

## VALIDATION OF TOOTH CEMENT ANNULATIONS FOR ASSESSING DENTAL AGE IN HUMANS

Minja Birimša<sup>1\*</sup>, Jelena Dumančić<sup>2</sup>, Marin Vodanović<sup>2</sup>, Sandra Anić Milošević<sup>3</sup>, Hrvoje Brkić<sup>2</sup>

<sup>1</sup> PhD Student at School of Dental Medicine, University of Zagreb

<sup>2</sup> Department of Dental Anthropology, School of Dental Medicine, University of Zagreb

<sup>3</sup> Department for Orthodontics, School of Dental Medicine, University of Zagreb

**Introduction:** Currently published research suggests that tooth cement annulations can be used for assessing dental age in humans. For the regular use in forensic practice the method should be properly validated. The purpose of this study was to examine the inter- and intra-rater agreement in the measurement of tooth cement annulations thickness.

**Materials and Methods:** The study sample consisted of 82 donor teeth of both sexes of which 26 were measured 10 months apart (two episodes (2E)) and 56 were measured during the same episode (1E). Additionally, 11 (of 82) teeth were randomly selected during the second episode by the second rater. The teeth used were extracted because of periodontal disease or for orthodontic or prosthetic reasons. The excluding criterion was the presence of tooth root lesions. Donors' age ranged from 20 to 69 years at the time of tooth extraction. Each tooth was embedded in a quick-setting autoacrylate, and the roots were cut with transverse incisions on an ISOMET 1000 cutter in the apical, middle and cervical third of the tooth's root. The thickness of each incision ranged from 0.3 to 0.5 µm. Cement thickness measurements were made with a light microscope and an Olympus EP50 camera, Version: V3\_20190202. The microscope was calibrated separately for each episode according to same specifications. The first rater was blinded for the measurements done during the first episode and measured the adjacent cuts in all three root thirds. The second rater was blinded for the measurements done by first rater. The correlation between the achieved estimated dental age, and chronological age was calculated using the Spearman correlation coefficient. Agreement between first and second episode measurements (inter-rater) was calculated using the coefficient of concordance (CC) with 95% confidence interval (CI), a for intra-rater agreement using a kappa test. Data was also presented using Bland-Altman plots.

**Results:** Statistically significant lower agreement level of the tooth cement thickness was found for adjacent cuts when they were measured in separate episodes (intra-rater agreement) compared to the agreement of same measures done in one episode: adjacent apical cuts,  $CC_{2E}=0.32$  (95% CI 0.0-0.59) vs  $CC_{1E}=0.84$  (95% CI 0.75-0.90); adjacent middle cuts  $CC_{2E}=0.38$  (95% CI 0.08-0.61) vs  $CC_{1E}=0.86$  (95% CI 0.76-0.91); and adjacent cervical cuts,  $CC_{2E}=0.48$  (95% CI 0.27-0.65) vs  $CC_{1E}=0.91$  (95% CI 0.86-0.94). Almost perfect agreement was found (mean whole root cement thickness, apical and middle third, kappa >0.86; cervical third cement thickness, kappa=0.815) for inter-rater agreement, yielding a very small measurement error of 0.89% (95% CI -0.21 do 1.98%).

**Conclusion:** Conducted validation of the measures of tooth cement annulations used in estimating the dental age of humans showed that there is a very high level of inter-rater agreement yielding a very small measurement error. Intra-rater agreement testing showed that the error could most probably be due to the error in microscope calibration which can be prevented by quality control measure using the measurement of standard tooth.

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**Key words:** forensic stomatology, age assessment, tooth cement thickness, transverse sections, validation

## VALIDACIJA IZMJERE DEBLJINE ZUBNOG CEMENTA U SVRHU

### UTVRDIVANJA DENTALNE DOBI ČOVJEKA

Minja Birimša<sup>1\*</sup>, Jelena Dumančić<sup>2</sup>, Marin Vodanović<sup>2</sup>, Sandra Anić Milošević<sup>3</sup>, Hrvoje Brkić<sup>2</sup>

<sup>1</sup> Studentica doktorskog studija Stomatološkog fakulteta Sveučilišta u Zagrebu

<sup>2</sup> Zavod za dentalnu antropologiju Stomatološkog fakulteta Sveučilišta u Zagrebu

<sup>3</sup> Zavod za ortodonciju Stomatološkog fakulteta Sveučilišta u Zagrebu

**Uvod:** Dosadašnja objavljena istraživanja pokazuju da se anulacije cementa zuba mogu koristiti za procjenu dentalne dobi. Kako bi se metoda mogla koristiti u redovitoj forenzičkoj praksi potrebno je provesti odgovarajuću validaciju metode mjerjenja debljine zubnog cementa. Svrha ovog istraživanja bila je ispitati podudarnost mjerjenja debljine zubnog cementa između dva nezavisna mjeritelja te u istog mjeritelja nakon određenog vremenskog intervala.

**Materijal i metode:** Uzorak studije sastojao se od 82 zuba donatora obaju spolova od kojih je 26 mjereno u dva odvojena navrata (uz vremenski odmak od 10 mjeseci - 2 epizode (2E)), a 56 zuba je mjereno u istom navratu (1 epizoda (1E)). Dodatno je 11 zuba (od ovih 82) odabranih slučajnim odabirom mjereno u drugom navratu od strane drugog mjeritelja. U istraživanju su korišteni zubi koji su izvadeni zbog parodontne bolesti i ortodontskih te protetskih razloga. Kriterij izuzimanja bili su zubi s lezijama korijena. Starost donatora u vrijeme vađenja zuba kretala se od 20 do 69 godina. Svaki je zub uklopljen u brzovezijući autoakrilat te su korijenovi rezani poprečnim rezovima na rezalici ISOMET 1000 u apikalnoj, srednjoj i cervicalnoj trećini korijena zuba. Debljina svakog reza iznosi je od 0.3 do 0.5 µm. Mjerjenja debljine cementa načinjena su pod svjetlosnim mikroskopom i kamerom Olympus EP50, Version: V3\_20190202. Mikroskop je za mjerjenje bažđaren u dva navrata (za prvo te za drugo mjerjenje) prema istim postavkama. Prvi mjeritelj je bio slijep za prethodne izmjere te je mjerio susjedni rez u odnosu na prvo mjerjenje u sve tri trećine korijena zuba. Drugi mjeritelj je bio slijep za izmjere koje je proveo prvi mjeritelj. Podudarnost između prvog i drugog mjerjenja izračunata je koeficijentom konkordanse (CC) uz 95%-tni interval pouzdanosti (CI), a podudarnost između dva mjeritelja izračunata je kappa testom te su obje prikazane Bland-Altman prikazom.

**Rezultati:** Utvrđena je statistički značajno manja podudarnost debljine zubnog cementa susjednih slojeva kada su isti mjereni u dvije vremenski značajno odvojene točke (2E) od strane istog mjeritelja u odnosu na podudarnost susjednih slojeva kada su mjereni u istoj vremenskoj točci (1E): susjedni slojevi na apsku  $CC_{2E}=0.32$  (95% CI 0-0.59) prema  $CC_{1E}=0.84$  (95% CI 0.75-0.90), u srednjoj trećini  $CC_{2E}=0.38$  (95% CI 0.08-0.61) prema  $CC_{1E}=0.86$  (95% CI 0.76-0.91) te u cervicalnoj trećini  $CC_{2E}=0.48$  (95% CI 0.27-0.65) prema  $CC_{1E}=0.91$  (95% CI 0.86-0.94). Utvrđena je gotovo potpuna podudarnost (kappa >0.86 za projek debljine zubnog cementa cijelog korijena, na apksu te srednjoj trećini te kappa=0.815 za cervicalnu trećinu korijena zuba) mjerena dvaju mjeritelja uz izrazito malu pogrešku mjerjenja od 0.89% (95% CI -0.21 do 1.98%).

**Zaključak:** Provedena validacija izmjerene debljine zubnog cementa u čovjeka u svrhu utvrđivanja dentalne dobi pokazala je da postoji visoka podudarnost izmjerenih vrijednosti između dva mjeritelja uz vrlo malu pogrešku mjerjenja. Utvrđena razlika između prvog i drugog mjerjenja u istog mjeritelja vjerojatno je posljedica razlike u kalibraciji mikroskopa te o navedenom treba voditi računa i napraviti provjeru korištenjem unaprijed utvrđenog standarda.

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**Ključne riječi:** forenzička stomatologija, određivanje dobi, debljina zubnog cementa, validacija