Assessment of gingival phenotype through periodontal and crown characteristics: a cluster analysis

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Abstract

Objective: To evaluate gingival phenotypes in 100 subjects (n = 100), with regard to maxillary central incisors and surrounding periodontium in an observational diagnostic study.

Materials and Methods: Individuals were grouped based on: Probing depth (PD); keratinized mucosa (KM); Ratio Height/Width Crown (CH/CW), papilla area (PA), gingival thickness (GT) and Gingival Volume (GV). ANOVA and chi-square tests were performed with a significance level of 5%. Results: PD (p=0.860) and CH/CW (p=0.086) were not statistically significant. Cluster analysis identified three groups: Cluster I (n = 32) had the lowest values: KM (4.54mm), GT (0.83mm), PA (15.64mm^2) and GV (3.80mm^3); Cluster II (n = 43) presented KM (8.02mm); GT (1.40mm); GV (11.18mm^3) and PA (14.10mm^2); Cluster III (n = 19) exhibited an average KM of 5.57mm, GT (1.14mm), PA (20.08mm^2) and GV (6.48mm^3).

Results: Cluster I was characterized as a thin phenotype; cluster II as thick, and cluster III, as an intermediate. Significant associations were found when transparency on probing was compared among clusters ($p < 0.05$) and gingival exposure when smiling ($p < 0.05$).

Conclusion: Thin phenotype was found in 34.04% of the sample (cluster I), thick phenotype in 45.75% (cluster II) and intermediate phenotype in 20.21% (Cluster III).

Keywords: Connective tissue; Diagnosis; Periodontics; Phenotype.

Introduction

Healthy periodontal tissues present significant intra- and inter-individual clinical variations (Maynard and Wilson, 1980; Olsson and Lindhe, 1991; De Rouck *et al.*, 2009; Kahn *et al.*, 2013; Lang & Bartold, 2018). As such, the identification of a patient's gingival phenotype and clinical parameters has been shown to be important for the predictability of treatments performed in clinical practice in several fields of dentistry (Fu *et al.*, 2010; Cook *et al.*, 2011). In 1980, Wilson and Maynard proposed dividing gingival characteristics into four different groups, depending on the thickness and range of keratinized tissue and bone width. Later, the term "periodontal biotype" was suggested (Seibert and Lindhe, 1989) due to the existence of these different morphotypes and many other researchers have attempted to analyze and describe these specific gingival characteristics (Olsson et al., 1993; Müller and Eger, 1997; Müller et al., 2000; Maurer et al., 2001; De Rouck *et al.*, 2009; Anand *et al.*, 2012; Kahn *et al.*, 2013; Abraham *et al.*, 2014). However, Cortellini and Bissada (2018) proposed "gingival phenotype" as a new term in the new classification scheme for periodontal and peri-implant diseases and conditions. These studies analyzed the identifier parameters of periodontal phenotypes, by observing individuals with different morphometric combinations related to the gingival thickness, crown length/width of the crown ration, papillae width/papilla height ratio, probing depth and keratinized width (Maynard and Wilson, 1980; Seibert and Lindhe, 1989; Kao and Pasquinelli, 2002). However, a simple visual inspection of these morphometric parameters is unable to identify the gingival phenotype (Cuny-Houchmand *et al.*, 2013; Bhat and Shetty, 2013).

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Some authors have proposed classifications for periodontal phenotype. Maynard and Wilson (1980) used a rating for four types of periodontium; In types I and III, individuals presented width of the keratinized mucosa from 3 to 5mm and thick gingiva, differing in thickness of the underlying alveolar bone. In type I, the bone is thick on palpation; already, in type III, the alveolar bone is thin and the roots can be palpated. In types II and IV, individuals present the width of the band of smaller keratinized mucosa than 2 mm and thin gingiva, differing from each other by alveolar bone thickness, which is thin in type IV and more thick in type II. Conversely, Seibert and Lindhe (1989), Olsson *et al.* (1993) and Müller and Eger (1997) ranked the periodontium according to the characteristics of the clinical crown. They observed that square teeth have a thicker gingiva, a greater range of keratinized mucosa, shorter interdental papillae and increased pocket depth. Those with triangular teeth had thinner gingiva, a narrow zone of keratinized mucosa, more elongated interdental papillae and a lower pocket depth.

De Rouck *et al.* (2009) proposed the differentiation of periodontal biotype by a visual method, evaluating gingival thickness through probing transparency. Subjects were divided into three groups by a cluster analysis: A1, defined as thin biotype, wherein the probe was translucent over the free gingival margin; A2, defined as thick biotype, wherein the instrument was not seen during the probing; and B, defined as an intermediate biotype, in which characteristics of the two previous groups were found in a less-defined manner. More recently, other methods have been proposed to support biotype analysis, such as: determining bone thickness by radiographic assays using metal blades for improved measurement (Stein *et al.*, 2013); use of cone-beam CT to associate the clinical periodontal biotype with the thickness of the buccal and gingival bone plate (Fu *et al.*, 2010; Cook *et al.*, 2011; Januário *et al.*, 2008). In addition, bone loss assessment with digital radiography (Teeuw *et al.*, 2009), 3D laser scanner (Rosin *et al.*, 2002), and the use of digital photographs, tooth dimensions and soft tissue analysis by computer programs (Eghbali *et al.*, 2009; Kan *et al.*, 2003) have also been used.

According to Müller and Eger (1997), a clinical examination is essential for a correct periodontal phenotype diagnosis. This examination should include not only periodontal probing to determine gingival thickness, but must also associated with a complete analysis of all factors related to gingival morphology, as these all have a great impact on aesthetics and the final harmony of the smile. Since the use of simplified and effective methodology to produce such data have yet to be established, the aim of this study was to identify the existence of periodontal phenotypes in a volunteer sample, using probing depth parameters (PD); keratinized mucosa (KM); papilla area (PA); height/width ratio of the crown (CH/CW), gingival thickness (GT) and Gingival volume (GV) by cluster analysis.

Materials and Methods

Subjects

This study, characterized as an individual, observational, prospective and cross-sectional study, was approved by the Ethics Committee of the Federal University of Rio Grande do Norte (909,875). The sample was non-probabilistic and composed of dental students from the Dentistry Department, of the Federal University of Rio Grande do Norte (UFRN). Subjects included matched the following inclusion criteria: had all maxillary anterior teeth in a state of periodontal health – recently defined as a: 1) pristine periodontal health, defined as a total absence of clinical inflammation and physiological immune surveillance on a periodontium with normal support (no attachment or bone loss). 2) clinical periodontal health, characterized by an absence or minimal levels of clinical inflammation in a periodontium with normal support; 3) periodontal disease stability in a reduced periodontium; 4) periodontal disease remission/control in a reduced periodontium. (Lang & Bartold, 2018). Individuals with crown restorations in the upper central incisors and/or orthodontic appliances; pregnant or breastfeeding women; subjects undergoing drug therapy with known effects on periodontal soft tissues and/or subjects with clinical signs of periodontal disease (periodontal pockets and clinical attachment loss), smokers and diabetics were excluded from the study.

The sample size was not based on statistical considerations for inference tests, but on the size required to ensure a correct population representation and its inherent structure. In particular, in small groups, the relevance of each group to the research question and the confidence to characterize them was taken into consideration. Thus, the ratio of thick phenotypes, from the De Rouck (2009) study, was considered to obtain a proportion of 66% thick phenotypes, representing a total of 87 subjects in the sample.

Clinical Parameters

All patients were subject to anamnesis and clinical periodontal examination after approval of the ethics committee in February 2016 and ended in September of the same year. For gingival phenotype analysis, an intraoral clinical examination was conducted, specifically in the upper central incisor, by a and trained calibrated (kappa= 0.936; $p<0.05$) examiner to measure the following variables: probing depth (PD), height/width ratio of the crown (CH/CW), height/width ratio of the interdental papillae (HP/WP) and Gingival thickness, as described below:

(I) CH/CW ratio of the maxillary central incisors: Crown height (CH) was measured from the incisal edge of the crown to the free gingival margin or, if noticeable, at the cementoenamel junction, with the aid of a dry-tip compass and a digital caliper. The crown width (CW) was measured between the proximal faces, using the distance between the mesial and distal dihedral angles as a reference, at the border between the cervical and middle thirds. The same technique was used for measuring the length.

(II) Width of the keratinized mucosa band (KM): Distance from the mucogingival junction to the gingival margin, measured at the midpoint of the buccal surface of teeth with the aid of a digital caliper.

(III) Gingival Thickness (GT): Transversal measurement from the keratinized mucosa to the periosteum with a finger spreader and a silicon slider and digital caliper (Figure 1-2).

(IV) Papilla area (PA): PA was defined using data for papilla height (P1H) and papilla width (PW), according to the calculation: P1HxPW / 2 (mm^2) (Kao *et al.*, 2008). These values were obtained with North Carolina probes (Millennium-Golgran®, São Caetano do Sul, SP, Brazil), measuring from the top of the papilla to the line that touches the most cervical soft tissue margin of the buccal-mesial surface. The width was measured at the border between the middle and cervical portions of the papilla.

(V) Gingival volume (GV): GV was obtained by calculating: Gingival thickness X Width of attached gingiva band X 1 mm, in the mesio-distal direction, at the midpoint of the attached gingiva at the vestibular face of each tooth (Kao *et al.*, 2008).

(VI) Probing depth (PD): Distance between the gingival margin and the most coronal portion of the junctional epithelium, at the midpoint of the vestibular face of the teeth, measured with the aid of the North Carolina probe (Millennium-Golgran®).

During the examination, periodontal clinical characteristics were also identified; e.g. gingival margin transparency (positive transparency of the periodontal probe was defined as thin; negative transparency of the probe was defined as thick (De Rouck *et al.*, 2009; Dutra *et al.*, 2011); gingival exposure when smiling (distance from the most apical point of the marginal gingiva of the upper incisors to the lip line) was measured with the aid of the millimeter probe.

Statistical analysis

Data were analyzed using the SPSS 22.0 (Statistical Package for Social Science) statistical software. The examiner responsible for data analysis was not aware of any information from the subjects, characterizing the study as double-blinded. Hierarchical analysis was performed first to determine the presence of outliers. Subsequently, non-hierarchical analysis (k-means algorithm) was applied. After cluster formation, ANOVA was performed to compare the clusters with respect to each others' analyzed parameters. Cluster analysis was performed based on the Euclidean distance of six clinical parameters to detect different combinations of morphometric data to characterize individuals with common parameters. The Bonferroni post-test was performed when statistically significant differences were present. The unpaired Student's t test was used to identify significant differences between genders.

To analyze the qualitative data regarding gingival phenotype (transparency on probing) and its association with the clusters, as well as the relation with the independent variables (sex and gingival exposure when smiling), we used the Chi-square test or Fisher's Exact. For all tests, a significance level of 5% was established (alpha = 0.05).

Results

Clinical Parameters

The initial study sample consisted of 100 periodontally healthy young subjects that were divided equally to avoid gender interference in results. However, after the hierarchical analysis, only 94 patients remained in the study, of which 46 (48.93%) were males and 48 were females (51.07%), all between 19-26 years old.

Non-hierarchical analysis using six parameters showed that the PD (overall mean 1.3 and standard deviation 0.2) and CW/CH variables do not adequately explain the model, since there was no statistical significance for this group ($F = 0.151$, $p = 0.860$, $F = 2.53$, $p = 0.086$, respectively) and, therefore, these parameters were excluded from the first cluster analysis. Table 1 shows the descriptive statistics of the clinical parameters after the result for cluster analysis ANOVA. Thus, these variables were excluded from the analysis, which was then performed again using only those parameters with a significance level of below 0.05.

Figure 1- Gingival Thickness measurement with a finger spreader and a silicon slider and digital caliper

Figure 2- Transversal measurement from the keratinized mucosa to the periosteum with a finger spreader and a silicon slider

With regard to gender, all parameters showed a significant difference. When compared to females, males presented: the highest mean keratinized mucosa width (5.67 mm vs 7.05mm, respectively; p <0.001); greater average gingival thickness (1.08 vs 1.23mm, respectively, p = 0.024); lower average mean papillae area (16.62mm^2 against 14.6mm^2; p=0,006) and higher mean gingival volume (6.38 mm^3 vs. 9.12 mm^3, respectively; p <0.001). These values indicate a higher prevalence of males in the cluster with thick phenotype characteristics (Cluster 3).

Cluster Analysis

The division method separated the sample into three groups, using morphometric data obtained from the sample. Cluster I was composed of 32 individuals (34.04%), Cluster II was composed of 43 patients (45.75%) and Cluster III of 19 individuals (20.21%). The specific characteristics of each cluster are presented in Table 2. Cluster I, when compared to the other groups, presented a lower KM range, lower GT and lower GV, but presented an intermediate papilla area between the

Table 1. Results from non-hierarchical analysis, by ANOVA test for clinical parameters analyzed. (PD; KM; PA; CH / CW; GV; GT).

	Mean Square	Degrees of Freedom	Mean Square	Degrees of freedom		
Probing Depth	0.007	2	0.044	91	0.151	0.860
Keratinized Mucosa	124.521	2	1.090	91	114.221	<0.001
Papilla Area	112.422	2	5.111	91	21.997	<0.001
CH / CW	0.023	2	0.009	91	2.525	0.086
Gingival Volume	536.410	2	2.009	91	266.953	<0.001
Gingival Thickness	2.917	2	0.039	91	75.007	<0.001

Table 2. Mean and standard deviation from clusters and their periodontal clinical characteristics.

	Cluster I (thin) Mean (sd)	Cluster II (thick) Mean (sd)	Cluster III (intermediary) Mean (sd)
Keratinized Mucosa (mm)	4.54 (1.05)c	8.02 (1.02)b	5.57 (1.38)a
Gingival Thickness (mm)	0.83 (0.22)c	1.40 (0.14)b	1.14 (0.24)a
Papilla Area (mm2)	15.64 (3.35)b	14.10 (2.59)b	20.08 (1.78)a
Gingival Volume (mm3)	3.80 (1.30)c	11.18 (1.28)b	6.48 (2.32)a

*ANOVA test. Lower case in line means statistically significant difference. (p<0.05) sd: standard deviation

Table 3. Association between presence/absence of probing transparency as a validate method on clusters I, II e III.

		Cluster			
		I (n%)	**II (n%)**	**Total**	**p**
Probing transparency	Presence	29 (96.67%)	1 (3.33%)	30 (100%)	<0.001
	Absence	3 (6.67%)	42 (93.33%)	45 (100%)	
		32	43	75	
		Cluster			
		I	**III**		
	Presence	29 (78.38%)	8 (21.62%)	37 (100%)	<0.001
	Absence	3 (21.43%)	11 (78.57%)	14 (100%)	
		32	19	51	
		Cluster			
		II	III		
	Presence	1 (11.11%)	8 (88.89%)	9 (100%)	<0.001
	Absence	42 (74.24%)	11 (20.76%)	53 (100%)	
		43	19	62	

*Fisher's Exact Test.

two other clusters. These data may be associated with a thin phenotype type (Figure 3). On the other hand, Cluster II presented a larger KM, of greater GT and GV, and a smaller papilla area, presenting a significant difference from the other groups. This profile can be defined as the thick phenotype group (Figure 4). Cluster III, however, presented a higher PA in relation to the other clusters; however, all the other parameters presented average values between groups and the group was, therefore, defined as the intermediate phenotype (Figure 5).

Since the periodontal probe within the sulcus may vary in be visiblity, with regard to transparency, phenotypes were classified into thin and thick. The thin phenotype was found to be more prevalent in cluster I (30.9%), which demonstrated transparency at the moment of probing (defined as thin). Conversely, the non-visualization of the probe (defined as the thick phenotype) was more prevalent in cluster II individuals (44.7%). The association between presence /absence of probing transparency revealed a statistically significant association among clusters and served to validate the methods, with regard to the determination of phenotypes in the clusters. In other words, individuals classified in cluster I (thin phenotype) had transparency on probing and those classified in cluster II (thick phenotype) did not have transparency on probing. The intermediate biotype (cluster III) assumed the intermediate characteristics between one of the extreme clusters.These data are presented in Table 3.

The "gingival exposure when smiling" parameter was divided into two categories (up to 2 mm and 2 to 4 mm) for better statistical adjustment. The highest prevalence of gingival smile was observed in cluster II (31.9%), which was of a thicker phenotype, while the lowest prevalence was observed in cluster III (8.5%). This parameter presented a significant association among the clusters (p <0.001).

In order to propose a clinical application of the clusters formed from this study, a range was calculated from the parameters that most explain this model (Table 1) - gingival volume and gingival thickness, as described below:

(1) Gingival volume:
   a) 1.5 mm^3 - 4.0 mm^3: Thin phenotype
   b) 5.0 mm^3 - 8.0 mm^3: Intermediate phenotype
   c) 9 mm^3 - 14 mm^3: Thick phenotype

(2) Gingival thickness:
   a) 0.4 mm - 0.9 mm: Thin phenotype
   b) 1.0 mm-1.3 mm: Intermediate phenotype
   c) 1.3 mm-1.8 mm: Thick phenotype"

Discussion

Studies of periodontal phenotype may improve the management and prevention of secondary effects in response to aesthetic dental rehabilitations (Kahn *et al*., 2013; Kao and Pasquinelli, 2002), as well as improve the aesthetical benefits and outcomes for dental implant treatments (Cuny-Houchmand *et al*., 2013). As such, a strict rehabilitation plan must be developed based on the correct diagnosis and knowledge of dental morphology.

Even though parameters used in previous studies have proven to be reliable (Maynard and Wilson, 1980; Seibert and Lindhe, 1989; Müller and Eger, 1997; De Rouck *et al*., 2009), the periodontal architecture presents characteristics that are determined genetically and depend on unique features in the individual, such as growth and aging as well

as tooth type and profile, and tooth positioning (Bowers, 1963; Mazeland, 1980; Olsson and Lindhe, 1993; Müller and Eger, 1997; Esfahrood *et al.*, 2013). Diagnostic methods were used to assess whether groups of people with different periodontal morphometric combinations exist in a sample, using the central maxillary incisors as a reference. This group of teeth presents clear clinical characteristics, and the parameters analyzed have also been explored in other teeth (Olsson *et al.*, 1993; Müller *et al.*, 2000; De Rouck *et al.*, 2009).

De Rouck *et al.* (2009) found a prevalence of 29% of the thick periodontal biotype, when evaluating the transparency of a periodontal probe through the gingival margin in central maxillary incisors. Three groups were detected with different combinations of morphometric data for these teeth (Crown width/crown length ratio – CW/CL) and surrounding soft tissues (keratinized gingiva width – GW; papilla height – PH; transparency of the periodontal probe – GT; probing depth (PD), measured by cluster analysis, and defined as: A1 (thin), A2 (intermediate) and B (thick). However, the present study used other parameters for phenotype determination and different methods of assessment to those of De Rouck *et al.* (2009), such as gingival volume and gingival thickness, as well as papilla area. Non-hierarchical analysis demonstrated that the parameters, crown width/crown length ratio and probing depth, did not fit the model, and were therefore excluded from the analyses.

The present study also divided the sample into three groups, with a final prevalence of 45.75% for the thick phenotype (Cluster II), which presented aspects such as larger keratinized width, greater gingival volume and gingival thickness and smaller papilla area, when compared to the other groups. Cluster I, defined as thin phenotype (34.04%), demonstrated contrasting characteristics to those of Cluster II; while cluster III was defined as an intermediate phenotype (20.21%), as this group presented data that was intermediate between groups I and II.

In the present study, the transparency observed during the periodontal probe test was comparable to that of previous studies assessing periodontal morphology (Müller and Eger, 1997; Müller *et al.*, 2000; Anand *et al.*, 2012; Abraham *et al.*, 2014). In other words, the thick cluster, defined after grouping (cluster II), with a larger keratinized mucosa width, thicker gingiva, and shorter and larger gingival papilla (reduced papilla area), was not transparent on probing. Although cluster II was classified as a thin biotype (cluster I), the measurements in our study are not as thin as reported in the above studies. This observation can be explained by an ethnic difference or in the age range of the population studied. Even so, the transparency of the periodontal probe test was effective for detecting the gingival phenotype and can be applied on occasions during which a quick examination of the patient is required. Additionally, this technique was used in this study as a method to validate the outcomes of the results for the cluster profiles.

Statistical significant differences between genders were observed, with males found to present features compatible with a thick phenotype. Mazeland (1980) and Müller *et al.* (2000) have also noted that keratinized width mucosa was larger in men than in women. De Rouck *et al.* (2009) and Anand *et al.* (2012), who reported a high prevalence of upper teeth with a thin biotype, in women, and a thick biotype, in men, observed the same pattern. According to these authors, gingival thickness may be associated to the phenotypic characteristics of both genders, which may determine the arrangement of soft tissues and the human skeletal framework, which is usually tougher in males. In contrast, Bowers (1963), Müller *et al.* (2000) and Egreja *et al.* (2012) found no such statistical significant difference between genders with regard to the parameters studied. As such, data in the literature to justify the association of smile dimensions and gingival exposition with the gingival phenotype and genders is lacking.

According to Ainamo *et al.* (1981) and Camargo *et al.* (2001), the keratinized mucosa width is genetically determined, varying according to the region of the oral cavity and may be modulate throughout life. In our study, the cluster identified as thick (Cluster II) showed a larger keratinized mucosa (8.02 mm) and the Cluster identified as thin (Cluster I) showed a smaller range of keratinized mucosa (4.54 mm) in the groups assessed.

Weisgold (1961) categorized the interdental papilla into two periodontal morphotypes, based on its association with the bone structure adjacent to the tooth. The first periodontal morphotype presents a thin and contoured gingival tissue, with long interdental papilla. The second was defined as a thick gingival tissue, with a straight contour, short and large interdental papilla and a thick bone structure. The influence of periodontal biotype on the presence and absence of interdental papilla was assessed by De Lemos (2013), who reported that the thin biotype presented a significantly higher presence of long and thin papilla (71.1%), compared to the thick biotype group (59.6%). It was concluded that biotype has a direct influence on the presence and height of interdental papilla, justifying the inclusion of this parameter in our analyses. The present study aimed to assess a different quantitative parameter, determined by assessing the area (height x width/2), instead of the width divided by height or the categorization into thin and narrow or large and short. The thick phenotype (cluster II) was associated with a smaller papilla area (14.10 mm^2); the thin phenotype (cluster I) presented an increase in this parameter, when compared to the thick phenotype (15.64 mm^2), although no significant differences were observed. However, an increased papilla area was found in the intermediate phenotype, cluster III (20.08 mm^2). This may be explained by the fact that the height and width of this group, considered in the calculation of the papilla area, were of intermediate values.

Olsson *et al.* (1993) have classified gingival thickness as thin or thick, using the transparency periodontal probe test. De Rouck *et al.* (2009) included a third type, termed intermediate, which shows less pronounced characteristics of both the thin and thick groups. The transparency of the periodontal probe test was also used during vestibular probing depth (Fu *et al.*, 2010; Cook *et al.*, 2011). In contrast, Kan *et al.* (2003) quantified this parameter by classifying thickness as ≤1.0 mm for thin gingiva and > 1.0 mm for thick gingiva. This study also assumed that cluster I (thin phenotype) presented <1.0 mm thickness (0.83mm).

This study used methods previously used by Pendleton (1934), Olsson *et al.* (1993) and Frost *et al.* (2015), based on the use of a finger spreader. In a systematic review (Zweers *et al.*, 2014), the gingival width average, assessed by periodontal probing, ultrasound measurement or caliper, varied between 0.63mm (±0.11) and 1.79mm (±0.31), for the thin and thick phenotype, respectively. In our study, an average of 1.15mm was found for this parameter.

Gingival volume determination was studied as a tridimensional measure (Menezes, 2010); however, only the volume of the central maxillary incisors was considered, while Menezes (2010) assessed the canine to canine GV. This measurement was found to be better for the classification into the previously mentioned clusters. However, this volume assessment has not yet been applied in cluster models, making it difficult to correlate this parameter with those of previous reports. Menezes (2010) categorized the sample into three groups that presented means that were similar to those found in our gingival volume assessment.

The determination of functional phenotypes is one of the major methods to determine treatment predictability with the goal of measuring and diagnosing the clinical periodontal parameter of each individual, and subsequently assessing aesthetic and functional improvements during rehabilitation (Esfahrood *et al.*, 2013). Thus, despite using several different methodologies, the use of a standard method has not yet been determined. Therefore, a unique and universal strategy is essential for this specific diagnostic, even though transparency on probing was shown to have a strong association with the results found in this study.

In conclusion, we compared different parameters and characterized each phenotype in a sample population. The existence of three gingival phenotypes groups was confirmed. The thin phenotype was found in 34.04% of the sample (cluster I), the thick phenotype was found in 45.75% of the sample (cluster II). The remaining 20.21% were grouped as having intermediate characteristics (cluster III).

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The Ethics Committee of the Federal University of Rio Grande do Norte approved this study (909,875) and informed consent was obtained from all included individuals through written form.

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