Labial Gingival Thickness Assessment at Mandibular Incisors of Orthodontic Patients With Ultrasound and Cone-Beam Ct. A Cross-Sectional Study

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Abstract

Background: Quantitative and qualitative analyses of several periodontal parameters play an important role in several dental procedures. Aim of the current study was to assess gingival thickness (GT) at mandibular incisors of orthodontic patients with two methods and determine how these methods are compared to each other when assessing periodontal anatomy through soft tissue thickness.

Methods: The sample consisted of 40 consecutive adult orthodontic patients. GT was measured at both central mandibular incisors, mid-facially on the buccal aspect, 2mm apically to the free gingival margin with two methods: a) clinically with an Ultrasound device (USD) and b) radiographically with Cone Beam Computed Tomography (CBCT).

Results: CBCT measurements were consistently higher than USD measurements, with the difference ranging from 0.13 mm to 0.21 mm. No difference was noted between the repeated CBCT measurements at the right central incisor (Bias= 0.05 mm; 95% CI= -0.01, 0.11, p=0.104). Although the respective results for the left incisor indicated, statistically, that the measurements were not exactly replicated, the magnitude of the point estimate was small and not clinically significant (Bias= 0.06 mm; 95% CI= 0.01, 0.11, p=0.014). Small differences between CBCT measurements made by the 2 examiners at the left central incisor (bias= 0.06 mm, 95% CI= 0.01, 0.11, p=0.014) were detected. However, this difference was minor, and again, not clinically significant. The respective analysis on the right incisor showed no significant difference (bias= 0.05 mm, 95% CI= -0.01, 0.11, p=0.246).

Conclusions: In the present study GT was assessed clinically with *in-situ* measurements and radiographically after computer-aided appraisal. Based on the reproducibility, CBCT imaging proved to be at least as reliable as the ultrasound determination, but yielded higher values than the USD measurements.

Background

The assessment of gingival thickness (GT) is often an important element that should be taken into consideration during treatment planning and subsequent decision-making process before various dental treatments. Quantitative and qualitative analysis of several periodontal parameters plays an important role, not only in planning of periodontal procedures [1-3], but also in conventional prosthodontics [4], implant therapy [5-7] and orthodontics, when change of teeth inclination is anticipated [8, 9].

Several methods have been recommended for measuring GT. Visual appraisal of gingival phenotype may be considered as rather uncomplicated and time-saving. Nevertheless, it might not always be considered as an objective method; it has been demonstrated that, irrespective of the clinician's skill, gingival phenotype may be accurately identified in only about 50% of the cases [10]. Another straightforward and commonly used method is trans-gingival probing with a periodontal probe. Potential limitations of this technique include the angulation of probe insertion, and the invasive nature of the procedure; often local

anaesthesia is required, which, in turn, has a two-fold limitation: patient discomfort and a transient local volume increase in the case of injection of local anaesthetic solution [11].

An ultrasound device (USD) was proposed to resolve this limitation [12]. The reproducibility of this method has been reported to be high [13]. Another routinely applied procedure, to classify gingival phenotype as thin or thick, involves placement of a periodontal probe in the gingival sulcus; then, its transparency through the soft tissue is appraised [14]. This method has been also reported as highly reproducible, with 85% agreement between duplicate measurements [15].

Finally, the use of Cone Beam Computed Tomography (CBCT) has also shown a high diagnostic accuracy in assessing GT, demonstrating minimal discrepancy with clinical and radiographic measurements [2, 16]. A few studies have demonstrated CBCT as a standard method for determining gingival and bone thickness [2, 17-19]. Recently, technological developments have resulted in CBCT devices with lower emissions of radiation [20]; this renders CBCT applicable in almost all dental procedures, although each patient should be evaluated individually based on their unique treatment needs and set of circumstances.

Nevertheless, at present, it is still unclear which is the most applicable method to assess GT; the lack of a gold standard technique to assess GT in human studies does not allow for any clear recommendations for the clinician.

Since there is still limited evidence-based data to certify the accuracy of CBCT in evaluating the thickness of soft tissues in patients' oral cavity, the aim of the present study was to compare CBCT with USD and to determine the comparability and applicability of these methods as diagnostic tools for assessing GT, on a subset of patients from a larger prospective study assessing orthodontic treatment, GT and gingival recessions development.

Methods

Sample selection

This cross-sectional study has evaluated clinically GT in 40 white caucasian consecutively included orthodontic patients just before orthodontic treatment commencement, aged 16 years old or more, that visited the Department of Orthodontics and Dentofacial Orthopedics, 251 Hellenic Airforce Hospital, Athens, Greece.

The study protocol was approved by the 251 Greek Air Force Hospital 'Education, Ethics and Research Committee' (Approval Number: 076/7592/06.05.2015) and was executed in accordance with the guidelines of the Declaration of Helsinki.

All patients, or their legal guardian, provided written consent to participate prior to any measurements or CBCT execution. CBCTs were not performed primarily for evaluating GT; CBCTs were principally carried out in order to assess bone condition and turnover in the mandibular anterior region, in the frame of an

ongoing prospective controlled study assessing the occurrence of gingival recessions in orthodontically treated patients.

Exclusion criteria were as follows:

- (a) presence of crown restorations or fillings involving the cervical part of the anterior mandibular teeth,
- (b) pregnant or lactating females,
- (c) presence of obvious clinical signs of gingival conditions/diseases resulting in swelling of the gingiva (e.g. gingivitis), or presence of increased probing depths (e.g. > 3 mm) at the mandibular central incisors,
- (d) presence of labial gingival recessions at the mandibular central incisors,
- (e) intake of medication with any known effect on the gingiva, (e.g. Ca antagonists, etc.)
- (f) presence of congenital anomalies or dental structural disorders.

Sample size calculation

This cohort of patients derived from another ongoing prospective study assessing gingival recessions in orthodontically treated patients. Nevertheless, current sample size calculation was performed using the formula of comparing two means and included 90% power and statistical significance of 0.05. Standard deviation applied was 0.18mm according to previous research [21] and anticipated mean diff. was 0.20 mm.

Clinical and measuring parameters

All clinical procedures, as well as the CBCT imaging, were performed before bracket placement. Measurements were carried out at both central mandibular incisors, mid-facially on the buccal aspect of each tooth, and 2 mm apically to the free gingival margin, with the following two methods:

1. Ultrasound. A periodontist assessed the GT of each patient with the Ultrasound device (USD). Measuring GT with USD (Krupp SDM®, Austenal Medizintechnik, Cologne, Germany) is based on the ultrasonic pulse-echo-principle: ultrasonic pulses are transmitted through the sound-permeable tissue (1518 m/s), and are reflected at the surface of the hard tissue. By timing the received echo, GT is determined and digitally displayed. Measurements may range between 0.5 and 8.0 mm with a resolution of 0.1 mm. Ultrasonic frequency is 5 MHz and the diameter of the transducer probe is 3 mm with a weight of 19 gr. Measurements were performed by perpendicularly placing the transducer probe on the gingival surface without pressure, ensuring that the center of the transducer would be 2 mm apically to the free gingival margin.

2. CBCT Imaging. All patients underwent CBCT examination in a private clinic (Orofacial Radiodiagnosis, Athens, Greece). CBCT images were acquired using the Morita Accuitomo 80 3D Imaging System at 90kV and 7mA for 17.5sec and a single 360° image rotation. The CBCT scans were obtained with 6 x 6 cm field of view and 80 µ voxel size. Images were processed by I-Dixel-3DX software, 2.0 version (J. MORITA MFG. CORP., Darmstadt, Germany). During the examination, a cotton roll was used to retract the lip and enable the imaging of labial soft tissues.

GT Measurement using CBCT Imaging.

The GT in all CBCT images was measured by two authors (DK, LK) independently and recorded in data extraction forms without patient identification information. The first examiner (DK) conducted the measurements twice with an intermediate interval of one month in order to evaluate the intra-examiner repeatability.

The method for measuring GT in the software was standardised after calibration between the two assessors in ten randomly selected CBCTs. This was implemented to ensure reproducibility of the measurement location (2 mm apically to the free gingival margin), as was the case of the clinical measurements with the Ultrasound transducer probe. Measurements in the CBCT images were then performed perpendicularly to the tooth axis. (Fig. 1)

Statistical analysis

Descriptive statistics were applied for age, CBCT and USD gingival thickness measurements. The repeated CBCT measurements by the first examiner were tested for systematic differences (bias) using paired t-tests. Repeatability was quantified via the 95% repeatability coefficient [22, 23]. The presence of a magnitude related trend for the differences as well as for their dispersion was assessed graphically. Additionally, the presence of a trend for the differences was assessed statistically using Spearman's rank correlation coefficient. Normality assumption was assessed both graphically and via the Shapiro-Wilk test. The agreement between the two examiners on GT measurements from CBCT data was assessed both statistically and graphically. Paired t-tests were applied to test for systematic difference between the two examiners, while the reproducibility was quantified via the 95% reproducibility coefficient and in accordance with the repeatability coefficient. Again, normality assumptions and magnitude related trends were evaluated as above. Method agreement was evaluated between CBCT and USD measurements using two separate Bland-Altman analyses. Finally, the 95% Limits of Agreement (95% LOA) and the corresponding 95% CIs were calculated. Normality assumptions were evaluated graphically and by means of the Shapiro – Wilk test. Statistical significance was set to $\alpha = 5\%$. All statistical analyses and graphical plots were conducted using Stata 13.0/SE software (StataCorp LP, College Station, TX, USA).

Results

40 subjects (17 females and 23 males) participated in this study. The descriptive statistics for age, CBCT and USD measurements are reported in Table 1.

Table 1. Descriptive statistics of all gingival thickness measurements (in mm).

	Mean (Std.Dev.)	Min	Max
Age	24.48 (6.68)	18.00	45.00
Mandibular left central incisor			
<i>CBCT: Examiner #1</i>	0.93 (0.24)	0.55	1.51
<i>CBCT: Examiner #2</i>	1.01 (0.24)	0.61	1.59
<i>USD</i>	0.80 (0.26)	0.50	1.50
Mandibular right central incisor			
<i>CBCT: Examiner #1</i>	0.95 (0.27)	0.50	1.50
<i>CBCT: Examiner #2</i>	0.99 (0.25)	0.42	1.43
<i>USD</i>	0.80 (0.22)	0.50	1.50

Table Legends. CBCT: Cone Beam Computed Tomography, USD: Ultrasound Device

Repeatability assessment

The results of the paired t-tests for bias between the 1st and the 2nd CBCT measurements made by the first examiner (DK) are reported in Table 2. Repeatability of USD measurements were performed in a previous cross-sectional study with the same methodology and objective [21]. Normality assumption was not violated for any of the differences between the repeated measurements of USD in two time points (Mean diff. 0.00, 95% CI -0.05, 0.05, $p=1.00$).

Table 2. Results of the paired t-tests between the repeated CBCT measurements by examiner #1.

Tooth	Bias (Std.Er.)	95% CI	p-value
<i>Mandibular left central incisor</i>	0.06 (0.02)	(0.01, 0.11)	0.014
<i>Mandibular right central incisor</i>	0.05 (0.03)	(-0.01, 0.11)	0.104
Bias= 2 nd - 1 st measurements (in mm)			

Table Legends. CI: Confidence Interval

Statistical analysis indicated that the repeated CBCT measurements were not identical for the mandibular left central incisor (Bias= 0.06 mm, 95% CI= 0.01, 0.11, p -value= 0.014), whereas the respective for the mandibular right central incisor could be considered identical (Bias= 0.05 mm, 95% CI= -0.01, 0.11, p -value= 0.104). Nevertheless, clinically, a difference of 0.06 mm in repeated measurements can be regarded mostly as unimportant. The corresponding Bland – Altman plots are displayed in figures 2a & 2b. Neither a magnitude nor a dispersion related trend could be identified graphically. Absence of the former trend was also implied by the Spearman's rank correlation coefficient (Mandibular left central

incisor: Spearman's rho= 0.10, p-value= 0.554; mandibular right central incisor: Spearman's rho= -0.14, p-value= 0.377). The Shapiro – Wilk test results showed that the normality hypothesis was valid for both left and right mandibular incisors (p-value= 0.595 and 0.614 respectively).

Reproducibility assessment

The results of the paired t-tests for bias between the two examiners (DK, LK) are reported in Table 3. Again, statistical analysis indicated that the repeated measurements were not identical for the mandibular left central incisor (Bias= 0.06 mm, 95% CI= 0.01, 0.11, p-value= 0.014), whereas the respective for the mandibular right central incisor could be considered identical (Bias= 0.05 mm, 95% CI= -0.01, 0.11, p-value= 0.246). Nevertheless, clinically, a difference of 0.06 mm in different doctors' measurements can be regarded mostly as unimportant. The corresponding Bland – Altman plots are displayed in figures 2c & 2d. Neither a magnitude nor a dispersion related trend could be identified (Mandibular left central incisor: Spearman's rho= -0.03, p-value= 0.836; mandibular right central incisor: Spearman's rho= -0.12, p-value= 0.377). The normality hypothesis was valid for both left and right mandibular central incisors (Shapiro – Wilk test p-value= 0.542 and 0.475 respectively).

Table 3. Results of the paired t-tests between the CBCT measurements made by the two examiners.

Tooth	Bias (Std.Er.)	95% CI	p-value
<i>Mandibular left central incisor</i>	0.06 (0.02)	(0.01, 0.11)	0.014
<i>Mandibular right central incisor</i>	0.05 (0.03)	(-0.01, 0.11)	0.246
Bias= examiner #2 – examiner #1 (in mm)			

Table Legends. CI: Confidence Interval

Method agreement (comparability)

The results of the paired t-tests between the two GT measuring techniques as well as the estimated corresponding 95% LOA and the respective 95% CIs are reported in Table 4. The respective Bland – Altman plots are displayed in Figure 3a-d. There was no evidence of a magnitude related trend for either the differences or for their dispersion after graphical evaluation.

Finally, all normality assumptions could not be rejected after either graphical evaluation or using Shapiro-Wilk tests (Mandibular left central incisor, CBCT measurements from examiner #1: p-value= 0.163; CBCT measurements from examiner #2: p-value= 0.561; Mandibular right central incisor, CBCT measurements from examiner #1: p-value= 0.157; CBCT measurements from examiner #2: p-value= 0.097).

Table 4. Results of the paired t-tests (Bias, Std.Er., 95% CI, p-value) between the CBCT and USD measurements along with the corresponding 95% LOA and the respective 95% CI for the LOA.

	Bias (Std.Er.)	95% CI for bias	p- value	95% LOA	95% CI for the LOA	
					Lower LOA	Upper LOA
<i>Mandibular left central incisor</i>						
CBCT measurements from examiner #1	0.13 (0.03)	(0.07, 0.19)	<0.001	(-0.26, 0.51)	(-0.35, -0.17)	(0.42, 0.60)
CBCT measurements from examiner #2	0.21 (0.04)	(0.14, 0.28)	<0.001	(-0.22, 0.64)	(-0.32, -0.12)	(0.54, 0.75)
<i>Mandibular right central incisor</i>						
CBCT measurements from examiner #1	0.14 (0.03)	(0.08, 0.21)	<0.001	(-0.26, 0.54)	(-0.35, -0.16)	(0.45, 0.63)
CBCT measurements from examiner #2	0.18 (0.04)	(0.11, 0.25)	<0.001	(-0.25, 0.62)	(-0.36, -0.15)	(0.52, 0.72)
Bias= CBCT – USD (in mm)						

Table Legends. CBCT: Cone Beam Computed Tomography, CI: Confidence Interval, LOA: Limit of Agreement, USD: Ultrasound Device

Discussion

The objective of the current study was to assess GT with non-invasive methods. Lower incisors were in focus since change in their inclination or torque may introduce a risk factor to gingival recessions marking this as an area of major concern, not only in functional, but also in aesthetic respect.

Direct measurement is regarded as a fairly objective method for GT assessment. Nevertheless, since it involves tissue penetration, its clinical applicability is associated with some limitations; [24] these are often linked with measurement errors, probably originating from instruments' rounded tips and thickness [21].

An USD showing a high reproducibility [12, 13, 21, 25], was selected as the first, non-invasive method, for measuring GT. The second selected method was CBCT imaging that has been shown to have a high

diagnostic applicability [2, 16].

According to the present results, the difference between USD and CBCT measurements of gingival thickness was not zero. In general, CBCT measurements were consistently higher than the USD measurements. This difference was independent of the magnitude of GT measurement. CBCT measurements were constantly higher than USD, with the difference ranging from 0.13 mm to 0.21 mm (Table 4). It is difficult to attribute the difference reported to one methodology or the other. If a possible explanation was to be given, It could possibly be the ultrasound procedure, due to measuring imprecision such as misangulation of the ultrasound transducer or over-compression of the soft tissue.

There was no evidence of significant differences between the repeated measurements made by the first examiner on the mandibular right central incisor (p -value=0.104). Although the respective results for the mandibular left central incisor indicated that the measurements were not exactly true replicates from a statistical point of view, the magnitude of the point estimate of bias was small and possibly not clinically significant (Bias= 0.06 mm; p -value=0.014).

Moreover, there was evidence of a small systematic difference between the CBCT measurements made by the two examiners on the mandibular left central incisor (bias= 0.06 mm, 95% CI= 0.01, 0.11). However, this difference was minor, and again, clinically unimportant. On the other hand, the respective analysis on the mandibular right central incisor showed no evidence of a significant difference between the two examiners (bias= 0.05 mm, 95% CI= -0.01, 0.11).

Numerous dental procedures require accurate measurement of GT, since respect of gingival phenotype is vital and appears to influence the outcomes of various treatment strategies. Gingival phenotype evaluation through simple visual appraisal is shown to be inaccurate [10, 26], mostly due to its subjective nature; it relies, at least to a great extent, on clinical competence. Thick gingiva, i.e. more than 0.8- 1mm of thickness, is shown to be relatively resistant to gingival recession following surgical or restorative therapies [27-30], whereas thin-scalloped gingiva is considered at risk because it has been associated with a compromised response following the same treatments [5, 27-32]. These findings point clearly to the need of a thorough diagnosis, through a straightforward and reproducible method, of these high-risk patients, before various interventions involving the gingiva. At this point it has to be outlined, as far as accuracy is concerned, that this term refers to closeness of the measurements to the true value of gingival thickness. By definition, true value cannot be measured by methods, as those in the present study. There is only an estimation of the true value. This is the reason why it is important to describe repeatability, reproducibility and the correlation of the methods tested.

Our study is not free of limitations, although efforts were made to minimise them. Firstly, the clinical measurements did not take into account potential differences in dental arch crowding or tooth inclination that may influence the clinical handling of the USD transducer probe, although it was anticipated that this wouldn't lead to a large method error. Secondly, at present, CBCT conducting for assessment of GT might not be justified due to the associated amount of radiation, as CBCT includes higher doses than two-dimensional imaging. Moreover, CBCT Images have a certain degree of inaccuracy attributed primarily to

image generation, processing, voxel size and various types of artefacts that might be present. In general, the smaller the voxel size, the higher the precision/resolution of the information provided. Larger voxels may include different tissues, and thus, the subsequent grayscale value may not indicate clearly one specific tissue, such as bone. This issue is primarily evident at the limits between neighbouring tissue types of different radio-density. However, at the same time, the smaller the voxel size the higher the motion artefacts. Thus, based on the above considerations and also on the need to keep the radiation exposure as low as possible, a specific CBCT image can only reach a certain degree of detail on the information it provides [33-35].

Finally, it has to be pointed out that the lack of a gold standard measuring procedure of soft tissue thickness may downgrade the clinical significance. Finding a gold standard measurement for humans is, nevertheless, almost impossible, because all clinical or imaging methods present an inherent measurement error, which is not always easy to assess during implementation. On the other hand, significance of the current study lies in the fact that it includes both imaging and clinical procedures and provides robust data for the comparison of the tested methods.

Conclusions

The clinical significance of the present study lies in the investigation of GT assessment with two distinct methods: clinically with *in-situ* measurements and radiographically after computer-aided appraisal. The present results demonstrate the differences between the tested methods. Based on the reproducibility, CBCT imaging proved to be at least as reliable as the ultrasound determination, but yielded higher values than the USD measurements.

List Of Abbreviations

GT : gingival thickness

USD: Ultrasound device

CBCT: Cone Beam Computed Tomography

CI: Confidence Interval

LOA: Limit of Agreement

Declarations

Ethics approval and consent to participate

All procedures were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the 251 Greek Air Force Hospital 'Education, Ethics and Research Committee' (Approval

Number: 076/7592/06.05.2015). All patients, or their legal guardian, provided written consent to participate prior to any measurements or CBCT execution.

Consent for publication

Not applicable.

Availability of data and materials

All data used and/or analyzed during this research are available from the corresponding author on reasonable request.

Competing interests

All Authors declare no conflict of interest. The authors state that they have no commercial relationship or conflict of interest with any of the products used in the present study and designed the study on their own initiative.

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Author Contributions

DK, LK and GK performed all clinical and radiographic measurements. DK, LK and ASt wrote the main manuscript text and prepared all figures. ASc and CK oversaw the project and assisted with the writing of the manuscript. GK assisted with the interpretation of statistics. All authors reviewed the manuscript.

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Figures

Figure 1

Measurement procedure in the CBCT images.

Figure 2

2A. Bland-Altman plot. Repeatability assessment for CBCT measurements. Mandibular left central incisor
 2B. Bland-Altman plot. Repeatability assessment for CBCT measurements. Mandibular right central incisor
 2C. Bland-Altman plot. Reproducibility assessment for CBCT measurements. Mandibular left central incisor
 2D. Bland-Altman plot. Reproducibility assessment for CBCT measurements. Mandibular right central incisor

Figure 3

3A. Bland-Altman plot. Method agreement. Examiner #1. Mandibular left central incisor 3B. Bland-Altman plot. Method agreement. Examiner #1. Mandibular right central incisor 3C. Bland-Altman plot. Method agreement. Examiner #2. Mandibular left central incisor 3D. Bland-Altman plot. Method agreement. Examiner #2. Mandibular right central incisor