ORIGINAL RESEARCH PAPER

Volume-8 | Issue-3 | March-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

AGE ESTIMATION FROM TEETH USING MODIFIED GUSTAFSON'S METHOD AND CEMENTAL ANNULATION LINES - AN IN VITRO STUDY.

Forensic Science

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ABSTRACT

Estimating age from teeth is reliable as they are preserved long after all the tissues and even bones have disintegrated. This resistance to decay has rendered teeth a useful tool for calculation of age.[1,2] In this era, where DNA is used in forensics, conventional age estimation methods are still fruitful due to their ease and cost effectiveness. The purpose of the study was to evaluate the reliability of age estimation of an individual from teeth using Modified Gustafson's method and cementum annulation lines.

Age estimation was done using teeth of known age and sex. Ground sections were observed under light microscope and age estimation was done using two methods. It was observed that both methods were reliable as the estimated age was close to actual age. However, the former method was more accurate as it had less margin of error (±0.18 years) than the second (±0.21 years). Thus, these methods may be reliably employed in age estimation.

KEYWORDS

Age estimation, Modified Gustafson's method, cementum annulation lines.

INTRODUCTION

Age estimation is one of the essential factors of forensic odontology and is a prime factor in establishing identity of a person. Age can be estimated using structures like skull, long bones and teeth. Gradual changes in teeth throughout life are the basis for age estimation which has been an archaic exercise, and since decades dentists have contributed to this science with several methods.[3] The enamel, dentin and cementum that comprise teeth have been used for this purpose.

Due to mastication and as a part of normal ageing process various age changes take place in teeth. Most common change is attrition or tooth wear which is physiologic wearing away of tooth as a result of tooth to tooth c pulp and represents dentin formed after root completion. In sclerotic dentin apatite crystals are filled in the dentinal tubules.[5]

Age changes are reflected in cementum also. The change that occurs is increase in thickness of secondary cementum or cellular cementum, which is usually found at the apical third of the tooth.[5] Cementum annulations lines also known as incremental lines of Salter also help in age estimation.

The present study was undertaken to co-relate age of an individual from teeth using Modified Gustafson's method and cementum annulations lines. Accuracy and precision of age changes were estimated using these two methods and an attempt was made to understand which method is more reliable.

MATERIALS AND METHODS

The present study was conducted in our institute. The sample comprised of 100 permanent teeth (48 males and 52 females) in age group of 18-65 years, which were procured during routine dental extractions from Department of Oral and Maxillofacial Surgery. Ethical clearance for conducting the study was obtained from the Institutional Ethical Clearance Committee. An informed consent was obtained from study subjects selected to be part of the study.

Longitudinal ground sections of 70-80 micrometers thickness were prepared using stone trimmer and combination stone (37C fine/ coarse). These ground sections were observed under light microscope and age estimation was done using Modified Gustafson's method based on the formula given by **Kashyap and Koteshwar Rao (1990)** using four Gustafson's criteria.[6]

The formulae of the various index values for each parameter were as follows:

1. Attrition (A): Incisor and canine attrition index value

(A)= a/Ax100

Where **'a'** is the width in mm of the attrited teeth.

'A' is the width in mm of the teeth at the cervical margin

Premolar and molar attrition index values:

(A) = a1+a2/Ax100

Where **'a1 and a2'** are the width in mm of the attrited tip of buccal and lingual sides.

'A' is the width of teeth in mm at the cervical margin (figure 1)

Different age changes take place in dentin as well. Secondary dentin is another age change where there is a narrow band of dentin bordering the pulp.

2. Secondary dentine (D): The dentine index was measured in terms of percentile value of secondary dentine deposited and total length of the pulp cavity according to the following formula:

(D) = d/Dx100

Where**'d'** is the length of the secondary dentine deposition in the pulp cavity.

'D' is the length of the entire pulp cavity of the tooth (figure 2)

3. Cementum apposition ©: The cementum apposition index was measured in terms of percentile values of cementum thickness on either side of the tooth in relation to the total width of the tooth at the point of thickest cementum deposition.

(C) = ce1+ce2/CEx100

Where **'ce1'** is the cementum apposition on one side. **'ce2'** is cementum.

apposition on other side in mm at the thickest point of the tooth.

'CE' is the width in mm of teeth of cementum at the thickest point (figure3)

4. Translucency of the dentin (T): Translucency of dentin was calculated for each tooth by the following formula:

(T) = t/Tx100

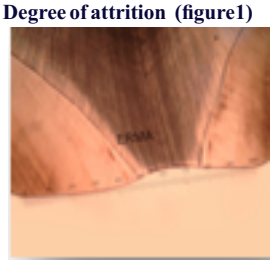
Where **'t'** is the length of the region of the tooth in mm

'T' is the length in mm of the entire tooth (figure 4).

Age was calculated by the formula:

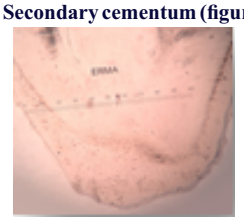
Estimated age: (A) + (D) + (T) + (C)/4[6]

Degree of attrition (figure1)



Secondary dentin (figure 2)

Secondary cementum (figure 3)



Translucency of dentin (figure 4)

Age estimation using cementum annulation lines

First the total width of cementum from dentin- cementum junction to the surface of cementum was measured using a micrometer in 10x view (figure 5). Later distance between two adjacent parallel incremental lines was calculated. Number of incremental lines was obtained by dividing total width of cementum by distance between two adjacent cemental lines.

Number of incremental lines = X/Y

X = total width of cementum

Y = width of cementum between two incremental lines

The number of incremental lines obtained were added to the chronologic age of eruption. The calculated age by using cementum annulations lines was obtained using the following formula. E = n+t

E = estimated age

n = number of incremental lines

t= eruption age of tooth[2]

Cementum annulation lines (figure 5)

The age estimated from above two methods was compared to actual age and the data obtained was subjected to statistical analysis.

RESULTS

The purpose of the study was to estimate age of an individual from teeth using Modified Gustafson's method, cementum annulation lines and to compare the age estimated by these two methods in their accuracy using same set of teeth.

The data obtained from the study was compiled, tabulated and subjected to statistical analysis, which was done using SPSS software version 16. Actual age was compared to age estimated by Modified Gustafson's method and cementum annulations lines using paired t test.

Age was estimated by Modified Gustafson's method using four parameters like attrition, secondary dentine, secondary cementum and translucency of dentin. When this age was compared to that of actual age, it was observed that actual age (Mean -29.31 years) did not differ significantly (Mean -29.13 years) (p-value -.821) with an error of ±0.18 years (table 1 bargraph 1).

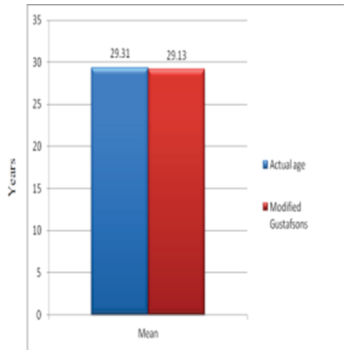
However when the same sample was analyzed to estimate age by studying cementum annulations lines, it was observed that the estimated age (Mean -29.1 years) was not significantly different than that of actual age (Mean -29.31 years) (p-value- 0.504) and had an error of ±0.21 years (table 2 bargraph 2).

Analysing these two methods of estimating age, it was observed that age estimation by Modified Gustafson's method was more accurate as it has less margin of error (±0.18) than cementum annulations lines (±0.21). But there was no statistically significant difference of age estimated by these two methods (table 3 bargraph 3).

Method	Mean (years)	N (No. of teeth)	SD	Mean Difference		p-value
				Mean	SD	
Actual age	29.31	100	11.79	0.18	8.05	0.821 NS
Modified Gustafson's method	29.13	100	9.36			

p- 0.821 (Not significant)

Paired t- test



Bar graph 1

Table 2

Method	Mean (years)	N (No. of teeth)	SD	Mean Difference		p-value
				Mean	SD	
Actual age	29.31	100	11.79	0.21	3.13	0.504 NS
Cementum annulation lines	29.1	100	11.20			

p- 0.504 (not significant)

Paired t- test

Bar graph 2

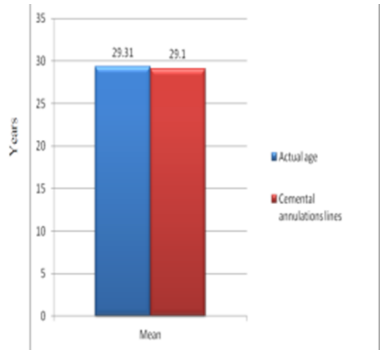


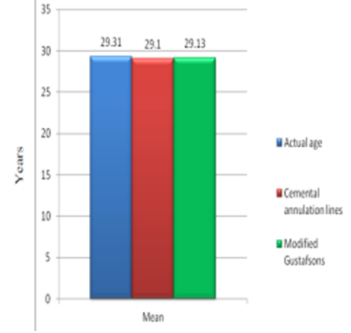
Table 3

Method	Mean (years)	SD	N (No. of teeth)	p-value
Actual age	29.31	11.79	100	0.859 NS
Modified Gustafson's method	29.13	9.36	100	
Cementum annulation lines	29.1	11.79	100	

p- 0.859 (not significant)

Repeated measures ANOVA

Bar graph 3



DISCUSSION

Human identification is of paramount importance in forensic dentistry. It has application in establishing the identity of living or deceased

persons and is also useful in living individuals whose chronologic age is under dispute.[7] The greatest advantage in using teeth over bone for forensic investigations is that they can be clinically inspected in living individuals. These benefits probably place teeth above other skeletal parameters and amongst the organs of choice in forensic age estimation and identification.[8]

The physiological changes in teeth due to ageing are the basis of age estimation. The enamel, dentin and cementum that comprise teeth have been used for this purpose.

Originally **Gustafson (1950)** used six parameters like attrition, periodontal disease, cementum apposition, secondary dentine deposition, root translucency and root resorption for age estimation. In our study age was estimated by Modified Gustafson's method using four of six Gustafson's criteria like attrition, secondary dentin, secondary cementum and translucency of dentin. Longitudinal ground sections were analysed using method given by **Kashyap VK and Koteswara Rao NR (1990).** Modification to the original method was intended to minimise the inherent difficulties in quantifying regressive changes in attrition, secondary dentine, root translucency and cementum apposition, by using index values based on actual physical measurements, which were measured using micrometer eyepiece.[6]

In present study mean error of ± 0.18 years was noted when compared to that of actual age. However, in original **Gustafson's method (1950),** a mean error of 3.6 years was seen. Later many studies were done using all or few of Gustafson's criteria like that of **Vlcek E (1977)** used four of Gustafson's criteria like degree of attrition, secondary cementum, secondary dentin and root apex resorption and noted an error of 3 years.[9]

Kashyap VK and Koteswara Rao NR (1990) studied four of six Gustafson's and noted a mean error of 1.59 years.[6] **Singh A et al (2004)** conducted a study to evaluate physiological changes of teeth by considering six Gustafson's criteria and found a mean error of 2.16 years.[10] **Rai B et al (2006)** used five markers like secondary dentine, the secondary cementum the degree of attrition, the resorption and the transparency of dentin and noted maximum error of estimation is 4.95 years at 95% confidence interval.[11] **Bajpai M (2011)** used six Gustafson's criteria to estimate age and found a mean error of ±4.86 years.[12] **Mangesh S et al (2012)** used only attrition to estimate age and noted a mean error of 1.25 years.[13] **Bajpai M, Mishra N and Sharma P (2012)** studied age estimation using physiologic changes of teeth according to Gustafson's criteria and found the mean error to be ±4.52 years.[14] **Kashyap VK and Koteswara Rao NR (1990)** and **Rai B et al (2007)** were the only two authors who considered the same four criteria as we have taken in our study. But, they too reported high mean error in their study.[6,15]

However in our study the error was as less as ±0.18 years. This can be attributed to firstly increased sample size (100), secondly unlike **Kashyap VK and Koteswara Rao NR (1990)**, who took teeth from cadavers and stored in water till they were sectioned, we used freshly extracted teeth stored in formalin and finally the methodology used. **Rai B et al (2007)** used 13 point scale to estimate age using the same criteria as ours and in our study formula given by **Kashyap VK and Koteswara Rao NR (1990)** was used. So there is change in methodology. Thus minimized error in our study can be justified.

The first use of cementum in human age estimation began with measurements of width of total cementum layer, rather than number of incremental lines. **Stott (1982)** was the first researcher to test the correlation between known age at death and a count of the number of increments, rather than the thickness of cementum.[2] In our study we estimated age using cementum annulations lines from longitudinal ground sections as it allows viewing the whole root surface as advocated by **Klevezal and Kleinenberg**[2] and also because age was estimated using Modified Gustafson's method which required longitudinal ground sections only.

The accuracy is reflected in the error bounds obtained from the statistical analysis which are no more than ±0.21 years. **Backofen UW et al (2003)** studied transverse sections of teeth from mid root and found a mean error of 2.5 years using light microscopy.[16] **Aggarwal P et al (2008)** studied longitudinal ground sections of teeth using polarizing and light microscopy to estimate age using cementum annulation lines and found a mean error of < 2 years using polarized microscopy.[2] **Pundir S, Saxena S and Agarwal P (2010)** studied longitudinal ground sections of teeth using light, polarized and phase contrast microscopy and found that cementum annulations were best viewed in phase contrast microscope than in light and polarized microscope.[1]

The decreased error of ±0.21 years in our study compared to that of previous studies can be attributed to firstly larger sample size, secondly method employed. In our study total width of cementum was measured and distance between two parallel lines was calculated. Number of cementum annulations lines were obtained by dividing the total width of cementum by distance between two lines. Age was calculated by adding number of TCA to chronologic age of eruption. So in this method each cementum annulation line was not counted. Other authors except **Agarwal P et al (2010)** counted number of cementum annulation lines. However, there can be a discrepancy while counting number of lines, hence this was ruled out.

Finally in our study, mean age of the sample was 29.1 years. **Agarwal P et al (2010)** in their study also found that annulation counts in younger age group (<55 years) appear to be close to actual age.[2] This is also supported by another study conducted by **Obertová Z and Francken M (2009)** where it was found that reasonably accurate age estimates based on TCA counts were only obtained in young adults. Under estimation of age occurred above the age of 40.[17] So, the reduced mean age of the sample also contributed to the accuracy of age estimation.

There are no studies in the literature that compare age estimation by Modified Gustafson's method and cementum annulation lines. In our present study, when age of an individual was estimated by these two methods, it was found that Modified Gustafson's method was accurate and more reliable than that of cementum annulation lines.

CONCLUSION

Thus from our study it can be concluded that age estimated by Modified Gustafson's method was more accurate and nearer to actual age than cementum annulation lines. There was no statistically significant difference of age estimated by these two methods. Thus, these methods may be reliably employed in age estimation from teeth. However, more studies with larger a sample size are required in future to establish Modified Gustafson's method as the best method of age estimation.

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