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Diagnostic Application of Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance in Dental Caries - An In vitro Study

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Abstract

Purpose: The study is to analyse the significance of Fourier transform infrared spectroscopy-Attenuated Total Reflectance (FTIR-ATR) in detecting the dental caries in human extracted teeth. The molecular information about collagen and hydroxyapatite structural changes associated with enamel, dentin, pulp of human tooth can be obtained using FTIR. **Materials and Methods:** Forty extracted tooth samples were obtained and divided into four groups as non-carious teeth, early carious teeth, advance carious teeth, carious deciduous teeth. Carious and non-carious tooth enamel, dentin, and pulp was analysed using FTIR-ATR. **Results:** The FTIR absorption spectrum reveals that carious teeth contained ester groups which is detected in higher amount when compared with sound tooth and also the intensity of absorbances increases with carious tooth. **Conclusion:** The study reveals that the ester group are important and unique to carious tooth and can be used as diagnostic tool in caries detection and for forensic evidence.

Keywords: Spectroscopy, Fourier Transform Infrared, Dental Caries

1. Introduction

The major components of tooth are made up of Enamel, dentin and cementum. Enamel is the most abundant and hardest tissue that has hydroxyapatite crystals, which is a calcium phosphate mineral and dentin is a complex structure that has highly mineralized nano crystallites and a protein matrix which is made up of type I collagen, this type of different composition and properties of tooth plays vital role in restorative dentistry[1]. FTIR-ATR method is widely practiced technique in infrared spectroscopy[2]. The sample preparation for this technique is quite easy, convenient and consistent results can be obtained[3]. In FTIR-ATR method the required sample is made contact with a sensing element and spectral image is obtained as a result of that contact[4]. Unlike the other alternative sampling techniques, radiation is no longer transmitted though the sample using infrared spectrometry; consequently, for the transmission of incident radiation the sample does not have to be very thin enough, with no band having an absorbance greater than 2.0AU (Astronomical Unit)[4].

2. Materials and Methods

2.1 Sample size

Totally forty tooth samples in both gender in different age groups are collected after extraction. Group A (n-10) non-carious extracted teeth of mandibular anterior teeth, Group B (n-10) early carious extracted teeth of premolars and anterior teeth, Group C (n-10) Advanced carious teeth of maxillary and mandibular molars, Group D (n-10) of Carious deciduous extracted teeth of maxillary and mandibular molars. Early carious extracted teeth had pit and fissure caries, advanced and deciduous tooth had smooth surface caries.

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2.2 Study Method

Ethical Clearance was duly taken from the Institutional Review Board.The Freshly extracted teeth samples were prepared, using sterile gauze to remove debris and soft tissue remained after tooth extraction has been removed. The extracted teeth has been stored in distilled water for 5 mins. Then the extracted teeth is air dried for 10 mins which has been sectioned into small block using diamond burs in cross section in parallel to the tooth. The small block of extracted teeth is placed on Arkansas stone then ground sectioned into 1-2mm thickness approximately and ground sectioned tooth samples has been collected in sterile container/ sealed packet. Processed tooth samples are kept in the diamond pointed plate in the FT-IR ATR (JASCO- 6600) before which has been sterilized using 70% of ethyl alcohol to avoid error and analysed for spectral analysis of tooth samples.

2.3 FTIR-ATR Spectroscopy

FTIR spectrometer (JASCO-6600) coupled to a microscope JASCO IRT-5200 and a Infrared spectrum are collected in the standard wavenumber range from 10cm^{-1} to 7800cm^{-1} at the resolution of 0.4cm^{-1} using halogen lamp and MCT detector (Figure 1a & 1b).

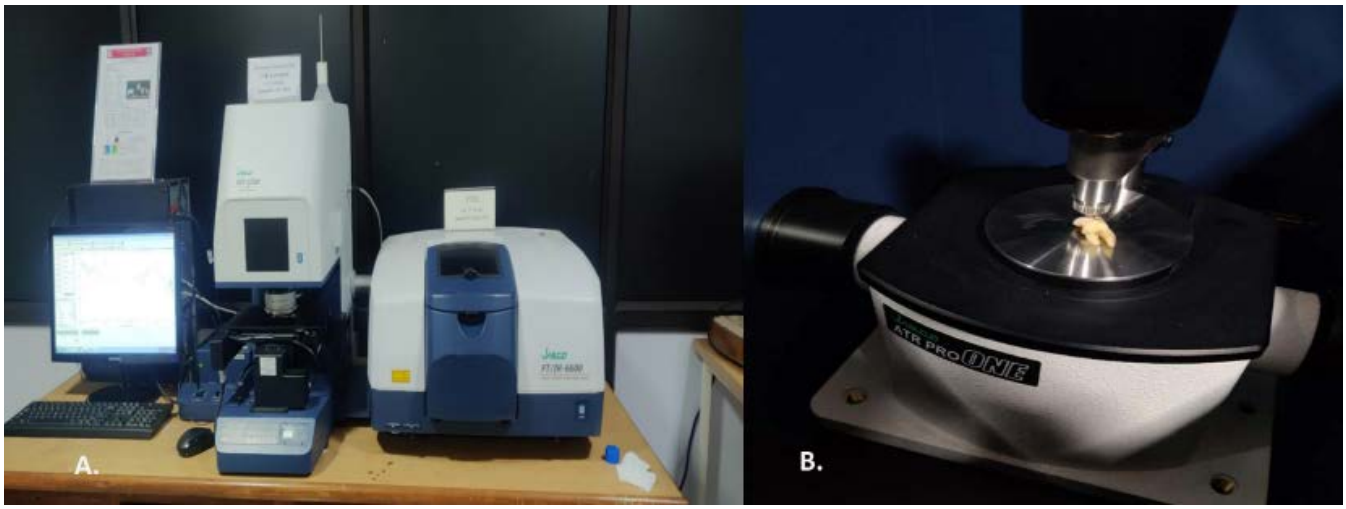


Figure 1. **a).** Stabilized tooth sample is analysed using FTIR-ATR (Fourier transform infrared spectroscopy), **b).** Sectioned tooth sample placed in FTIR.

Table 1. Intra-group and inter-group Analysis of spectral range using One-way ANOVA

ANOVA			Sum of Squares	Df	Mean Square	F	Significance
A) Peak1010		Between the Groups	.972	3	.324	5.125	.005**
	Within the Groups	2.276	36	.063			
	Total	3.248	39				
B) Peak2376		Between the Groups	.492	3	.164	4.393	.010*
	Within the Groups	1.344	36	.037			
	Total	1.836	39				
C) Peak2384		Between the Groups	.493	3	.164	4.367	.010*
	Within the Groups	1.355	36	.038			
	Total	1.848	39				

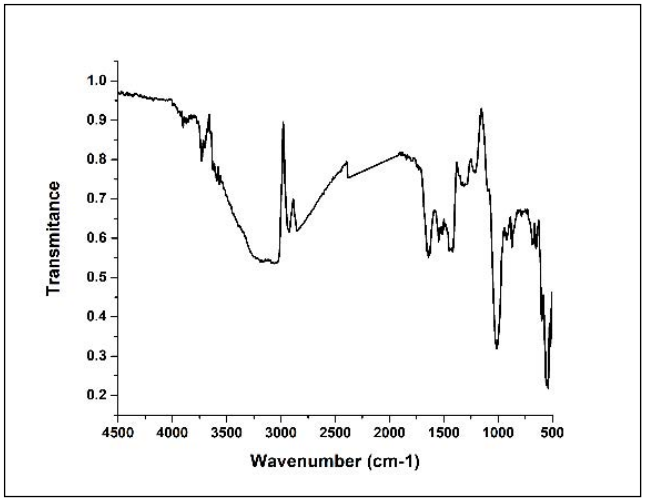
Infrared spectral region from 800-1200cm^{-1} is dominated by mineral bands whereas the region from 1200-1800cm^{-1} contains mainly collagen bands. Major FTIR peaks detected for amide I band is (1650cm^{-1}-1780cm^{-1}), phosphate vibration from (959-1030cm^{-1}) which were directly related to degree of mineralisation. Peak absorption was significant at 1010cm^{-1} which indicates that presence of amide band I and Phosphate group for sound tooth & peak 2376, 2384cm^{-1} indicates that presence of stretching of ester groups (C-H) alkanes in carious tooth.

3. Statistical Analysis

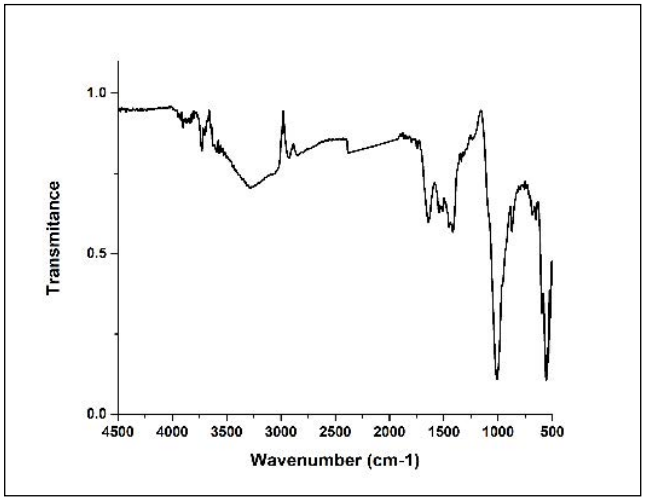
Since the data followed an asymmetrical data distribution non parametric tests were applied. Descriptive analyses were done using One-way ANOVA to know the difference between the mean of spectral values that is obtained from the four groups and Post hoc test for multiple comparison among the groups. The results were statistically significant at p-value<0.05 Analysis were done using SPSS Software 22.0.

4. Results

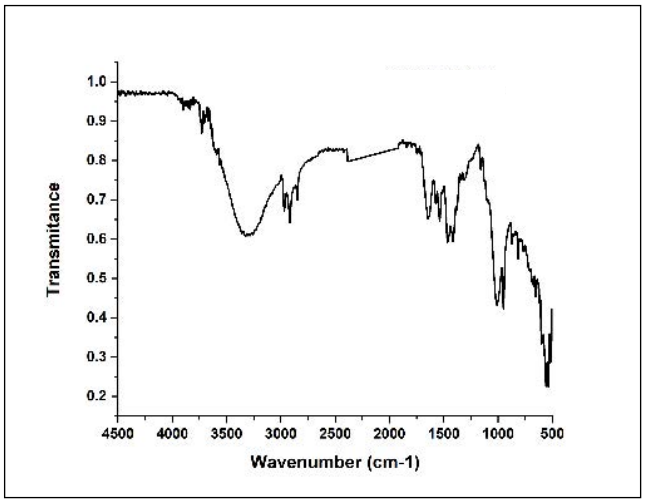
The results were significant <0.05 at three peaks 1010cm^{-1}, 2376cm^{-1}, 2384cm^{-1} when spectral range were analysed by One-way ANOVA (Table 1) & Post-Hoc test (Table 2). The demineralisation process which occurs in the carious tissue is the difference in the mineral/protein ratio found between sound and carious tooth. Peak values are significant at 1010cm^{-1}, 2376cm^{-1}, 2384cm^{-1} spectral band. (Figure 2) represents the graphical image of FTIR spectra recorded for each tooth samples of four different groups.



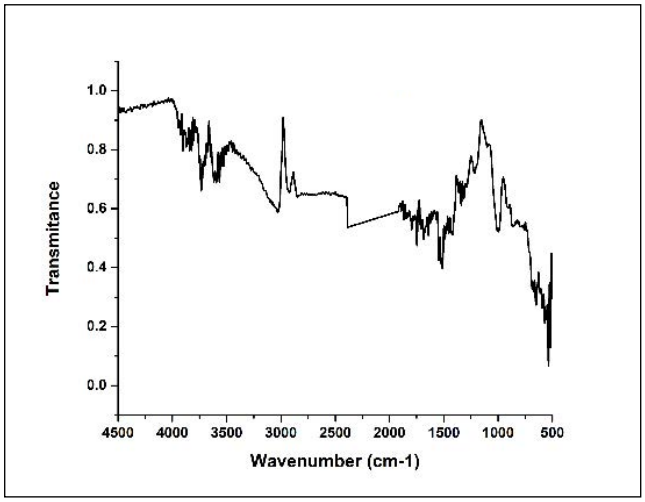
a. Advanced carious teeth.



b. Early carious teeth.



c. Deciduous carious teeth.



d. Non - carious teeth.

Figure 2. FTIR spectrum recorded in the 400-4500 cm^{-1} region. **a).** Represents spectra from tooth sample of Advanced carious teeth with Peak absorption at wavelength 2384 cm^{-1}. **b).** Early carious teeth with Peak absorption at wavelength 2376 cm^{-1}. **c).** Deciduous carious teeth with Peak absorption at wavelength 1750 cm^{-1}. **d).** Non- carious teeth with Peak absorption at wavelength 1010 cm^{-1}.

Table 2. Multiple comparisons made within groups at three significant peak value to assess the significance of differences between pair of groups. At peak 1010cm^{-1} significant differences present between non carious teeth and early deciduous carious teeth, at peak 2376cm^{-1} differences between non carious teeth, and early, deciduous carious teeth, at peak of 2384cm^{-1} advanced carious and non-carious teeth

Dependent Variable	Mean Difference (I-J)			Significance
	I group	J group	(I-J)	
peak1010	No Caries	Early Caries	.418294853*	.004**
		Advanced Caries	.207684218	Ns
		Deciduous Caries	.094820930	Ns
	Early Caries	No Caries	-.418294853*	.004**
		Advanced Caries	-.210610634	Ns
		Deciduous Caries	-.323473923*	.033*
	Advanced Caries	No Caries	-.207684218	Ns
		Early Caries	.210610634	Ns
		Deciduous Caries	-.112863289	Ns
	Deciduous Caries	No Caries	-.094820930	Ns
		Early Caries	.323473923*	.033*
		Advanced Caries	.112863289	Ns
peak2376	No Caries	Early Caries	-.276577980*	.015*
		Advanced Caries	-.216208060	Ns
		Deciduous Caries	-.260289834*	.023*
	Early Caries	No Caries	.276577980*	.015*
		Advanced Caries	.060369920	Ns
		Deciduous Caries	.016288146	Ns
	Advanced Caries	No Caries	.216208060	Ns
		Early Caries	-.060369920	Ns
		Deciduous Caries	-.044081774	Ns
	Deciduous Caries	No Caries	.260289834*	.023*
		Early Caries	-.016288146	Ns
		Advanced Caries	.044081774	Ns
peak2384	No Caries	Early Caries	-.277190584*	.015*
		Advanced Caries	-.216239676	Ns
		Deciduous Caries	-.260156820*	.024*
	Early Caries	No Caries	.277190584*	.015*
		Advanced Caries	.060950908	Ns
		Deciduous Caries	.017033764	Ns
	Advanced Caries	No Caries	.216239676	.058*
		Early Caries	-.060950908	Ns
		Deciduous Caries	-.043917144	Ns
	Deciduous Caries	No Caries	.260156820*	.024*
		Early Caries	-.017033764	Ns
		Advanced Caries	.043917144	Ns

5. Discussion

Carious tooth is shown to have an increase in the number of destruction and deformation of valence vibrations for N-C-O, N-H(amide) and C=O(carbonyl) bonds, there will be reduction in the crystallinity index and loss of orientation hydroxyapatite crystals within the affected enamel. This indicates the occurrence of destructive processes in the organic matrix of hard structure of tooth[5]. The clinical examination can only detect the visual deterioration of the tooth but cannot reveal the structural modification. So, such changes can be put on evidence only by microscopic methods of analysis as Fourier Transform Infrared Spectroscopy[6].

FTIR analysis identifies the vibration modes of the molecular bonds. As in previous studies the presence of carbonate group was higher in the deciduous teeth than the permanent teeth[3]. According to Infrared absorption frequencies for common functional Groups in the Infrared spectra the most intense band of peptide, which is known as amide I band a type of protein band, that is centred near 1500cm^{-1}-1660cm^{-1} range. The integration of band is determined in each band area instead of determining the band maxima. And studies showed that presence of amide band I and II, phosphate groups were present between the peaks from (235 cm^{-1}, 1450 cm^{-1} and 963 cm^{-1}) in non-carious tooth[7].

Energy dispersive X-ray spectroscopy also detects the advanced mineral-to-matrix ratio and rate which is harmonious with increase in calcium and phosphorous concentrations in permanent teeth than primary teeth[3]. Fourier-transform infrared spectroscopy a vibrational spectroscopy method that provide unique spectra for each element and molecules. The crystallinity index of phosphate group of enamel has been used in the analysis of various biological tissues for diagnosis and identification of fossiled traces of teeth[6,8-10].

In this study vibrational spectrum of the samples from enamel, dentin and pulp were measured in the 400–4000cm^{-1} spectrum. The demineralisation mechanism occurring in the carious tooth have difference in the mineral/protein ratio found between sound and carious tooth[2]. The observed absorbances between 1010cm^{-1} and 2384cm^{-1} for non-carious and carious tooth indicates the differences between these tissues. It is suggested that the increased absorbance at 2384cm^{-1} for the carious dentin is a consequence of a higher in the number of aliphatic side-groups of various amino acid residues and also presence of ester group around 1750cm^{-1} in carious teeth was slightly higher when comparing to non-carious teeth.

When comparing the peak differences between early and advanced carious teeth the peak values were at the range of 1750cm^{-1}-2384cm^{-1}, multiple comparison between these two groups using mean values were not statistically significant. peak values between non carious and early carious teeth shows slight variation in spectral range from 1010cm^{-1} to 1750cm^{-1} which are not significant statistically. Spectral range from 900cm^{-1} to 2000cm^{-1} were chosen for the analysis because only in this field the main features determining the nature of enamel, dentin can be observed[3].

In previous studies, ATR-FTIR spectroscopy has been used to evaluate the concentration of phosphate group in hydroxyapatite present in enamel and crystallinity characteristics to determine the degree of mineralization and demineralization process in the teeth[11] and also ester groups were found in carious tooth which can be used as diagnostic tool[11].

The Conclusion of the study is peak value of 1010cm^{-1}-2384cm^{-1} can be used as diagnostic spectral range for non-carious tooth and carious tooth. The presence of higher amount of ester as functional group in carious tooth when comparing with non-carious tooth plays a significant role in diagnosis of dental caries. Furthermore, studies are required with larger samples with defined chemical treatment of samples prior to analyses using ATR-FTIR for precise diagnosis and formulating treatment plan for dental caries.

Ethical approval: The study protocol approved by the Institutional ethical committee.

Vide Reference No:5/IERB/2021 TNGDC.

Competing Interests: Authors have declared that no competing interests exist.

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