

Raman Spectroscopy of tissues Collected at Different Fractions of Radiotherapy: Response Assessment to Radiotherapy in Cervix cancers



*M.S.Vidyasagar¹, K.Maheedhar^{1,2}, B.M.Vadhiraja¹, Donald J Fernandes¹, V.B. Kartha² and *C. Murali Krishna²*

¹ Department of Radiation Oncology, Shirdi Saibaba Cancer Hospital, KMC, MAHE, Manipal - 576104

² Centre for Laser Spectroscopy, KMC, Manipal, Manipal – 576 104

E mail: pittu1043@gmail.com, krishna.murali@univ-remis.fr

Introduction

One of the leading cancer among women and accounts for 23% mortality (one fourth of global total are reported in India).
Radiotherapy - main stay of treatment for locally advanced stages (stage IIB and above)

- **RT Regimen** - External Beam Radiation Therapy of 45 Gy / 20 fractions / 4 weeks → 2 weeks rest → 2 doses of Brachytherapy, 8.5 Gy to point A region once a week → One month rest
- Clinical assessment of tumor response to radiation (Degree of tumor shrinkage) – only at the end of treatment

☞ (Radiation treatment spans over 4 months from the first fraction of RT)

- ☞ Radiation resistance is serious hurdle
- ☞ Tumors of same clinical stage and histological type may respond differently to radiotherapy

No established method to monitor the radioresponse

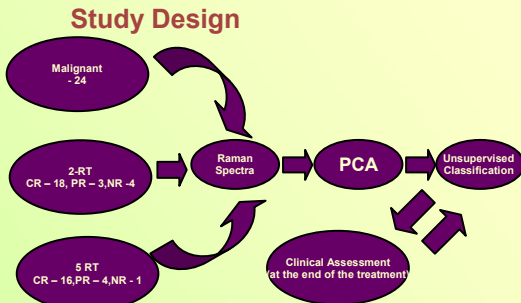
Optical spectroscopy methods (Fluorescence[1,2], FTIR [3] and Raman[4]) can discriminate cervical cancer.

Based on biochemical features rather than morphological features
Interpretation is highly objective
Minimally skilled technician

Our previous studies demonstrate the feasibility of classification between malignant and RT tissues as well as classification among CR, PR and NR

Objective

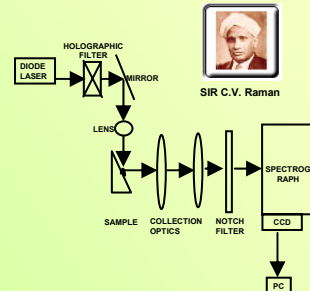
To explore the feasibility of the Raman Spectroscopic prediction of Radioresponse using samples collected at the early stage of treatment



Malignant - Samples collected before patient is exposed to Radiotherapy
2-RT - Samples collected 24 hours after patient is exposed to 2nd fraction of Radiotherapy
5-RT - Samples collected 24 hours after patient is exposed to 5th fraction of Radiotherapy

COMPLETE RESPONSE (CR) – 100% decrease
PARTIAL RESPONSE (PR) – ≥ 65% decrease
NO RESPONSE (NR) – < 65% decrease [6]

Experimental Set up



Raman setup

Excitation source – Diode laser (785nm wavelength)
Detection system – HR 320 Spectrum one liquid nitrogen cooled CCD
Accumulation time- 2 seconds for normal recording
30 seconds for a good spectra

Data Analysis

Data pretreatment: Spectra were baseline corrected, smoothed, calibrated and normalized to 8282 band. Pretreated spectra are subjected to Principal Components Analysis (PCA).

Classification of tissue types by PCA: PCA was carried out in unsupervised mode
Unsupervised analysis : Score of factor was discriminating parameter.

Results and Discussion

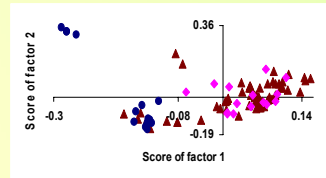


Figure 1. PCA of malignant tissues corresponding to 2-RT (▲complete response ♦Partial response ●No response)

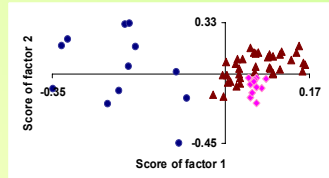


Figure 2. Classification of tissue types on the lines of 2-RT response using PCA - resulted in three clusters corresponding to ▲complete response ♦Partial response ●No response

No clear separation among the malignant spectra corresponding to three tissue types (CR, PR and NR) of 2-RT is observed. Though NR spectra exhibit some tendency of separation, it is out weighed by the overlap with CR spectra

Unsupervised analysis resulted in three cluster corresponding to CR, PR and NR. Score of factor 1 provided good classification between responding (CR and PR) and non-responding conditions. Score of factor 1 for NR spectra is largely negative compared to responding conditions. Based on score of factor 2, partial response spectra come out as a separate group from complete response spectra

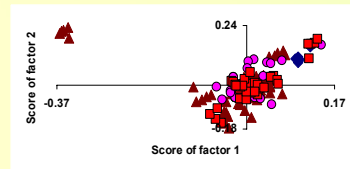


Figure 3. PCA of malignant tissues corresponding to 5-RT (▲complete response ♦Partial response ●No response ■ Response awaiting)

As in the case of 2-RT, here also no clear classification was observed among the malignant spectra corresponding to three tissue types of 5-RT (CR, PR and NR)

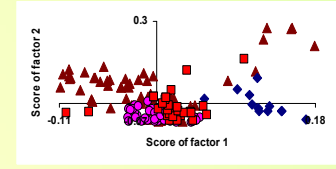


Figure 4. Classification of tissue types on the lines of 5-RT response using PCA (▲complete response ♦Partial response ●No response ■ Response awaiting)

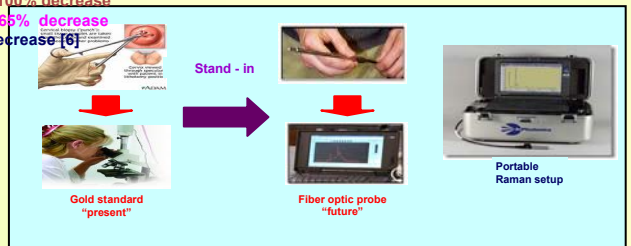
In this case also unsupervised analysis based on score of factor 1 produced clear classification between responding conditions. However analysis using spectra of 5-RT could not able to classify among responding conditions more clearly. Based on score of factor 2 some tendency of separation among responding conditions was observed. However clinical assessment of few more samples is still awaiting.

Conclusions and Prospective

This suggest that prediction of radiation response using malignant tissues may not be possible. Available data shows good correlation between clinical assessment and Raman spectroscopic prediction using 2 and 5-RT tissues.

This study demonstrates the feasibility of Raman spectroscopic prediction of tumor response to radiation therapy in cervical cancers

Prospectively study would be continued using 5-RT tissues to explore the feasibility of classification among responding conditions



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