormonal changes could result in gingival appearance changes.

Thin gingival phenotype was associated with higher buccal and proximal gingival recession measurements after treatments such as immediate implantation [8] and coronally positioned flap surgeries, with the highest root coverage found in thick gingival phenotype [7]. However, the great variety of methods in defining gingival phenotype may lead to misdiagnosis and unnecessary over-treatment or complicate the establishment of treatment protocols. Moreover, there is no consensus on which gingival thickness measured at which vertical level should be considered important/critical for clinical decision-making. Although the phenotype classification of the present study was made according to the evaluation at the marginal point, the correlation analysis was performed between transgingival probing-CBCT by considering the quantitative measurements of 3 points varying according

to the amount of KTW. This caused each tooth's gingival thickness/BBT points at the middle and apical points in the vertical direction on the alveolar bone to vary uniquely. The present study focused on interpreting the methods and techniques for their correct use. Ideal gingival points and cutting values should be determined in different oral regions using an easy-to-apply, harmless, and simple method for clinical use. Ignoring tooth numbers and individual or regional differences can be a study limitation. No comparison was made between the central, lateral, and canine regions, nor was a comparison made between the posterior regions of the mouth and the mandible regions. The fact that comparisons are not made regionally and including the whole mouth can be considered as a limitation.

Although the radiation dose is high, the clinical use of CBCT to determine gingival phenotype should be questioned. Transgingival probing is an invasive method that causes patient discomfort but is more objective, straightforward, and accepted as the gold standard than TRAN [21]. In this study, the agreement between transgingival probing and CBCT was high, gingival thickness/BBT measurements had moderate/poor correlation, and intra- and inter-observer agreement was excellent. Digital measurement with CBCT is a repeatable, reliable, and non-invasive method comparable to transmucosal measurements performed with an endodontic spreader [13, 14, 16]. The authors reported that the best correlation was observed between transgingival probing and CBCT measurements, supporting the findings of the current study [35, 36]. Therefore, CBCT evaluation can be requested before implant and orthodontic treatments to estimate the treatment prognosis and take precautions. Accurate determination of the CBCT settings, such as kV, mA, and voxel size (less than 0.3 mm), should be selected to obtain reliable results.

Although CBCT can measure gingival thickness, it is limited to cases requiring 3D imaging. Radiation-free, short-wavelength, high-frequency US has been reported as a repeatable and reliable method for examining superficial structures such as the gingiva. Studies evaluating high-frequency US in soft tissue imaging have not found a significant difference with the gold standard [37, 38]. Some studies have found no difference in ultrasound measurements compared with direct measurements for the anterior gingiva of the jaws [24]. The authors recommended ultrasound for anterior region measurements, but they also stated that it is not suitable for posterior regions due to the diameter and profile of the ultrasound probe [24]. In addition, differences in vestibule depths of the teeth and curvature of the bone structure make the use of the probe difficult [37]. Recently, smaller and more practical intraoral probes have become available. Thus, it is quite possible to evaluate periodontal structures in situ

without exposure to radiation in a short time, but more research is needed on this subject.

Consistent with the present study, TRAN methods have been reported to have low accuracy and reproducibility when a threshold of 1 mm was used [15, 39]. So, a cutoff value of 0.8 mm was suggested [34, 40]. The observed inconsistencies were attributed to ethnic origin differences, heterogeneous methodology, and lack of standardization of the distance between the measurement point and the gingival margin [4, 15, 30, 31, 34, 41, 42]. This current study similarly evaluated probe visibility and found 66.6% thin phenotype with SPP and 35.5% thin phenotype with CPP. Only 16.6% of the thin phenotype was found in transgingival probing and 27.7% in CBCT. The fact that the areas diagnosed as thin by TRAN were more than in transgingival probing suggests that transparency methods have a higher upper threshold for thin phenotype categorization, supported by a recent study [39]. This finding is consistent with a previous study [15], which observed that 73% of the areas classified as thin by SPP were thick phenotypes. Thin phenotype diagnosis may be overdiagnosed with the TRAN method, as in the present study. Since the risk of aesthetic complications of treatments is known to be high in thin phenotype [43, 44, 45], the low specificity of the TRAN method may lead to false thin phenotype diagnoses and, hence, overtreatment. Thin phenotype rates of 59.8% [46], 40.3% [36], and 43.2% [47], have been reported with the TRAN method in the literature. Some authors find CPP and SPP equally effective for the definition of maxillary thin phenotype but do not recommend them because of their low specificity in the diagnosis of thick phenotype and their inability to distinguish gingival thickness categories correctly [15, 39]. At the same time, some studies recommend their use in clinical practice by finding gingival phenotype measured using CPP to be highly correlated with transgingival probing [16, 48] and emphasize the importance of distinguishing thin- moderate-thick phenotypes. In addition to heterogeneous gingival thickness values, the authors also defined "moderate phenotype" [4, 16, 42]. However, although the thin phenotype gave poor esthetic results in terms of total root coverage, there was no significant difference between moderate-thick and very thick phenotypes [7], and the effect of moderate phenotype in terms of peri-implant clinical parameters is unclear. Therefore, evaluating more clinical treatment results is necessary before recommending more than two phenotype classifications.

Gingival phenotype includes gingival thickness and KTW by definition, but most clinicians use only gingival thickness. Periodontal phenotype definition is characterized by KTW, gingival, and bone morphotypes [3]. The finding of thicker phenotype in wider KTW in the present study is supported by several studies [4, 5, 10, 39, 47],

while there are also studies that reported wider KTW in thin phenotype [46]. According to the reported means of 4 and 4.3 mm for thin/thick KTW, respectively [39], the thin/thick KTW values of the presented study were measured as 6.7 and 7.7 mm. The weak positive correlation between KTW and gingival thickness in the present study is consistent with previous studies [4, 6, 39, 40, 41, 48]. Also, the moderate positive correlation between marginal gingival thickness and apical/middle-BBT is similar to the previous study [40]. Recently, authors [1] reported that periodontal phenotype varies among different individuals or regions of the mouth. It has been suggested that KTW and gingival thickness are independent of each other, although they are affected by tooth position [2].

Furthermore, the results of this study are consistent with previous studies that found facial gingival thickness to be positively correlated with BBT and KTW, indicating that the thicker the gingival tissue, the thicker the alveolar bone [5, 16, 49]. Although the determination of thin and thick gingival phenotype is related to the apical-coronal level and the cutoff value [31], it was observed that the thicker the gingival thickness, the thicker the BBT and the wider the KTW, regardless of the vertical assessment level [49], the results were consistent with the present study. Since a wide methodological diversity exists for assessing gingival thickness and BBT [12, 30, 35, 50, 51], a standard definition and anatomical landmarks of gingival phenotype and periodontal phenotype should be established to provide adequate and reliable direct comparisons between studies. This study did not examine embrasure shape, tooth crown type, tooth position, or individuals with a history of periodontitis, like some studies [46, 49]; only the facial surfaces of the maxillary anterior teeth of gingivally healthy individuals from a single and limited population were taken into account. These can be considered limitations of this study.

The authors found that the prevalence and severity of gingival recession were higher in areas with thin gingival phenotype [1]. This is of great importance for the maintenance of periodontal health and for better predicting the clinical outcome of different treatment procedures. Some studies did not find a statistically significant difference between gingival recession and gingival thickness [10]. Although some studies associate smoking with a thicker biotype, no significant difference was found between smoking and gingival thickness [52].

According to this study, BBT averages are around 1 mm in the middle-apical regions. Another study found that 70% of the BBTs measured from 3 to 6 mm apical were less than 1 mm, which is very thin despite the differences in methodology [53]. BBT is important for tissue healing after tooth extraction [54]. Since it has been reported that a minimum thickness of 2 mm is required for immediate

implant placement in the esthetic zone [55], it should be considered that bone augmentation techniques or late implant placement should be considered in cases where thin BBT is present.

In the present study, there was no significant difference between thin and thick phenotypes in terms of gender and age distributions. Some studies found thin phenotype to be more common in women [5, 31, 40, 56], and some authors reported higher gingival thickness and BBT measurements in men [31, 56], whereas others found no difference between age or gender and gingival phenotype [6, 10, 33, 46, 47, 52, 53]. The discrepancies between studies may be explained by differences in the method used to measure gingival and periodontal phenotype, sample size or cutoff points used to classify thin and thick phenotypes [31, 49].

Conclusion

Apart from the differences between the tested methods, the best agreement was found between transgingival probing and CBCT. CBCT images taken before implant placement or orthodontic treatment planning can be considered a noninvasive technique that does not expose the patient to an extra effective radiation dose to evaluate periodontal phenotype. Additionally, CBCT is indicated when deemed necessary on a case-specific basis to examine the periodontal phenotype status. Due to the extremely thin phenotype prediction of the TRAN method, clinicians should use caution to avoid overtreatment. Finally, determining that the KTW is wider and the BBT is thicker in thicker gingiva may guide clinicians in treatment planning. It is recommended that future studies should follow the long-term results of treatments with different phenotype classifications.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12903-025-06007-0>.

Supplementary Material 1

Acknowledgements

Not Applicable.

Author contributions

Conceptualization: M.A.T., D.İ.A., B.Ç., Methodology: M.A.T., D.İ.A., B.Ç., Formal analysis: D.Ö., Investigation: M.A.T., D.İ.A., Data Curation: M.A.T., D.İ.A., Writing—original draft preparation: M.A.T., B.Ç., K.K., D.Ö., Writing—review and editing: M.A.T., B.Ç., K.K., D.Ö., Visualization: M.A.T., B.Ç., K.K., D.Ö., Supervision: M.A.T., Funding acquisition: No funding.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Data availability

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the ethics committee at Ankara Yıldırım Beyazıt University, clinical ethics committee (no: 2637996/08.07.06.2023-07). Clinical procedures were carried out in conformity with the Declaration of Helsinki. Individuals who agreed to participate in the study signed informed consent forms.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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Received: 2 January 2025 / Accepted: 15 April 2025

Published online: 23 April 2025

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