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Original Article

Root Dentin Transparency: A Reliable Method of Age Estimation in Nepalese Population

Bhoj Raj Adhikari[1]*, Mamata Shakya[2], Chandan Upadhyaya[2], Swagat Kumar Mahanta[3], Nitesh Chaurasia[2], Pratibha Poudel[1], Pankaj Kumar Singh[4], Ritesh Srii[1], Simant Lamichhane[5]

[1]Department of Oral Medicine and Maxillofacial Pathology, Kathmandu University School of Medical Sciences, [2]Department of Oral and Maxillofacial Surgery, KUSMS, [3]Department of Community and Public Health Dentistry, KUSMS, [4]Department of Forensic Medicine, KUSMS, [5]Department of Periodontology and Oral Implantology, KUSMS, Dhulikhel, Nepal

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Abstract

Background

Root dentin transparency, one of the age changes in dentin, is considered to be a stable parameter not affected by environmental and pathological factors. The effect of age changes in thetransparency of root dentin in Nepalese population have not been established so far. In the present study, we aimed to determine the age of an individual using the root dentin transparency in the Nepalese subjects and attempt to derive the formula to estimate the age applicable to the Nepalese population.

Materials and Methods

A cross sectional analytical study was conducted to measure the length of root dentin transparency in the tooth of patients of known age. Correlation of the age and the length of transparent root dentin was determined. A linear regression analysis was carried out to derive a formula applicable to the Nepalese population.

Results

The length of transparent root dentin increased linearly with the age of an individual. The newly derived formula was used to calculate the estimated age from the length of transparent root dentin. The difference between the actual age and the calculated age was within 6 years in 63.2% of the sample.

Conclusion

A simple and relatively inexpensive method for calculating age by measuring root dentin transparency is described.

Keywords: *Dentin, Forensic dentistry, Nepal, Tooth*

***Corresponding Author:**
Dr. Bhoj Raj Adhikari
Lecturer
Email address: bhojraj@kusms.edu.np
ORCID: https://orcid.org/0000-0002-2716-879X

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Introduction

Information related to the identification of a living person can be obtained by various ways [1-4]. There are limited means to do so in a deceased person. Forensic method of identification of a person including both morphological and morphometrical methods have been studied since decades [2, 3]. However, very few of them are applicable in situations where the body of the deceased one is decomposed, skeletonized, or burnt. During such situations, the roots of teeth of the dead person serve as an important tissue to provide the necessary information [5, 6].

Root dentin transparency (RDT), one of the age changes in dentin, is considered to be a stable parameter not affected by environmental and pathological factors [5]. The effect of age changes in the transparency of root dentin in Nepalese population has not been established so far. A strong need of reliable and accurate method of determination of age in human based upon RDT in Nepalese population has been looked for, for a long time.

In the present study, we aimed to find a relation between age and the RDT in the Nepalese subjects and attempt to derive the formula to calculate the age applicable to the Nepalese population.

Materials and Methods

This is a cross sectional analytical study using the extracted teeth of 144 Nepalese population between the age of 10 years and 80 years who visited the department of dental surgery in Dhulikhel Hospital between July and September 2022 (IRC-KUSMS Approval No & date: 53/22, 19th June 2022). Informed written consent was taken from each participant for the surgical procedure and the publication of the data. Teeth extracted for therapeutic reasons including periodontally compromised situation, endodontically untreatable teeth and those extractions indicated for orthodontic treatment were included in this study. Teeth lost due to trauma, grossly decayed condition, root canal treated teeth and fractured during surgery in which cemento-enamel junction could not be determined were excluded from the present study. Tooth affected by developmental disorders that may affect the histological structure of dentin were also excluded from this study. The sample size was calculated using the following formula [7], minimum Sample Size (n) = $Z^2p(1-p) / e^2$, prevalence of cause of extraction of teeth (p) = 17.44, Z = 1.96 at 5% of Confidence Interval; Margin of error (e) = 7%, n = 112.88 (rounded up to 113).

The age and gender of the patient were recorded prior to commencement of the treatment. The age was recorded by one investigator, and blinded to the other investigator who scored the transparency of the teeth. The teeth subjected to extraction as indicated in the inclusion criteria were extracted using standard, aseptic surgical protocol. Following extraction, the teeth were fixed in 10% neutral formaldehyde (formalin) solution, dried and mounted on acrylic autopolymerizing media (self-cure resin) and encoded. Teeth sectioning were done in buccolingual direction near the center of the tooth using air-rotor high speed handpiece and diamond coated burs and disks. Teeth sections of final thickness measuring 250 µm (+/-10 µm) were obtained by manually grinding on Arkansas stone. The final thickness was measured on digital vernier caliper (Ingco, China). Prepared sections were cleaned on xylene, dried and pasted on a scaled graph sheet [Fig 1] and the picture was taken using a high-resolution semi-professional DSLR camera (Canon KissX9i, Japan) at a resolution of 600 dpi, and the images were stored in a laptop (MacBook Pro, Apple, CA, USA).

The root dentin transparency (RDT) of each tooth was measured from the apex to the coronal direction as described in previous literature [8]. In short, the horizontal borderlines formed between the transparent dentin (upto which the graph lines were clearly visible) and the opaque dentin on each of the buccal and lingual sides were marked as the coronal extent of the measurement. Only the length of the dentin was studied and measured, while a distinct zone of cementum at the tip of the root apex on the longitudinal sections was not included in the transparent root length. If the borderline or junction between the transparent dentin and opaque dentin is not a relatively horizontal line then both the minimum (Tmin) and the maximum (Tmax) extension of transparency was measured. In cases where the junction was found to be a fairly even horizontal line or situations where transparency could be appreciated only on one side (i.e., buccal or lingual), only one measurement was taken as transparency level (T). For any un-uniformity in transparency between the buccal and lingual aspect of teeth the mean value (Tm= [Tmin + Tmax]/2) was recorded. The correlation between the transparency length and the actual age (a) of the individual was statistically calculated. A regression equation was developed statistically to obtain the formula specifically for the Nepalese population. Following this, the sections were decoded. The calculated age (A) was obtained using the newly

developed regression equation. The actual age (a), calculated age (A), difference between two (a-A), transparency mean (Tm=RDT), and Standard Error (SE) values were tabulated using MS Office Excel Spreadsheet (Microsoft Corp. Redmund, Washington, USA). The calculated age (A) of the subjects after using the newly developed formula was compared with the actual age (a) in the study samples and the difference between them were statistically evaluated to find the standard error. Furthermore, RDT was statistically evaluated in smaller groups based on gender, arches and teeth from each side in the oral cavity. Statistical analysis was performed using IBM Statistical software Statistical Package for Social Sciences for iOS (SPSS, Version 23; SPSS Inc, USA). Pearson's correlation test was used to determine correlation between age and RDT. Linear regression was used to derive the equation between age and RDT applicable to the Nepalese population. Statistical significance was set at 0.05 at 95% confidence interval.

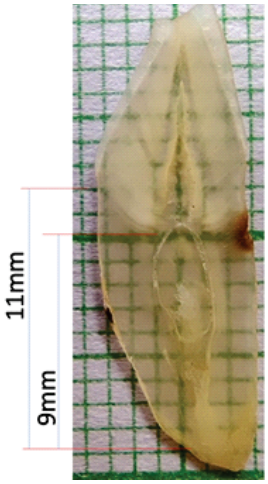

Figure 1: Root dentin transparency and its measurement in mm.

Results

Total number of teeth sample included in this study is 144, with 72 each from male and female participants between the age of 10 and 80 years [Table 1 and 2]. Root dentin transparency is in the range 0 to 12 mm (5.406 +/-0.293). There is a strong correlation (r = .933) between the length of root dentin transparency (RDT) and the actual age (a) of the patient and was statistically significant at $p<0.05$. A linear regression analysis of the actual age and RDT yielded a formula $y=4.788x+20.188$, where "y" is the calculated age (in years) for the given length of root dentin transparency "x" (in mm) [Table 3]. This newly derived formula was used to calculate the age of the individual to test the reliability of the formula.

The calculated age (A) is highly correlated to the actual age (a) for each of the length of transparent dentin and is statistically significant. The difference between the actual age and the calculated age (a-A) is determined in an average to be 5.57 (+/-0.273) years. Application of linear regression equation showed that our method can estimate age to within 6 years of actual age in 91 cases (63.2%). Similarly, this method can estimate age within 7 to 10 years of actual age in 38 cases (26.4%) and above 10 years in 15 cases (10.4%) [Table 4]. Additionally, cross−validation is performed in smaller groups based on gender, arches and teeth from each side in the oral cavity. The RDT is highly correlated in each of the groups and statistically significant [Table 5]. The result suggested that all the models considered the whole cohort of samples and provided, in essence, the maximum possible prediction power of the approach we have implemented.

Table 1: Demographic details of the teeth sample

Age Group	No of samples
Above 10 and below 20	13
Above 20 and below 30	24
Above 30 and below 40	22
Above 40 and below 50	21
Above 50 and below 60	20
Above 60 and below 70	36
Above 70 and below 80	8

Table 2: List of teeth sample used in the study

Teeth Group	Right	Left	Total
Maxillary Incisors	18	18	36
Mandibular Incisors	16	18	34
Maxillary Canines		2	2
Mandibular Canines		4	4
Maxillary Premolars	15	19	34
Mandibular Premolars	18	16	34
Total	**67**	**77**	**144**

Table 3: Newly derived regression formula to calculate the age of an individual based on length of root dentin transparency, especially applicable to the Nepalese population.

$$y = 4.788\,x + 20.188$$

y: calculated age of the individual (years); x: length of root dentin transparency (mm)

Table 4: Accuracy of age estimation

Sample	Accuracy of estimated age [% (number)]		
	Upto 6 years	7 to 10 years	Above 10 years
	63.2% (91/144)	26.4% (38/144)	10.4% (15/144)

Table 5: Correlation of age and root dentin transparency in each of the smaller groups

Parameters (Age vrs RDT)	n	Pearson Correlation coefficient	SE
Overall sample	144	.933	1.504
Females only	72	.942	2.212
Males only	72	.908	1.848
Right sided teeth only	67	.909	2.028
Left sided teeth only	77	.949	2.196
Maxillary teeth only	72	.954	1.969
Mandibular teeth only	72	.924	2.285

RDT: Root Dentin Transparency; n: number of samples; SE: Standard Error; $p<0.05$

Discussion

The result from the present study showed that the root dentin transparency is directly proportional to increase in age and thus would serve as a reliable tool to aid in age estimation in both living and the dead. It is also clear that there is an area of linear increment in transparency that starts from the apical region and extends coronally with advancement in age. A strong correlation (r = .933) between RDT and age advancement is shown in the present study and, these results are strongly concomitant with previous studies [8-11]. The present study tested the correlation of RDT with the age of the patient not only in an overall sample, but also based on gender, arch and sides. The result suggested that there is highest correlation of RDT with the age of the patient with respect to the teeth on the maxillary arch (r = .954) and on the left sides of the face (r = .949), and the result is consistent with the studies in Indian population [8]. The results suggest that this approach is key for accurate and efficient age estimation based on RDT analysis. Furthermore, based on linear regression, a formula is derived to calculated the age applicable to the Nepalese population. It is clear that for every 4.7 years of increment of age, after 20-years-of-age, there is 1mm increase in root dentin transparency. Thus, there is a direct proportional relationship between the age and the length of root dentin transparency after 20-years-of-age of an individual. The lower cut-off age using the newly derived formula is 20 years, provided that age estimation below this age can be done accurately using other parameters including the eruption pattern of teeth, the length of formed root and the extent of root apical closure. The newly derived formula is used to calculate the age for the given length of root dentin transparency, in order to validate the newly obtained equation. Comparison of the actual age (a) and the calculated age (A) is done. The difference (a-A) between the actual age and the calculated age is determined in an average to be 5.57 (+/-0.273) years. The result suggests that there is high degree of accuracy within 6 years difference between the actual age and the calculated age. Out of 144 samples in our study 63.2% of the samples yielded the calculated age within 6 years of the actual age and was in consistent with the previous studies in Indian population [8].

Identification of an individual stands out to be of prime importance in modern civic society which recognizes the need of establishing the individuality of a living or dead person. During identification process of human remains, age-at-death is one of the major screening factors that helps reduce the universe of possible matches [1, 12]. Information related to the identification of a living or a conscious person can be obtained by various ways [1-4]. There are limited means to do so in a deceased person including the cases where victims have suffered mass disasters, road traffic accidents, fire accidents, criminal insults and more. Forensic odontology is a branch of forensic medicine dealing with study of odontogenic structures in identifying criminal suspects or the remains of a dead person. Forensic method of identification of a person including both morphological and morphometrical methods have been studied since decades. A number of previous literatures have described various methods to determine age, gender and stature that have always proved to be pivotal in post-mortem identification of deceased individual. Various soft tissues including viscera, parameters in skull, pelvic bone, and other peripheral skeletal structures have been time-tested in estimation of the age of an individual [2,3]. However, among the number of methods that are available, very few of them are applicable in situations where the body of the deceased one is decomposed, skeletonized, or burnt. Such situations warrant a stable and consistent marker of age estimation. During such situations, the roots of teeth of the dead person serve as an important tissue to provide the necessary information [5, 6]. Unlike bones and soft tissues, teeth are extremely resilient and often well-preserved without significant loss of microstructure, even in the extremes of the situation impending the demise of an individual. In addition to sex, stature, and population affinity, teeth constitute baseline information in the identification process of deceased individuals. Dental age, one of the measures of physiologic development, is applicable from intrauterine period to adulthood [1]. Teeth continue to undergo age related

changes making age estimation possible even in late adulthood. Human vital teeth subjected to functional loading suffer various physical and chemical stimuli that may cause collagen fibers and apatite crystals to begin appearing in the dentinal tubules leading to sclerosis of the tubules. Sclerosis reduces the permeability of the dentin and may help prolong pulp vitality. As these changes are considered as a defensive reaction of the dentin they continuously increase with increasing age of an individual. Initially there are a few apatite crystals in the dentinal tubule but gradually the tubule becomes filled with a fine meshwork of crystals. With advancing age, the tubular lumen is obliterated with mineral, which appears very much like the peritubular dentin so that the refractive indices of dentin in which the tubules are occluded are equalized, and such areas become transparent in transmitted light. Dentin transparency is least deviated by pathologic processes and environmental changes, so it is considered as a sole significant parameter for dental age estimation.

One of the well-known concepts of RDT was proposed by Gustafson [5]. RDT was included as a part of six different parameters to estimate the age of an individual. The included variables were attrition, secondary dentin deposition, apical migration of periodontal ligament attachment, cementum deposition at the root apex, root resorption at the apex and root dentin transparency. Among them, the former five parameter are highly variable and may be subjected to change rapidly due to stimuli including masticatory load, parafunctional habit, dental caries, oral hygiene status among many others. RDT however, resists these stimuli to a larger extent and is mainly determined by consistent changes in the histological environment of the dentin primarily dictated by the advancing age of the individual. Previous studies have shown that the histological methods of age estimation using RDT have been accurate across the different ages and least deviated by pathological or demographical factors [1, 8]. Accounting to this reason, RDT is considered as the strongest and the most reliable parameter of age estimation among the six variables in Gustafson method. Based on this, the present study showed a strong relation between RDT and age of the individual and the data is consistent with the results from previous studies. There are few limitations of the present study. The present study can estimate the age in an individual above the age of 20 years. There are other reliable age estimation methods in individuals below the age of 20 years.

Conclusion

Age-at-death assessment is a crucial step in the identification process of human remaining in forensic odontology. This task in an individual is particularly difficult to achieve with reasonable accuracy due to high variability in the senescence processes. A simple and relatively inexpensive method for estimating age by measuring RDT has been described. Even with a destroyed crown and an intact root, age can be determined reliably using the level of root dentinal transparency. To improve the accuracy of age estimation, here we proposed a formula applicable to the Nepalese population. Based on cross-validation and computational experiments, our results demonstrate that age estimation from root dentin transparency can be accurately inferred across the entire adult age span.

Recommendation

The present study gave the age estimate based on conventional method of examination of root dentin transparency length. The digital methods are getting more emphasis in the present scenario. Digital methods are highly recommended for increased accuracy of age estimation.

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Conflict of interest: None

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