ORIGINAL ARTICLE

Ascertaining of age by Raman spectroscopic analysis of apical dentin – A forensic study

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Abstract

Context: Age estimation in forensics employ various methods of which Raman microspectroscopy provides a noninvasive method by assessing various parts of dentin.

Aim and Objectives: The aim of this study is to ascertain the age of carious tooth using Raman spectra of apical dentin and to correlate the similarity of the spectra in a defined age group. **Settings and Design:** Teeth of known age from Indian population (n=48) and morphology (incisors, canine, premolars, molars) indicated for extraction due to caries were allocated into four groups, i.e., Group A (21-30 years), Group B (31-40 years), Group C (41-50 years) and Group D (51-60 years). **Materials and Methods:** Teeth were sectioned and the apical 2 mm of dentin was examined by a Raman microspectroscopy machine for spectra from 400 cm^{-1} to 1500 cm^{-1}, and peak at phosphate group at 963 cm^{-1} was taken for statistical analysis. **Statistical Analysis Used:** The data were analyzed using IBM SPSS version 20.0. **Results:** Pearson's correlation to test the strength of linear relation of the spectra of the teeth within an age group showed an "*r*" value approaching 1. One-way ANOVA to test the difference between means of spectrum values obtained between the four age groups was statistically significant at $P < 0.05$. **Conclusion:** Raman spectroscopic analysis of apical dentin of teeth affected by caries can also be used to estimate the age and the Raman spectra obtained differed for different age groups, and the same can be advocated as an alternative method to ascertain age in forensic dentistry.

Key words: Age determination, dentin, forensic dentistry, Raman spectrum

Introduction

Age estimation plays an important role in forensic medicine and teeth are sometimes the only valuable remains along with skeleton when other external features have been destroyed. Furthermore, teeth can also be used to determine age of a living individual in association with crimes and other purposes. Age estimation from teeth includes various methods such as clinical, radiological, histological, and physical-chemical methods. Physical and chemical methods include evaluation of the ionic and crystalline structure of the mineral components of the human tooth. Tissues in the oral cavity are capable of producing spectra when analyzed by optical spectroscopic systems.[1] Raman microspectrometry is one among the recently developing areas for age estimation by giving

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a molecular fingerprint of its chemical structure and is a highly selective and noninvasive technique. It can provide a quantitative and qualitative mapping of the chemical components of any analyzed specimen.[2] The various bands obtained in Raman spectra are molecule specific and provide direct information about the biochemical composition of any analyzed specimen, and focused areas can be analyzed by means of optical microscopes coupled to the spectroscopic machines.[3]

Gustafson was the first to provide a formula for calculating the age by assessing six variables – attrition, periodontitis, secondary dentin deposition, cementum apposition, root resorption, and root dentin translucency.[4] Of these six factors, relative translucency of root dentin has been established as the most reliable aging characteristics[5,6] and could be used to estimate age by image processing systems.[7] Root dentin starts to appear translucent in the apical region by the third decade of life and starts progressing coronally with advancing age. This is due to the deposition of peritubular dentin within the tubules causing a reduction in diameter of the tubules with advancing age.[8] Various studies have been done to estimate the age of an individual by Raman spectroscopic analysis of human dentin.[9,10] However, these studies involve Raman spectroscopic analysis of multiple areas in the coronal, mid, and root dentin, acquisition of multiple values from each region, and assessment of various groups such as phosphates, carbonates, and amides I and II, and the teeth assessed were noncarious. The present study was done with the aim to overcome the shortcomings of the previous studies and to ascertain the age of a specimen by assessing if the spectra obtained from a single age group correlated with each other and spectra obtained among the different age groups were different from each other.

Materials and Methods

Teeth indicated for extraction due to dental caries from patients of Indian origin and known age group from both genders were extracted in such a way that they belonged to four groups – Group A, Group B, Group C, and Group D [Table 1] with 12 specimens in each age group. Within each age group, teeth were extracted in such a way that 3 were incisors, 3 were canines, 3 were premolars, and 3 were molars. However, root canal-treated teeth and those with periapical pathology were excluded from the study. The extracted teeth were uniformly stored in formalin (10% buffered) until the previous day of assessment. They were then debrided with hydrogen peroxide solution IP (6% w/v 20 volume) and sectioned perpendicular to their occlusal surface into two halves with a diamond disc and straight handpiece [Figure 1]. The sectioned teeth were then stabilized on a glass slide with wax with the cut flat surface facing the microscope lens of a Raman spectroscopy Horiba Jobin Yvon LabRAM HR micro Raman system [Figure 2]. A ×100 confocal microscope was used to examine only the apical 2-mm dentin. The excitation source was a laser at a wavelength of 785 nm. Spectra were obtained from 400 cm^{-1} to 1500 cm^{-1} under the same conditions for each tooth. Various peaks were observed for amides I, amides II, and phosphate group at 1235 cm^{-1}, 1450 cm^{-1}, and 963 cm^{-1}, respectively, and were plotted on a graph [Figure 3]. The peak for phosphate group at 963 cm^{-1} was alone quantified and taken for statistical analysis.

Results

The data were analyzed using SPSS version 20.0 (IBM SPSS, IBM Corp., Armonk, NY). Table 2 shows the descriptive statistics for the samples included in the study. The mean spectrum values were plotted against age groups for the four groups of teeth. As the graph shows [Figure 4], the peak of the spectrum for incisor showed a 10.9% increase after 20 years reaching a peak at 31–40 years, followed by a decrease of 16.1% for 41–50 years and a further decay of 39.38% as it reached 51–60 years. Premolar and molar showed a similar trend with a 13.8% and 11.4% increase on approaching 31–40 years, followed by a decrease of 14.09% and 22.5% in 41–50 years and a further decay of 15.02% and 27.15% in 51–60 years, respectively. However, for the canine group, the peak of spectrum was obtained for the age group of 41–50 years, with an increase of 10.1% after 21–30 years, further increase of 10.8% at 41–50 years, and decrease of 14.03% as it reaches 51–60 years. Pearson's correlation was done to test the strength of correlation of the spectra of the specimen within an age group. The spectra

Table 1: Sampling

	Incisor	Canine	Premolar	Molar
Group A (21-30 years) $n=12$	3	3	3	3
Group B (31-40 years) $n=12$	3	3	3	3
Group C (41-50 years) $n=12$	3	3	3	3
Group D (51-60 years) $n=12$	3	3	3	3

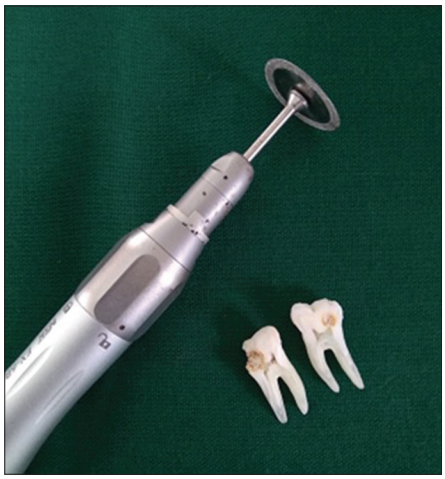

Figure 1: Tooth sectioned into two along the long axis perpendicular to the occlusal surface with a straight handpiece and diamond disc

obtained for different groups of teeth correlated with each other with a "*r*" value approaching 1 [Table 3]. A one-way between-groups ANOVA was conducted to compare the effect of age on the values of spectrum obtained for these specimens of different age groups. There was a significant effect of age on the spectrum values at $P < 0.05$ level for the four age groups ($F[3,12] = 4.53$, $P = 0.023$). This shows that the spectra between the age groups were significantly different [Table 4].

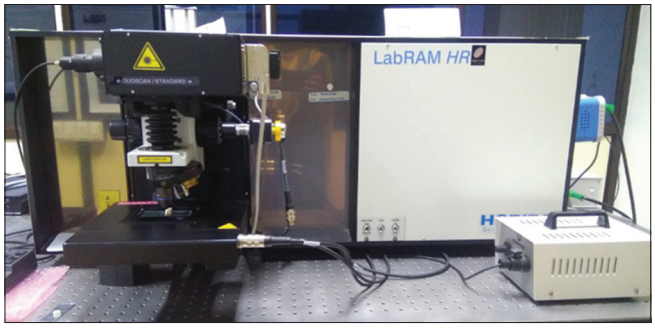

Figure 2: Sectioned tooth stabilized by wax on a glass slide with the cut flat surface facing the microscope lens of a Raman spectroscopy Horiba Jobin Yvon LabRAM HR micro Raman system

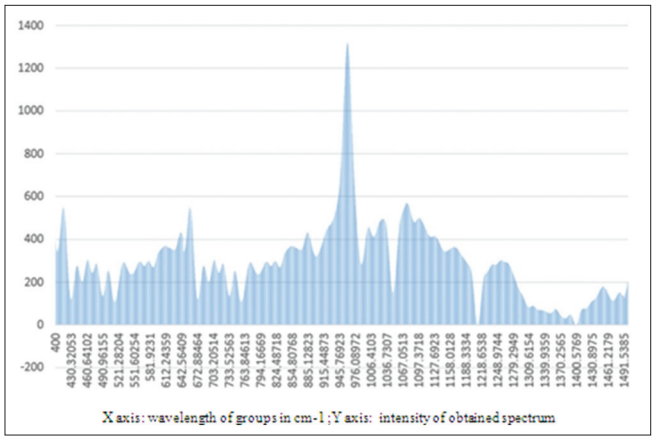

Figure 3: Raman spectra obtained for apical dentin showing peak at 963cm^{-1} for phosphate group

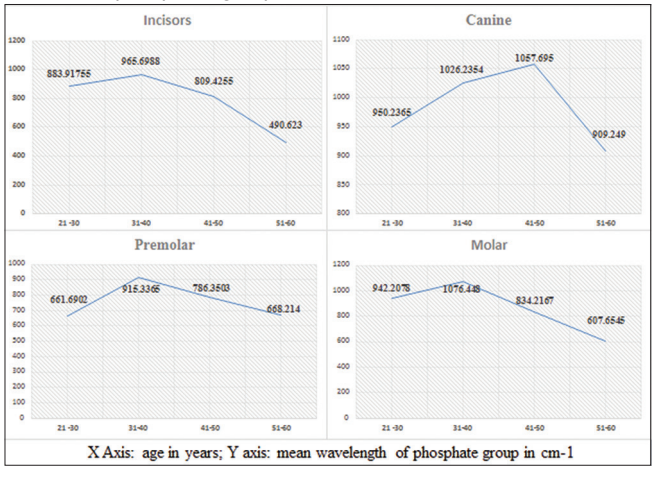

Figure 4: Age and tooth anatomy-based variations in the Raman spectra obtained from apical dentin

Discussion

Age estimation has long posed a challenge in forensics. The various parameters from the human tooth that can be used for age estimation are the number of teeth in the dentition, tooth color and fluorescence, attrition, periodontal recession, cementum apposition, root resorption, secondary dentin, root translucency, peritubular dentin, racemization,

Table 2: The summary statistics for the different ages of the sample teeth obtained from different age groups of the study

Descriptive	Group A	Group B	Group C	Group D
Mean (age)	25.16667	35.08333	44.75	55.25
Median	25	35	45	56
Mode	25	32	42	56
Standard deviation	2.405801	2.609714	2.562846	2.73446
Sample variance	5.787879	6.810606	6.568182	7.477273
Skewness	0.18716	0.07203	0.138525	0.28211
Range	8	8	8	8
Minimum	21	31	41	51
Maximum	29	39	49	59
Count	12	12	12	12

Table 3: Pearson's correlation coefficient (r) for specimens between different age groups

	Incisor	Canine	Premolar	Molar
21-30 years' age group (Group A)				
Incisor	1	0.998726	0.970759	0.998527
Canine	0.998726	1	0.981635	0.994517
Premolar	0.970759	0.981635	1	0.956305
Molar	0.998527	0.994517	0.956305	1
31-40 years' age group (Group B)				
Incisor	1	0.980158	0.883810	0.120491
Canine	0.980158	1	0.959009	0.314873
Premolar	0.883810	0.959009	1	0.314873
Molar	0.120491	0.314873	0.570927	1
41-50 years' age group (Group C)				
Incisor	1	0.815167	0.782157	0.682211
Canine	0.815167	1	0.998474	0.962968
Premolar	0.782157	0.998474	1	0.976311
Molar	0.682211	0.962968	0.976311	1
51-60 years' age group (Group D)				
Incisor	1	0.989785	0.972677	0.955144
Canine	0.989785	1	0.972677	0.955144
Premolar	0.972677	0.972677	1	0.860295
Molar	0.955144	0.955144	0.860295	1

'*r*' value >0.7 indicates strong correlation

Table 4: One-way ANOVA

ANOVA (one-way ANOVA)						
Source of variation	**SS**	**Df**	**MS**	***F***	***P***	**F critic**
Between groups	416618.8	3	138872.9	4.537438	0.023968	3.490295
Within groups	367264.2	12	30605.35			
Total	783882.9	15				

The test is significant at $P<0.05$ as P is 0.023. SS: Sum of squares, Df: Degrees of freedom, Ms: Mean square, *F*: *F* statistic, F critic: F critical value

and cementum annulation.[11] The most recent methods include physical and chemical methods such as amino acid racemization of aspartic acid[12] and Raman spectroscopic analysis. The developments such as highly sensitive spectral detectors, cheap and miniature laser diodes, and inexpensive microelectronics have made optical spectroscopic techniques such as Raman spectroscopy – an useful application in biomedical sciences. Raman spectrum can be used to obtain spectrum from human dentin which is composed of 70 wt% inorganic portion with the majority composed of calcium phosphate,[13] and hence, only the phosphate band from the Raman band was taken for analysis.

In the present study, we have obtained the Raman spectra only from the apical 2 mm of dentin after storage in 10% neutral formalin. Studies have shown storage for prolonged periods in formalin, do not cause changes in root dentin translucency.[8] Root dentin translucency also known as dentinal sclerosis starts appearing from the root apex and progresses coronally with advancing age. This starts apically by the third decade of life and is the most reliable dental aging characteristics that is least affected by any superimposed pathology on the coronal portion of the tooth. This translucency occurs as a result of matching of refractive indices of the intertubular dentin and peritubular dentin that occlude the dentinal tubules and causing a reduction in diameter with advancing age.[14] It has been proved that intratubular sclerosis occurring in the coronal dentin due to caries[15] is different from intratubular sclerosis occurring at the root apex with advancing age.[16] Furthermore, Raman spectroscopic analysis of various dentinal areas when exposed to lactic acid for a period of 30 days showed that apical dentin was most resistant to acid attack when compared to that of coronal dentin.[17] Root canal-treated and nonvital teeth were excluded from the study because these teeth become optically opaque in their dentinal portion due to the absence of stimulation from the pulpal tissue.[8] Hence, apical dentin can alone be measured by Raman spectroscopy as a reliable dentinal area in contrast to previous studies that have assessed multiple areas of dentin. Furthermore, the presence of bacteria within the tubules in the apical region will not cause any significant change in the chemical composition of the apical dentinal structure. Hence, carious tooth can be used for Raman spectroscopic analysis to determine the age.

The results of the present study show that the spectra obtained from teeth within the same age group correlated with each other. The spectra obtained for a particular tooth of an age group was different when compared to another age group ($P < 0.05$). This study proves that Raman spectra obtained in order to estimate the age of a specimen can be taken only from the apical dentin and can be done on carious teeth also. However, the reason for gradual increase in phosphate group reaching a peak at 31–40 years, followed by a gradual decrease with progressing age with the exception of canine, needs to be probed with further experimental studies and bigger sample size. The limitations of the study include (i) a small sample size and (ii) analysis has not been tried on incinerated teeth and endodontically treated teeth.

Conclusion

Raman spectroscopic analysis is a noninvasive technique for age estimation from the human tooth, and apical dentin in carious tooth can also be used as a reliable predictor for age estimation. With the presence of handheld Raman spectroscopic machines, determination of age of a specimen or a corpse can be done at the site of postmortem if we could in the future establish a database for standard spectra for various age groups and various tooth anatomies. Future research in Raman spectra of incinerated teeth also needs to be probed so that Raman spectroscopic analysis of human apical dentin can provide a valuable adjunctive or mainstay method to assess age in forensic odontology.

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Conflicts of interest

There are no conflicts of interest.

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