



Research article

Tooth shade variation in Indian population: An objective guide to age estimation

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ABSTRACT

Introduction: The color of the teeth is affected by chronological age due to the variations of the hard and soft tissue structure of the teeth. There are very few studies have assessed the shade of the Enamel and correlated it with the age changes. Hence the study aimed to assess the enamel shade with the aging in the individual.**Materials and methods:** The central incisors of 388 individuals (5–78 years) were assessed for tooth color using a VITA 3D master shade guide and the Hue, Value and Chroma were noted. Mean grey values of the teeth were obtained by image manipulation using Image J software and normalized using the values obtained from the 18% contrast grey card. Chi square tests and linear regression analysis is used to find associations with age and tooth shade variations.**Results:** The teeth had higher score of Hue, Value and Chroma in older individuals. The tooth shade tended to shift towards redder hue with older age group. Simple linear regression analysis showed a significant correlation of age with normalized grey value in association with the shade parameters. ($r = 0.717$, $SEE = 12.322$ years)**Conclusion:** Tooth color changes with age and the mean age in grey values and shade guides can be a useful tool for age estimation.

1. Introduction

Tooth colour is dynamic in nature and shows a trend with age. Research has shown that the color of teeth shows a trend with chronological age as the enamel, dentin and cementum gets modified with age. The shade of the teeth is dependent on intrinsic factors (like the optical property, quantity of hard tissue, genetics, race and gender) as well as the extrinsic factors of lighting and observer's perspective [1]. The color of enamel can also be dependent on environmental factors, nutrition, diet, vitamin deficiencies, fluorosis etc.

Dental tissues are highly resistant to degradation and putrefaction this makes them important evidence in forensics for age estimation [2]. Due to less variability, morphological characteristics of teeth form basis for most commonly used method of dental age estimation. The color of

teeth has been reported to be affected by chronological age as the enamel, dentin and cementum changes with age. The color of enamel can also be dependent on environmental factors, nutrition, diet, vitamin deficiencies, fluorosis etc. With increase in age the pulp chamber retracts, leaving secondary dentin, secondary dentin hardens and becomes less permeable, dentin Chroma becomes more saturated, and both tooth lightness and enamel thickness decrease [3].

There are currently 2 systems to assess shade of the enamel: shade guides and spectrophotometer [4]. Shade using shade guide system is used for esthetic restoration. Dental laboratories further compare the shade of the selected tooth, using a photograph taken by a standardized technique. We hypothesize that photograph of a tooth taken in a standardized manner can be used to digitally assess mean grey values of the

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tooth which can further be normalized using a standard contrast card, and then be used to correlate the age.

The purpose of study is primarily to find correlation between enamel shade and chronological age of an individual and secondly to estimate age of the individual from enamel shade. As a null-hypothesis, it was stated that there is no difference in the enamel shade with different age groups.

2. Methodology

The study included patients visiting the OPD unit of Department of Conservative Dentistry and Endodontics, of our college. Ethical approval was secured from the Institutional Ethics Committee, vide reference number:20020.

The photographs were taken following informed consent from subjects, which would solely be used for research purposes without disclosing their identification. The study consisted of 388 patients who were randomly selected among the outpatients visiting the department of conservative dentistry and endodontics. Randomization of the selection of a participant was developed by using random numbers generated from <https://www.random.org/>. For every 10 patients, one patient was selected as per the random number generated. The study consisted 388 patients who were randomly selected from outpatient department irrespective of gender and divided into 6 empirical groups as follows based on the decades, Group 1: <20 [5–20yrs] (n = 79); Group 2: 21–30 years (n = 84); Group 3: 31–40 years (n = 81); Group 4: 41–50 years (n = 61); Group 5: 51–60 yrs (n = 44); Group 6: >60 years (n = 39). Photographs of the maxillary central and lateral incisors teeth were taken which were free from cavities, fractures, restorations, endodontic tooth preparations or abnormal stains. Medically and physically handicapped patients or individuals with a history of tobacco chewing and smoking are excluded from the study.

All the photographs were taken by the same investigator throughout the study to reduce interobserver variation. Further, to minimize intra-observer variability, a custom-made apparatus was designed to standardize the distance and colour matching of each photograph. The photograph was taken from a standardized distance of 30 cm (with use of a custom-made jig) using Nikon DSLR model D1200. The jig consisted of an acrylic lath with a carbon armllet with a diameter equal to that of the DSLR camera lens. A provision was made to keep the distance of the camera to the teeth to be photographed, adjustable using a portable mount. At the other end of the carbon armllet, there was a cheek retractor attached which was used to stabilize the apparatus. We standardized the distance to be 30 cm in our study (Figure 1A). The standardized jig along with cheek retractor was positioned in the subject's mouth along with a standard 18% grey card (Figure 1B). Photographs taken were saved in a raw format and converted to tiff format for evaluation. The images were analysed in Image J version 1.42 and the region of interest was highlighted at a predefined dimension of 100 square pixels at the center of labial aspect of central and lateral incisor (without reflection of light). Corresponding 100 square pixels of the grey card was also marked and evaluated for mean grey value.

The photograph (saved in *.tiff format) of the maxillary anterior tooth captured along with a 18% grey contrast card was used for the grey value normalization procedure. The *.tiff image was analysed in Image J image software. A 100 square pixel area of the labial surface of the teeth (to assess tooth shade) and of the accompanying grey card was marked and measured for the average grey value using analyse > measure tool. The mean value of the grey values of the 18% grey card in 388 samples was obtained as 131.52 ± 17.412 units. The difference in the contrast card's "mean grey value" of each case with the mean (i.e 131.52) was then calculated. Further, the percentage difference from the actual mean grey value of the contrast card, was then estimated (difference of grey value *100/actual measured grey value). The mean grey values of the tooth surface measurements among 388 samples was derived as 209.24 ± 15.135 units. The conversion factor for each case was calculated as; $209.24 \times \text{percentage difference of the contrast card grey value}/100$. The normalized tooth shade was obtained by then adding the conversion factor to the actual tooth mean grey value measured (Figure 2).

The enamel shade was evaluated using VITA SYSTEM 3D Master guide by a single examiner who is an experienced restorative dentist practicing tooth coloured restoration for 10 years. The VITA system 3D categorizes the enamel shade on the basis of Hue, Chroma and Value. The hue represents the dominant colour of the tooth, value represents the Lightness of the tooth and chroma represents the saturation of the tooth. The patient was made to sit on dental chair [near adequate natural light] the shade guide will be held parallel to selected tooth [maxillary central/lateral incisors] at an arms distance and middle third of labial surface will be matched with shade guide. Each tooth will be observed for 4–5 s. Care was taken to keep the surrounding would clear from bright colors to avoid bias and alteration in assessment. Shade will be selected closest to that of natural tooth (Figure 1B).

3. Results

The study comprised of 388 subjects who were randomly selected from the outpatients visiting the dental clinic. The cohort included 204 females and 184 males with a mean age of 35.52 years (5–78 years range). The patients were then divided into 6 categories based on their age into class intervals of decades. There was no association or significant difference observed in color of enamel in males and females in any age group. Intraobserver assessment of the shade assessment was done in 50 cases and showed a Kappa value of 0.754 ($p < 0.001$) indicating very good agreement of repetition of analysis. Association of the hue value and chroma of the tooth as assessed using VITA shade guide system showed values 4/5 show predominance in the age of 51 and above and Reddish hue is seen predominantly >41 years. There was significantly higher proportion of colour value grades of 3,4,5 in the age group of 51–60 (70.4% collectively) and red hue in the tooth showed a peak proportion of 44.5% at the age of 51–60 years (Table 1, Figures 3 and 4) The chroma grades also showed significant differences with age. Higher chroma scores of 2.5 and 3 were seen with increasing age group (χ^2 value of 50.849 and p value of <0.001) (Table 1 and Figure 5).

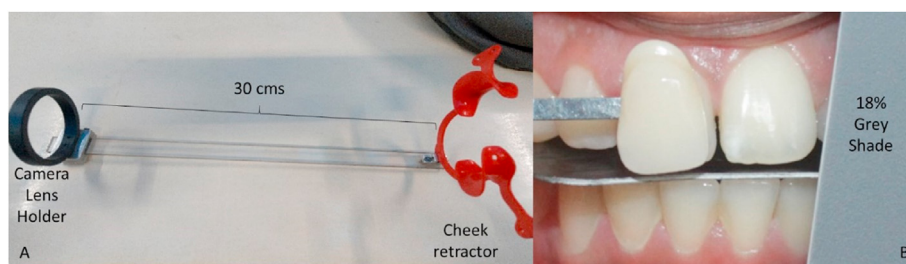


Figure 1. The custom Jig used for standardization of the Photograph. B: Photograph of the labial surface of the central incisor with the VITA shade and the 18% grey shade for standardization.

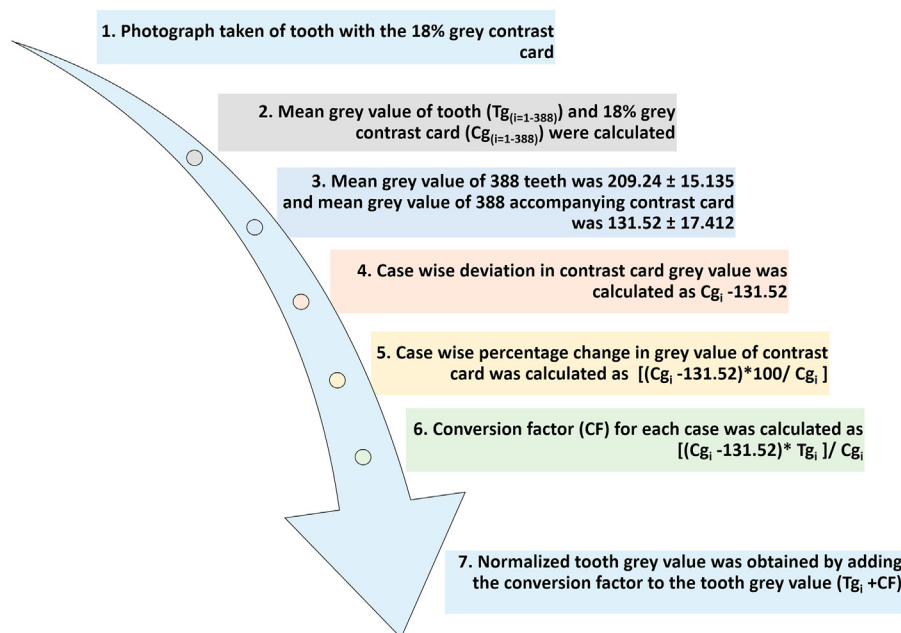


Figure 2. Flow chart of derivation of the normalized Mean grey value.

Table 1. χ^2 tests of association for the grades of Hue, Value and Chroma of the colour as assessed by shade guide with age.

		Age						Total	Chi square test and P value
		<20 (n = 79)	21-30 (n = 84)	31-40 (n = 81)	41-50 (n = 61)	51-60 (n = 44)	>60 (n = 39)		
Value	VALUE 1	46 (58.2)	20 (23.8)	6 (7.4)	2 (3.3)	0 (0)	0 (0)	74	Chi square value of 236.771 and p value of <0.001
	VALUE 2	28 (35.4)	56 (66.7)	52 (64.2)	23 (37.7)	13 (29.5)	8 (20.5)	180	
	VALUE 3	5 (6.3)	8 (9.5)	20 (24.7)	30 (49.2)	23 (52.3)	16 (41)	102	
	VALUE 4	0 (0)	0 (0)	3 (3.7)	6 (9.8)	6 (13.6)	14 (35.9)	29	
	VALUE 5	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.5)	1 (2.6)	3	
Hue	L	22 (27.8)	24 (28.6)	21 (25.9)	11 (18)	5 (11.4)	10 (25.6)	93	Chi square value of 58.681 and p value < 0.001
	M	55 (69.6)	51 (60.7)	44 (54.3)	24 (39.3)	19 (43.2)	15 (38.5)	208	
	R	2 (2.5)	9 (10.7)	16 (19.8)	26 (42.6)	20 (45.5)	14 (35.9)	87	
Chroma	1	19 (24.1)	27 (32.1)	21 (25.9)	8 (13.1)	5 (11.4)	8 (20.5)	88	Chi square value of 50.849 and p value < 0.001
	1.5	17 (21.5)	29 (34.5)	28 (34.6)	23 (37.7)	17 (38.6)	11 (28.2)	125	
	2	34 (43)	24 (28.6)	22 (27.2)	14 (23)	11 (25)	5 (12.8)	110	
	2.5	7 (8.9)	4 (4.8)	8 (9.9)	12 (19.7)	8 (18.2)	10 (25.6)	49	
	3	2 (2.5)	0 (0)	2 (2.5)	4 (6.6)	3 (6.8)	5 (12.8)	16	

Linear regression analysis was performed to estimate the age of the individual. The parameters entered included the Value, Hue, Chroma and the normalized tooth mean gray value. The regression equation showed an r value of 0.717 and standard error of estimate of 12.322 years (Table 2, Figure 6).

4. Discussion

Age related changes in tooth have been deliberated previously. The surface layer of enamel reflects most prominent changes during aging, the composition changes because of ionic exchange with oral environment occurs. The colour of the tooth is influenced by genetics, race as well as sex of the individual. Haralur et al (2014) have shown that there is a significant correlation between the skin and tooth colour across different ethnic groups proving the point that genetics play a role in tooth colour change [1]. To keep the study more appropriate to our population we have undertaken this study in the Indian Population alone. In a study on age related changes in enamel, the facial enamel thickness above CEJ significantly decreased while the physiologic dentin thickness and the incisal edge pulp distance increased. It is thus known that color of tooth

depends on 3 parameters – thickness of enamel as well as dentin and volume of the pulp chamber [3].

The results attained in present study showed that in younger age groups enamel shade is more yellowish hue compared to older groups [>45yrs]. The yellowish hue is due to not only the decrease in thickness of enamel but also increasing thickness of dentin (which is yellow in colour) with age. Atsu SS et al. have reported significant reduction in the thickness of the enamel with age as studied electron microscopically. They further showed that this reduction is significant after the age of 50 years [5].

This fact supports our finding of higher scores of Value, Hue and Chroma seen after the age of 50 years.

The shade of the teeth varies from the incisal edge to the gingival region, owing to the variations of the thickness of enamel and dentin and the shade is best assessed at the middle third of the tooth. The dentist usually employs a visual mode of comparison with a shade guide under natural light to assess the shade of the tooth. Among the shade guides the 3D master contains shade tabs more uniformly arranged and is known to improve repeatability among clinicians [3, 4, 6].

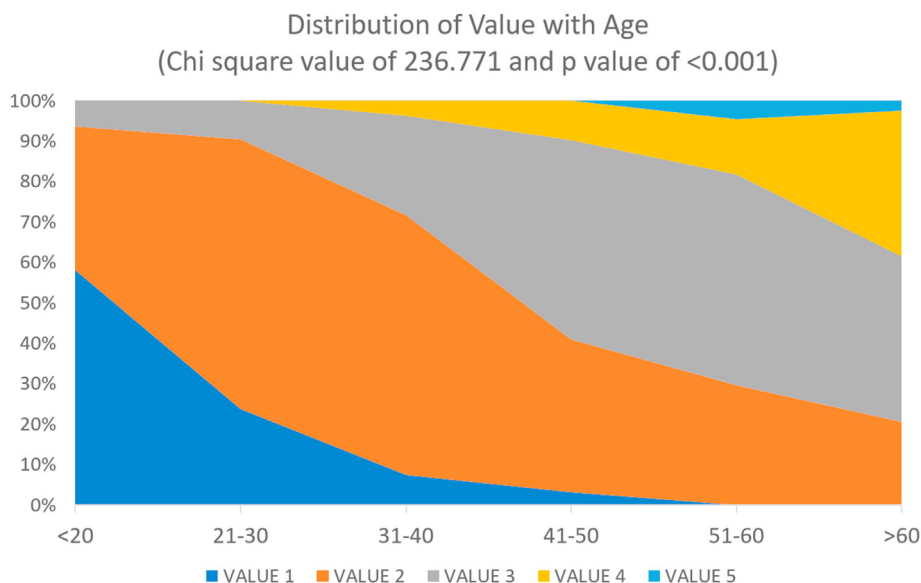


Figure 3. Area graph showing the proportion of distribution of different scores of Value over different age groups.

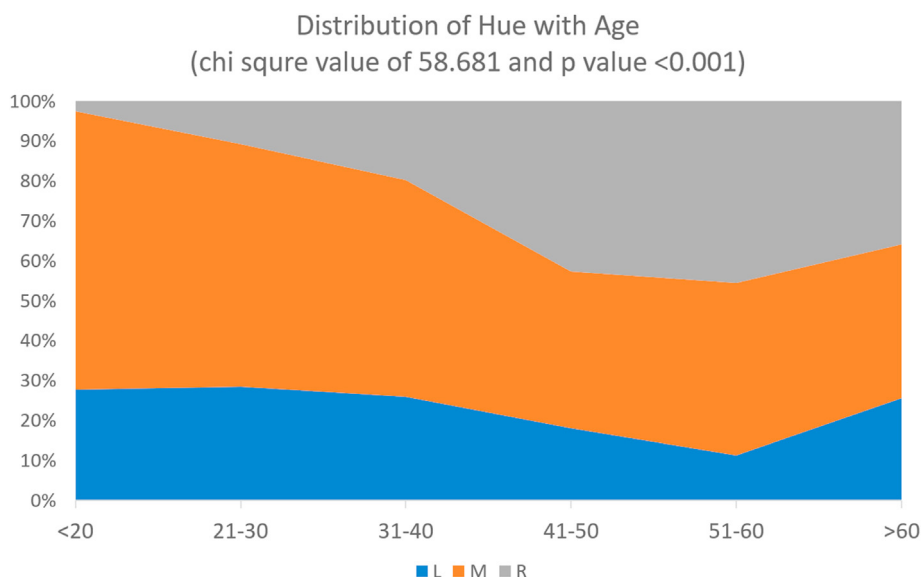


Figure 4. Area graph showing the proportion of distribution of different scores of Hue over different age groups.

Our preferred use of visual shade matching [VITA 3D SYSTEM] over conventional Shade system is justified because of its detailed classification ability of the colour.

Hassel et al in their study showed significant changes in color coordinates with age. A decrease of lightness (value) was observed in younger cohort whereas in older individuals showed increase in chroma. A significant shift to more reddish tooth colors was observed with age [7].

These results are similar to our results as we see more reddish hue with increased age. Gomez-Polo et al (2015), compared the central incisors of 1361 Caucasian Spanish individuals using spectrophotometer. He proposed that the maximum variation (45%) is due to Lightness of the colour (value) [8]. Hasegawa A et al found that the center site of the labial aspect of incisors become darker and more yellow in color as the age increases. The found that the L* (indicative of lightness coordinate) decreased with increasing age. The colour coordinate a* (indicative of chromaticity coordinate in red-green axis) did not correlate with age however b* (indicative of chromaticity coordinate in yellow-blue axis)

showed a significant positive correlation with age [9]. Polo CG et al (2015) showed similar results with the L* coordinate showing significant negative correlation with age (r = -0.674) whereas the a* and b* coordinates showed a significant positive correlation with r values of 0.46 and 0.417 respectively [10].

Odioso et al (2000) noted that for each year there was 0.10 a* units increase i.e. the range of yellow chromaticity and 0.22 L* units decrease in lightness value [11].

This supports our study findings also as the yellow colour increased with higher age groups with concomitant darker shades. In our study, we further found hue was also showing a significant shift to higher grades along with scores of value. Objective type of measurements definitely have an advantage over subjective type of measurements like shade guide analysis. Measurements obtained from spectrophotometer or software based colour space evaluation have lesser inter and intra observer variations. Visual shade selection varies, depending on clinician's color perception and experience, ambient light condition, background of tooth, the shade guide used. The

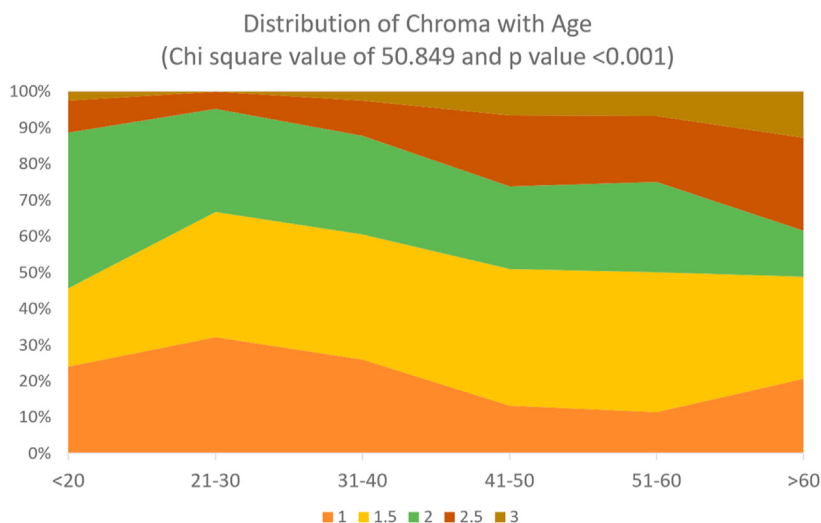


Figure 5. Area graph showing the proportion of distribution of different scores of chroma over different age groups.

Table 2. Linear regression analysis with age as a dependent variable shows significant association of the Hue, Value, Chroma as well as the Normalized tooth mean grey value.

Model		Unstandardized Coefficients		Standardized Coefficients	t	P value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
Enter	(Constant)	-23.168	4.741		-4.887	<0.001	-32.489	-13.846
	Value	13.007	.722	.647	18.026	<0.001	11.589	14.426
	Hue	4.623	.926	.179	4.992	<0.001	2.802	6.444
	Chroma	4.733	1.144	.148	4.137	<0.001	2.483	6.983
	Normalized tooth mean grey value	.059	.017	.124	3.472	.001	.026	.093

R value of 0.717, R square value of 0.514, standard error of estimate 12.322 years.

Equation age = -23.168 + 13.007(value)+4.623(Hue)+4.733(Chroma)+0.059(Normalized tooth mean gray value).

^a Dependent Variable: Age. All p values <0.05 are considered significant

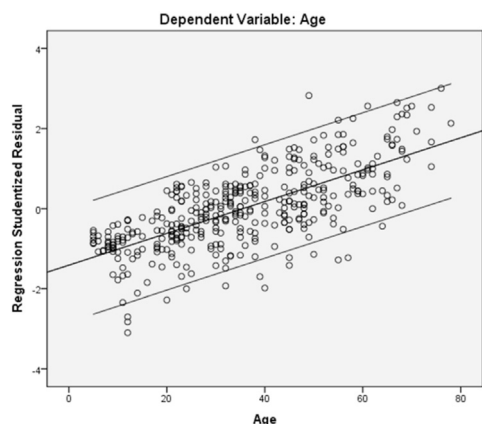


Figure 6. Scatter plot of the residuals of the equation shows a positive correlation with age.

commission international de l'Eclairage (CIE) defined a colour space: CIE Lab, that is known to represent the colour in three dimensions of L*, a* and b*. This colour space has been efficiently analysed using image analysis softwares in studying colour changes in various bleaching procedures [6, 12].

Karaman T et al have analysed the colour change in the CIE Lab colour space as well as the VITA master shade guide. Although the shade A2 was

the most frequent shade detected across age, the proportions of shades B2 and C3 increased in individuals above 45 years of age [13].

Digital assessment of the colour spaces can have an edge over the visual shade guid comparison techniques. In this study the color of enamel was analysed using the image analysis software Image J which was able to pick up the mean grey values in an 8 bit image. These subtle changes in colour cannot be evaluated objectively by human eye. The derived regression equation with the inclusion of the normalized grey values (obtained using 18% contrast grey card) along with the hue, value and chroma scores showed a R value of 0.717 and a standard error of estimate of 12.322 years.

5. Conclusion

Age estimation can be done using the tooth colour. With age due to the change in the thickness of enamel, dentin and pulp the tooth attains a reddish hue with higher value and chroma which can be established using the grey value following standardization. This could prove to be a useful technique in forensic science for age estimation.

Although the study explores the standardization of the tooth colour to estimate age, the practical solution is less feasible. There are too many confounding factors involved in such an assessment, for example, ethnicity, extrinsic and intrinsic changes in tooth color, examiner reliability, use of appropriate share card, amongst a few. With the geopolitical situations migrant individuals, individuals seeking asylum may be

from different ethnic background making such an estimation less reliable.

5.1. Clinical significance

Enamel shade is a composite result of the thickness, translucency and chemical nature of enamel as well as dentin. This optical character varies with age owing to the constant change in the properties and thickness of enamel and dentin. Enamel decreases and Dentin increases with age due to attrition and secondary dentin deposition respectively. In forensic sciences, age estimation is one of the four pillars of human identification. Use of enamel shade in age estimation by objectively measuring the optical properties would be an useful aid in this regard. The salient advantage of this method would be the no requirement for extraction of teeth, process of ground sectioning or radiographic evaluation of the specimen.

Declarations

Author contribution statement

Srikant N: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shweta Yellapurkar: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Annapurna S and Vaishnavi Gundeti: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nidhin Philip Jose and Vinod Jathanna: Performed the experiments; Wrote the paper.

Dilip Naik: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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