

Evaluation of the Reliability of Color-Based Phenotype Probes in the Determination of Gingival Phenotype

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ABSTRACT

Objective: This study aimed to determine gingival thickness with newly developed color-based phenotype probes and to compare the results with the traditional method (transgingival probing).

Methods: 100 individuals with a mean age of 38.37 ± 11.03 years who had Miller I class gingival recession in the anterior region were included in the study. In measurements performed with color-based phenotype probes, white (thin), green (medium), and blue (thick) colored tips were used. In the transgingival probing method, a digital caliper with a penetration depth of 0.01 mm sensitivity was used.

Results: Of the teeth included in the analysis, 45% were in the maxillary anterior region, and 55% were in the mandibular anterior region. The mean tissue thickness was 0.76 ± 0.17 mm in the mandibular jaw and 1.22 ± 0.36 mm in the maxillary jaw ($p = .001$). A statistically significant relationship was found between the values determined with the transgingival method and the observed probe color ($p = .001$). The tissue thickness values of the cases whose observed probe color was white were significantly lower compared to those with green, blue, and no color ($p < .05$). When the mean tissue thicknesses were compared according to colors, tissue thickness significantly increased toward the blue color ($p = .001$). There was a statistically significant relationship with the gingival thickness measurement values ($p = .001$), and a low level of agreement was determined ($Kappa = 0.159$). In addition, it was determined that different colors were observed with the color-based phenotype probes in the same quantitative ranges.

Conclusion: Based on the assumption that color-based phenotype probes yield more subjective results, we believe that they can be used in clinical practice to determine gingival phenotype, but when quantitative data are required, preferring to use the transgingival method would give more accurate results.

Keywords: Phenotype, gingiva, tissue

1. INTRODUCTION

The integrity of the gingival tissues is necessary to ensure ideal treatments and long-term clinical results (1). The thickness of the gingival tissue and the width of the keratinized tissue are important in terms of protecting soft tissue health around the teeth and implants (2). Gingival recession is defined as atrophic periodontal changes. The term "atrophy" refers to a decrease in the volume and cellular population in an organ or tissue as a result of certain processes such as hypoxia, mechanical compression, and locally diminished vascularization (3). Gingival recession can be induced by periodontal diseases, dental plaque, wrong use of dental floss, aggressive toothbrushing, wrong occlusal relations, and off-arch teeth (4). Gingival recession can be seen in areas where the gingival phenotype is thin and tooth cleaning is difficult. In the 2017 World Workshop on Classification of Periodontal and Peri-Implant Diseases and

Conditions, the term periodontal phenotype was proposed to jointly evaluate the characteristics of soft tissue and bone morphology (5).

Measurement of periodontal soft tissue size is very important in terms of treatment planning, function, aesthetics, and prognosis (6). Aimetti et al. defined the periodontal phenotype as thin (<1 mm) or thick (>1 mm) (7). In addition, Kan et al. described thick gingiva as more dense and fibrous in appearance, and thin ones as more sensitive and almost transparent (8).

It is suggested that thin and thick gingival phenotypes respond differently to orthodontic treatment, periodontal treatment, surgery, and restorative dental treatment (9). Gingival recession may develop in individuals with thin phenotypes due to insufficient amount of soft tissue after orthodontic movements, implant surgery, crown prolonging

procedures, non-surgical periodontal treatment, and prosthetic treatment (10).

Since gingival phenotype is an important factor regarding periodontal health and treatment success, various measurement methods have been defined to determine it (11,12). One of the important parameters evaluated in the gingival phenotype is gingival thickness (5). Various methods such as visual examination (13), transgingival probing (12), ultrasound device (14), and visibility of the periodontal probe along the gingival groove have been used to evaluate gingival thickness (11). Recently, new types of periodontal probes have been mentioned in the literature to be used in determining phenotypes (15–17). There is no definite consensus in the literature on which method is the most accurate and appropriate for the measurement of gingival thickness. Although color-based phenotype probes offer a simple and visualized method for clinicians, very limited studies evaluating (6) their reliability (15,16) were encountered in the literature.

In this context, the present study aimed to determine the gingival thickness in cases with a gingival recession in the anterior region with the newly developed colored-tip color-based phenotype probe and to validate it with the transgingival probing method, which is the traditional measurement method.

2. METHODS

2.1. Patient Selection

100 patients between the ages of 19 and 65 who applied to the Department of Periodontology of Van Yüzüncü Yıl University Faculty of Dentistry with a complaint of gingival recession were included in our study. A written consent form was signed by all individuals who voluntarily participated in the study. The research was started with the approval of the Van Yüzüncü Yıl University Clinical Research Ethics Committee (17.06.2020/Decision no:15).

Inclusion criteria were determined as the (18) presence of Miller 1 class gingival recession in at least one of the mandibular and maxillary anterior teeth, volunteering for the study, being periodontally healthy, or the inflammation in the gingiva being limited to the gingiva (gingivitis).

Exclusion criteria were determined as the presence of systemic disease, smoking, pregnancy or breastfeeding, history of surgery in the relevant region (19), presence of significant melanin pigmentation, diagnosis of periodontitis, (20) and use of any drug affecting periodontal tissues (21).

2.2. Patient Data Records

After the systemic and dental anamnesis of 100 patients (46 females and 54 males) who met the study criteria were taken in detail, they were given detailed information about their diagnosis and the procedures to be followed.

2.3. Randomization

Before proceeding to the randomization process, all participants were evaluated in terms of gingival recession areas and jaws (mandible-maxilla). In cases with Miller 1 class gingival recession in the anterior region of a single jaw, the jaw with recession was included in the study. In cases with Miller 1 class gingival recession in both jaws, the jaw to be included was determined by the coin toss method. If there was more than one Miller 1 class gingival recession in a jaw, the tooth to be included was again randomly selected. Teeth with Miller 1 class gingival recession were written on a piece of paper in a way the patients could not see them. Each tooth was assigned a letter, and the patients were asked which letter they chose. The tooth corresponding to the letter chosen by the patient was included in the study, and its measurements were made. All measurements were made by a single clinician and based on a single tooth with Miller 1 class gingival recession.

2.4. Gingival Thickness and Phenotype Measurement

Gingival thickness was determined using a newly developed tool, color-based phenotype probe, and transgingival probing method.

When measuring with a color-based phenotype probe, the white-colored probe was first placed in the gingival sulcus with a force of less than 0.25 N. If the color appeared, that is, if the probe was reflected from the gingival tissue, the phenotype was recorded as thin. If the white color was not visualized, the green-colored probe was used in the same way, and if the color was reflected, the phenotype was recorded as medium thickness. If the green-colored tip was not visualized from the gingival tissue, the blue-colored probe was used, and if the only color seen was blue, the phenotype was classified as thick. If the blue tip was not visualized, the gingival tissue was recorded as very thick (12,17) (Figure 1).



Figure 1. Color-based phenotype probe

In a recent study investigating the reliability of color-based phenotype probes, (22) cases were divided into four categories according to tissue thickness measured by an endodontic file [< 1 mm (thin), ≥ 1 to < 1.25 mm (medium), ≥ 1.25 to < 1.5 mm (thick), and ≥ 1.5 mm (very thick)], and part of the analysis was performed according to these values (22). It was also examined whether the data in our study were consistent with these ranges presented by the literature.

In the transgingival probing method, measurements were made for each tooth from 2 points: the apical of the free gingival groove and the coronal of the mucogingival junction. After the gingival thickness measurement points were determined with a marker pen, a topical anesthetic spray (Xylocaine® spray; Vemcaine 10%, lidocaine) was applied so that the patient did not feel pain. The 15-point endodontic spreader (G-STAR Medical Co.,Ltd.,Guangdong,China) with a silicone stopper on it was advanced perpendicularly to the gingiva until contact with hard tissue was felt, and gingival thickness was determined. The penetration depth between the stopper and the tip of the file was measured using a digital caliper (Mitutoyo Corporation, Kanagawa, Japan) with a precision of 0.01 mm (16, 23) (Figure 2).

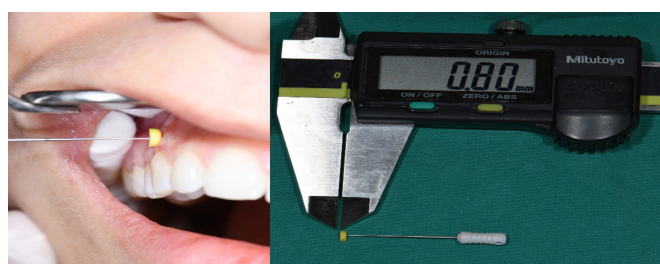


Figure 2. Transgingival probing method

The averages of the gum thicknesses obtained from both measurement points were taken and recorded as the first measurement. After 10 minutes, after the same procedures were repeated for the second time by the same researcher, the average of the two measurements was taken, and the final gingival thickness of the included tooth was determined.

2.5. Statistical Methodology

G*Power 3.1. software was used to calculate the sample size of the study. Based on the theoretical power value of 80% with a 5% margin of error, 95% confidence level, and moderate effect size, it was determined that at least 100 observations were required, and the research was conducted on 100 participants in total. The data obtained in this study were analyzed by the Licensed IBM SPSS Statistics Version 21 software. While the normal distribution of variables was being researched, the Shapiro-Wilk test was employed because of the unit numbers. While interpreting the results, 0.05 was used as the level of significance, and in the case of $p < .05$, it was suggested that the variables did not have a normal distribution, but in the case of $p > .05$, the variables had a normal distribution.

The Chi-Square analysis was applied to examine the correlation between the groups of nominal variables. If the nominal values did not display sufficient volumes in the cells of 2X2 tables, Fisher's Exact Test was used. The Pearson Chi-Square test was used to analyze RxC tables with the help of the Monte Carlo Simulation. When examining the differences between the groups, the Mann-Whitney U Test and the Kruskal Wallis H Test were used in intergroup comparisons if

the variables did not display a normal distribution. In case of a significant difference in comparisons with more than two groups, groups with significant differences were determined with the help of Post Hoc (Mann Whitney U test with Bonferroni correction) tests.

Spearman's Correlation Coefficient was used to examine the relationships between non-normally distributed variables.

Kappa coefficient was used to evaluate the agreement with the gingival thickness scale given in the literature.

3. RESULTS

46 of the individuals participating in the study were female, and 54 were male ($p = .276$). The mean age of the patients was 38.37 ± 11.03 ($p = .079$) with a range of 19-65 years. 45% of the teeth with gingival recession were located in the maxillary anterior region and 55% in the mandibular anterior region ($p < .05$).

There was a statistically significant difference between the visualized probe color and the jaws ($p < .05$). The visualized probe color of 65.45% of the regions included in the mandible and 62.22% of those included in the maxilla was green, that is, medium thickness. No probe color was visualized in 8.89% of the regions included in the maxilla (very thick). The distribution of the measured clinical parameters of the individuals is presented in Table 1.

Table 1. Relationship between jaws in terms of visualized probe color

		Included Jaw						Chi-Square Test	
		Mandible		Maxilla		Total		Chi-Square Test	p
		n	%	n	%	n	%		
Probe Color Visualized	White	19	34.55	1	2.22	20	20	-	.001*
	Green	36	65.45	28	62.22	64	64		
	Blue	0	0	12	26.67	12	12		
	None	0	0	4	8.89	4	4		
	Total	55	100	45	100	100	100		

n: number of patients; %: percentage; significance: * $p < .05$

While the mean tissue thickness of the teeth in the mandibular jaw was 0.76 ± 0.17 , the mean tissue thickness of the teeth in the maxillary jaw was 1.22 ± 0.36 mm ($p < .05$) (Table 2).

There was a statistically significant relationship between all tissue thickness values measured regardless of the included jaws and the visualized probe color ($p < .05$). The tissue thickness value of the cases with visualized white color was significantly lower than the ones with green color, blue color, and no visualized color, and the tissue thickness value of the cases with visualized green color was significantly lower than the ones with blue color and no visualized color ($p < .05$) (Table 3).

Table 2. Relationship between jaws in terms of tissue thickness values

	Included Jaw	Included Jaw						Mann-Whitney U Test		
		n	Mean	Median	Min	Max	Sd	Mean Rank	z	p
Tissue Thickness (mm)	Mandible	55	0.76	0.77	0.33	1.1	0.17	31.84	-7.115	.001*
	Maxilla	45	1.22	1.13	0.79	2.08	0.36	73.31		
	Total	100	0.97	0.88	0.33	2.08	0.36			

n: number of patients; Sd: standard deviation; mean: mean; median; median, Min: minimum; Max: maximum; *p<.05

Table 3. Relationship between tissue thickness measurement values and visualized probe color

	Tissue Thickness (mm)	Tissue Thickness (mm)						Kruskal–Wallis H Test		
		n	Mean	Median	Min	Max	Sd	Mean Rank	H	p
Probe Color Visualized	White	20	0.66	0.68	0.33	0.91	0.17	20.55	50.708	.001*
	Green	64	0.92	0.88	0.52	1.77	0.23	50.09		
	Blue	12	1.41	1.29	1.14	1.8	0.25	87.33		
	None	4	1.87	1.97	1.45	2.08	0.29	96.38		
	Total	100	0.97	0.88	0.33	2.08	0.36	*W-G, *W-B, *W-H, *G-B, *G-H		

n: number of patients; Sd: standard deviation; Min: minimum; Max: maximum; significance *: p<.05, W: white, G: green, B: blue, H: no color

Figure 3 shows the average values of the recorded tissue thickness values in cases where white, green, and blue colors were seen and no color was seen. The tissue thickness value increased significantly from white to green, from green to blue, and where no color was seen (p<.05).

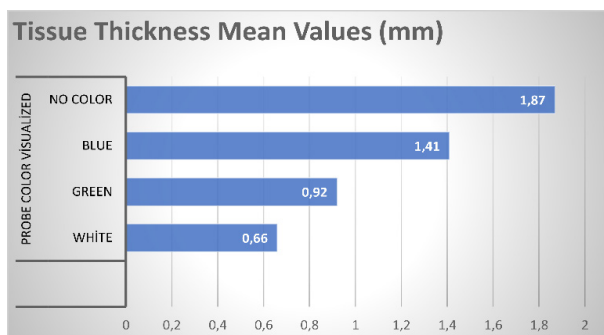


Figure 3. Tissue thickness trend by probe color visualized

The minimum and maximum values of the measured tissue thickness values following the recording of the Visualized Probe Color are shown in Figure 4. It was determined that the white-colored probe was observed at a gingival thickness within the range of 0.33-0.91 mm. It was observed that the green-colored probe was visualized at a minimum thickness of 0.52 mm and a maximum thickness of 1.77 mm. Measurements of other colors are also expressed in the graph.

According to the scale provided by Bertl et al. in their study, the cases were divided into four categories according to the measured tissue thickness [(<1 mm (thin), ≥ 1 to < 1.25 mm (medium), ≥ 1.25 to <1.5 mm (thick), and ≥ 1.5 mm (very thick)], and these values were associated with the visualized probe color (22). Since our study aimed to determine the reliability of color-based phenotype probes, an additional statistical analysis was performed to show its consistency with the values in this literature (Table 4). Although the relationship between the relevant literature and our research results was statistically significant (p<.05), it was determined

that there was a low level of agreement when the kappa level was taken into consideration (Kappa=0.159).

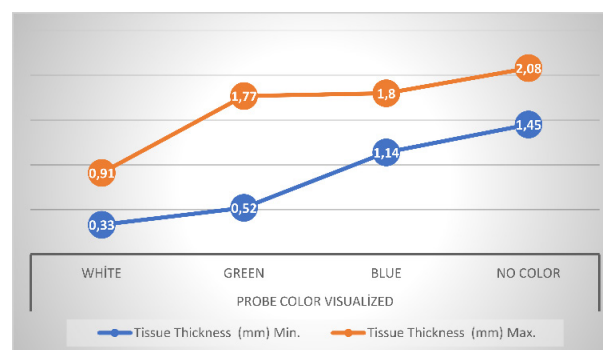


Figure 4. Minimum and maximum tissue thickness values of visualized probe colors measured with the caliper

According to our research results, it was observed that the cases with the visualized probe color white (n=20) were compatible with the given scale by 28.99%. The fact that 49 cases, which were visualized to be green, had a thin phenotype according to the scale decreased the compatibility rate.

It was observed that the cases with the green probe color (n=10) were compatible with the given scale by 71.43%, and the 4 cases determined as blue were also in the medium thickness phenotype class according to the scale.

It was determined that those with blue probe color (n=3) were 50% compatible with the scale. It was observed that 2 cases that were visualized as green and 1 case where no color was visualized (very thick) were blue according to the scale.

According to our study, it was determined that cases in which no color was seen (n=3) were compatible with the scale by 27.27%. It was observed that 3 cases seen as green and 5 cases seen as blue had very thick phenotype (no color was seen) according to the scale. Since the agreement increases with the approximation of the Kappa value to 1, our analysis result indicates a low compatibility (Kappa=0.159).

Table 4. Analysis of the compatibility between gingival thickness scale and probe color visualized

		Phenotype Given in Gingival Thickness Scale										Kappa Compliance Test	
		Thin (white)		Moderate (green)		Thick (blue)		Very Thick (no color)		Total		Kappa	p
		n	%	n	%	n	%	n	%	n	%		
Probe Color Visualized	White	20	28.99	0	0	0	0	0	0	20	20	0.159	.001*
	Green	49	71.01	10	71.43	2	33.33	3	27.27	64	64		
	Blue	0	0	4	28.57	3	50	5	45.45	12	12		
	(No color)	0	0	0	0	1	16.67	3	27.27	4	4		
	Total	69	100	14	100	6	100	11	100	100	100		

n: number of patients; %: percentage; significance * $p < .05$

4. DISCUSSION

Gingival phenotype is a critical factor that significantly affects the clinical decision process of dentistry and aesthetic results (11, 24, 25). Determination of gingival phenotype is necessary to manage periodontal health and plan restorative or orthodontic treatment, especially in areas with thin-narrow gingivae (11).

In the literature, there is a probe transparency method in which the periodontal probe is advanced in the direction of the mucogingival line and the transparency of the gingiva is determined (26). In the probe transparency method, the gingival phenotype is characterized as thin if the contour of the probe can be seen from the gingival edge, and thick if it cannot be visualized (27). Accordingly, Rasperini et al. introduced easy-to-use (17), non-invasive color-based phenotype probes for the evaluation of gingival phenotype. In our study, the evaluation of the gingival phenotype was performed using the aforementioned color-based phenotype probe.

Transgingival probing, which is considered the current gold standard for ensuring the use of the gingiva, is likely to affect patient comfort and provides the advantage of providing lens data, although anesthesia in some areas is an invasive method (28). This method, which provides accurate quantitative data, was used as a reference to determine the reliability of the probes investigated in our study.

When the tissue thickness of the mandible and maxilla were evaluated, our study results were similar to many studies in the literature and it was confirmed that the tissue thickness detected in the mandibular anterior teeth was lower than that of the maxillary anterior teeth (29). In the measurements made within the current population limits, it was observed that the color-based phenotype probes and the transgingival method were generally compatible (the probe color changed from white to blue as the thickness increased), and the mandible tissue thickness was significantly lower than the maxilla.

In a study analyzing the relationship between gingival phenotype and gingival thickness with ultrasound, visual evaluation, and color-based phenotype probes, it was reported that ultrasound measurements were consistent with measurements made with color-based phenotype

probes and that color-based phenotype probes were adequate to determine different gingival phenotypes (30). In another recent study, the gingival thickness of 86 (16) periodontally healthy teeth was measured by transgingival probing and cone beam computed tomography (CBCT). In addition, color-based phenotype probes were also used in the study to explain the relationship between gingival thickness and gingival phenotype. As a result of the study, a strong significant relationship between transgingival probing and CBCT and a significantly strong correlation with color-based phenotype probes were reported (16). Similar to the literature, in our study, it is seen that as the value of gingival thicknesses measured by caliper increased, the tendency towards the blue color expressing the thick phenotype increased.

A recent clinical study compared the diagnostic accuracy of two different transparency methods using steel and color-coded probes to identify (31) thin and thick gingival phenotypes. In the study, which accepted the transgingival probing method as a reference, it was stated that probe transparency methods were highly sensitive to diagnose the thin phenotype but showed a weakness for the thick phenotype (31). In an animal study using the probe transparency method, 3 different probes were used to determine the gingival phenotype in a total of 24 sections with different tissue thicknesses (32). It was attempted to determine the phenotype by making thin/thick evaluation with a periodontal probe, thin/medium/thick evaluation with a double-tip periodontal probe, and thin/medium/thick/very thick evaluation with a color-based phenotype probe. With color-based phenotype probes, the transition threshold from thin phenotype to medium phenotype was shown in the range of 0.4 to 0.5 mm, and the transition range from "medium" to "thick" was not observed. However, a very thick phenotype was observed starting from the threshold of 0.7 to 0.8 mm. Gingival phenotype classification is presented as thin (< 0.5 mm, high risk), medium (0.5-0.8 mm, medium risk), and thick (> 0.8 mm, low risk). In the study, it was stated that these probes could be easy and reliable tools to use for routine clinical practice (Fischer ve ark., 2021). Another recent study expressed gingival phenotype reference ranges as < 1 mm (thin), ≥ 1 to < 1.25 mm (medium), ≥ 1.25 to < 1.5 mm (thick), and ≥ 1.5 mm (very thick) (22). When our study results were compared with the literature data, it was

determined that the colors visualized and the measured minimum-maximum caliper values were in a variable range, that is, different colors could be seen in the same value ranges. This finding is consistent with the literature. However, in the thin phenotype in which the white color was seen, although it seemed statistically significantly correlated with the gingival thickness scale values given by Bertl et al., the low level of compliance (28.99% (Kappa=0.159, $p < .05$) conflicts with the study of da Costa et al (31). These differences may develop due to anatomical features such as gingival pigmentation, collagen content, and blood flow and circulation of individuals (32). In addition, it is thought that the subjective evaluations made by clinicians through inspection may have led to different results.

When the results are examined in detail, it is pointed out that color-based phenotype probes are not sensitive in quantitative measurements and are a subjective technique due to different colors seen in different ranges in both thin phenotype and thick phenotype, different clinicians presenting different colors in the same range, and even a single clinician seeing different colors in the same ranges. However, the tendency of the color observed from white to blue in parallel with the increase in tissue thickness measured by the caliper seems to provide easy and applicable advantages in clinical practice.

The limitations of this study are that only Miller 1 class gingival recession was included for standardization purposes, the depth of recession was not taken into account, the gingival color differences of individuals were not evaluated, and the effect of gender and age-related variables on the gingival phenotype was ignored. It may be beneficial to conduct more comprehensive research including these aspects. Another limitation of the study is that the presence of fenestration and dehiscence defects may cause erroneous results in transgingival thickness measurement, and these factors were not evaluated.

5. CONCLUSION

In parallel with the increase in tissue thickness values detected by the transgingival method, it was observed that the measurement of color-based phenotype probes changed in accordance with white, green and blue colors, respectively. It was determined that different colors were seen in the same quantitative ranges as color-based phenotype probes and proved weak compared to the traditional method in terms of providing quantitative values. Therefore, in the gingival phenotype evaluation, it was observed that the use of the transgingival method yielded more accurate results when quantitative data were required. In routine clinical practice, it is thought that color-based phenotype probes may be an alternative to the traditional method as they offer a non-invasive method and ease of application in cases where phenotype detection would be performed.

In this study, the usability of color-based phenotype probes in gingival thickness and phenotype detection was

investigated, and since this method is new, its diagnostic accuracy was examined in comparison with the transgingival probing method. Our study findings showed that color-based phenotype probes were weak in quantitative measurements in terms of presenting different colors at different intervals based on the values determined by transgingival measurement; however, they may provide an advantage in clinical practice in terms of the similarity of measurement trends in both methods.

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Author Contributions:

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Design of the study: NZA

Acquisition of data for the study: ETS

Analysis of data for the study: ETS, NZA

Interpretation of data for the study: ETS, NZA

Drafting the manuscript: ETS

Revising it critically for important intellectual content: NZA

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