MICRO RAMAN SPECTROSCOPIC ANALYSIS ON BLOOD SERUM SAMPLES OF DUCTAL CARCINOMA PATIENTS

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ABSTRACT

Objective: Identification of biochemical changes in ductal cancer patient's serum samples using micro Raman spectroscopy.

Methods: Micro Raman spectroscopy was used for the identification of Raman shift bands. Data analysis was done using K-means clustering.

Results: Micro Raman spectroscopic analysis of human breast cancer patient's serum samples was done. Biochemicals present in the samples were identified from the peak evaluations. K-means clustering analysis was used to differentiate the biochemicals present in the samples.

Conclusion: From the study, we conclude that Raman spectroscopy has the potential to differentiate the biochemical changes occurring in the human body, and the differentiation can be done using K-means clustering.

Keywords: Ductal cancer, Spectroscopy, Blood serum, Scattering.

INTRODUCTION

Raman spectroscopy is an optical technique based on inelastic scattering of monochromatic light, interacting with a biological specimen. While light beam is embattled on sample molecules, a minute portion of the sprinkled light undergoes shifts in its frequency. These Raman shift amplitudes correspond to the vibrations of intermolecular bonds. Each molecule has an exclusive vibrational energy state that corresponds to an explicit Raman shift. As such, the Raman spectrum is a direct function of the molecular composition of the samples, and functional and structural changes may be evidenced by a change in Raman optical signature. The wealthy information contained in Raman spectra may be sufficient to recognize molecular changes in a sample compared to reflectance or fluorescence spectroscopy. Every molecule has its own vibrational pattern that can provide as Raman marker to provide the significant biochemical information hidden in the sample [1-4].

Frontline of defense for the treatment of cancers is surgery. Intraoperative assessment of tissue or demarcation of tumor margins has relied on conventional histopathological analysis of biopsied tissue or physical inspection based on pre-operative images. These conventional techniques are defective for the discovery of minute or premalignant lesion which results later in tardy analysis and supplementary surgery. Raman spectroscopy is supposed to have the perspective to pick up the diagnosis of a large number of malignancies, together with a lot of common and lethal cancers [5].

Raman spectroscopic technique might sense the variations of molecular arrangement and chemical composition. Throughout the development of a tumor, considerable changes will occur in the configuration and accumulation of the main biomolecules which make up the cell and tissue, such as, carbohydrates, lipids, proteins, and nucleic acids. Since biochemical variations happen in advance, then in the clinical indication of manifestation and tumor medical imaging discovery, molecular spectroscopy shows the prospective early analysis of a tumor. Sharp peak characteristics, which are free from the interference of water fewer samples are required for sample chemical treatment. Raman spectroscopy is a promising tool to comprehend real-time and non-invasive discovery at the molecular level [6-16].

In the present study, we are trying to identify the biochemical changes occurring in the breast cancer patient's blood serum samples which will lead to the detection of various malignancies in the early stage.

METHODS

Preparation of sample
Protocol approved by the Human Ethical Committee at Karpagam Faculty of Medical Sciences and Research, Coimbatore (IHE/77/PHYSICS/03/2017), was followed for the procedures conducted. Serum samples from 15 ductal carcinoma patients before surgery and five normal persons were collected. All the serum samples were prepared by the same person with the same instruments.

Blood sample collection
Blood serum specimen was collected from 15 cases that were clinically diagnosed with cancer and five healthy volunteers. Serum was split from the blood by steadily stirring it with the use of a centrifuge at the speed of 1500 rotations/s. Samples were then stored at −80°C to avoid exterior interface. The use of the samples for investigation did not have an effect on the course of the operation or treatment of the patient.

For Raman spectral measurements of the serum sample, a drop of 40 μL was sited onto a silicon substrate positioned on the X-Y stage setting of a Witec microscope, attached to the Raman system. Raman spectrometer, bearing a laser wavelength of 785 nm having an irradiation power of 17 mW, was used. The laser beam was fixed to the plane of the specimen with a 100x point. Raman system was calibrated with a silicon semiconductor using Raman peak at 529/cm.

Processing of raw Raman spectra was done by carrying baseline modification, smoothing and normalization to eliminate noise, sample

fluorescence, and short noise from cosmic rays to be finally analyzed using IBM SPSS software.

RESULTS AND DISCUSSION

Baseline correction

Origin 6 software was used for the baseline correction and identification of the peaks. The fast Fourier transform filter setup was used for smoothing. Number of points considered for smoothing were five. After this baseline, correction was done and the peak's values corresponding to the peaks were identified. The resulting graph is given below.

Normal serum samples show high intensity peaks at 753, 844, 949, 1008, 1168, 1333, 1453, 1659, 1899 cm^{-1}, etc. Each peak indicates the presence of particular biochemical variation occurring in the human body (Fig. 1). The main band assignments found are explained, as 753 cm^{-1} corresponds to the presence of tryptophan and 844/cm represents the presence of tyrosine. At 949 cm^{-1} band for amide III is present and at 1008 cm^{-1} it shows symmetric C-C aromatic ring breathing, at 1168 cm^{-1} C-C stretch in amino acid, 1333 cm^{-1} CH$_2$ twist and bend, CH$_3$ deformation (protein/lipid), nuclide acid, 1453 cm^{-1} δ (CH2) bending, δ (CH3) out-of-phase, 1659 cm^{-1} C=O stretch in amide I, and amino compound I alpha-helix.

In ductal carcinoma serum sample, main peak assignments are 856, 908, 949, 1005, 1171, 1263, 1330, 1453, 1659, 1750, 1864, and 1899 cm^{-1}. 856 cm^{-1} corresponds to side chain vibrations of amino acid in proline and hydroxyproline as well as c-c vibrations of collagen backbone and tyrosine, 1005 cm^{-1} corresponds to phenylalanine, 1171 corresponds to C-C stretch amino acids, 1263 corresponds to N-H bending in amide III, 1330 cm^{-1} corresponds to CH2 twist and bend, CH3 deformation (protein/lipid), nuclide acid, and 1750 cm^{-1} C = O stretch (lipid). These are the main bands differentiated from normal serum samples (Table 1 and Fig. 2).

K-means clustering of serum spectral data

K-means clustering analysis was used to group the spectral data based on the similarity in forming clusters and region of similarity in biochemical properties. Biomolecular assignments considered for K-means clustering are protein, tryptophan, amide III, C-C stretch, NH amide III, and CH$_2$ twist.

From Graph 1, the presence of CH2 twist is predominant and the occurrence of NH amide III is seen. Even though NH amide III is absent in cluster 2, its presence in cluster 1 and 3 indicates the significance of NH amide III in the sample. f-values of protein (8.162), stretch (35.185), NH amide III (2.866), and CO stretch (12.328) show lower values in the normal samples than others in malignant samples. It indicates that among the four parameters all of them have a highly significant impact on the formation of clusters.

In malignant sample clustering, represented in cluster Graph 2, all the biochemical assignments show high f-values compared to normal that is protein (26.005), CC stretch (216.339), NH amide III (8.172), and CO stretch (11.438). This indicates all the parameters have less impact on the formation of the cluster. K-means clustering clearly differentiates between normal and malignant breast cancer samples.

From the literature survey, it is clear that the presence of proteins and amino acids is predominant in cancer patients when compared to that of the normal persons.

CONCLUSION

Analysis of blood serum samples of healthy persons and ductal breast cancer patients was done using micro Raman spectroscopy. Band assignments corresponding to the bands in the Raman spectra are identified. K-means clustering was used to find out the significance of biochemicals such as proteins, tryptophan, amide III, CC stretch, NH amide III, CH2 twist, and CO stretch of amino acids in the formation of clusters. From the study, it is clear that the malignant sample clearly differs from a normal sample. It is because all the biomolecules are in homogeneously distributed in the malignant sample.

AUTHOR'S CONTRIBUTION

All the authors have attributed directly to the planning, execution, or analysis of the work reported or to the writing of the paper.

ACKNOWLEDGMENTS

Table 1: Assignment of serum Raman spectra for fingerprint region

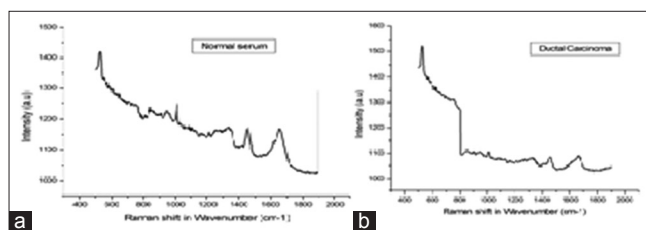| Wavenumber (cm^{-1})		
Healthy person	**Ductal carcinoma patients**	**Major assignments**
	578	Tryptophan
753		Tryptophan, Thymine
	761	Tryptophan
844		Tyrosine lipid
	856	Amino acid side chain vibrations of proline and hydroxyproline as well as c-c vibrations of collagen backbone, tyrosine
949	949	Amide III
	953	Protein (C-C stretch protein backbone, alpha-helix), lipid
	1005	Phenylalanine
1008	1008	Symmetric C-C aromatic ring breathing
1168		C-C stretch amino acid
	1128	Protein, lipid
	1171	C-C stretch amino acids
	1263	N-H bending in amide III
	1330	CH$_2$ twist and bend, CH$_3$ deformation (protein/lipid), nuclide acid
1333	1333	CH$_2$ twist and bend, CH$_3$ deformation (protein/lipid), nuclide acid
1453	1453	δ (CH$_2$) bending, δ (CH$_3$) out-of-phase
	1564	Guanine, adenine, tryptophan, and deformation mode of C=C
1659	1659	C=O stretch in amide I, amino compound I, alpha-helix
	1750	C=O stretch (lipid)

Fig. 1: (a and b) Unprocessed Raman plot of normal serum samples and ductal carcinoma serum samples, respectively
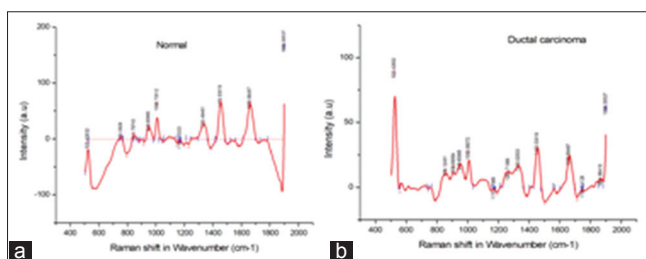

Fig. 2: (a and b) Raman shift to intensity plot of normal serum sample and ductal carcinoma serum sample, after baseline correction and smoothing

Graph 1: K-means clustering plot of normal serum sample

Graph 2: K-means clustering graph of ductal carcinoma patient serum sample
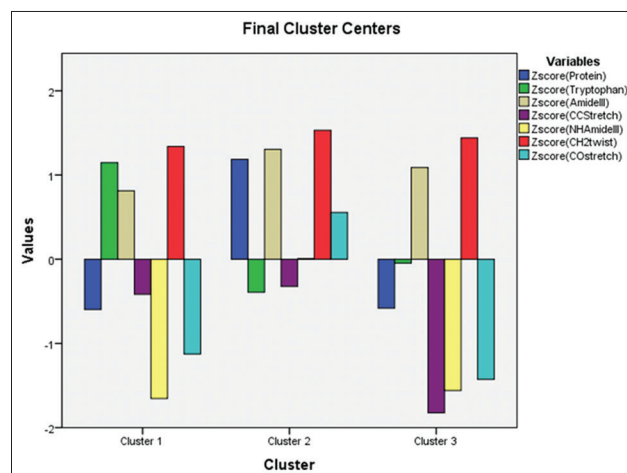
CONFLICTS OF INTEREST

We certify that regarding this paper, no actual or potential conflicts of interest exist. The paper is original, has not been accepted for publication nor is concurrently under consideration elsewhere, and will not be published elsewhere without the permission of the editor.
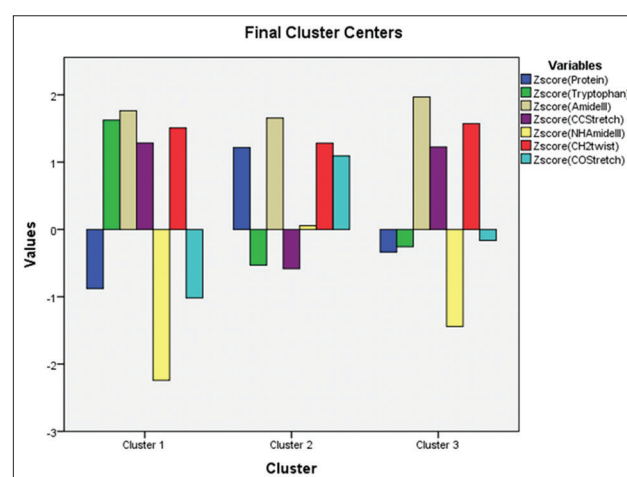
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