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ORIGINAL ARTICLE

Diagnosis of Hepatitis C Virus Infection in human serum using ELISA and Raman Spectroscopy

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ABSTRACT

In this study we have presented the optical detection of Hepatitis C virus and molecular changes in human serum through partial least square regression vectors obtained from their Raman spectra. 140 samples tested through enzyme-linked immunosorbent assay and PCR for confirmation were used to create a model by utilizing spectral variations in the Raman spectra of the positive and control groups. Regression coefficients of this model were obtained and analyzed. The regression vector yielded by this model is utilized to predict hepatitis C in unknown samples. This model has been evaluated by a cross-validation method, which yielded a correlation coefficient of 0.91. Moreover, 30 unknown samples were screened for hepatitis C infection using this model to test its performance. From these calculations, accuracy, specificity and sensitivity of this model were determined to be 86.67%, 93.75% and 78.57% respectively. The value of area under the ROC curve was found to be about 1 which shows that the model is accurate.

Keywords: hepatitis C, partial least square (PLS) regression, multivariate analysis, Raman spectroscopy, ELISA

INTRODUCTION

Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver. HCV infection causes hepatic inflammation, liver damage and progressive fibrosis leading to cirrhosis and hepatocellular carcinoma (HCC). HCV is a bloodborne virus and transmitted by the use of contaminated syringes, intravenous drugs, tattooing, dental procedures and barber razor[1]. HCV has infected almost 170 million people world-wide.[2] HCV has infected approximately 10 million people in Pakistan.[3] HCV seropositive frequency is 4.7% in Pakistan which is significantly higher as compared to the neighboring countries[1].

Diagnosis is usually done first by Immunochromatographic Technique (ICT) and Enzyme Linked Immunosorbent Assay (ELISA). RNA or viral core detection through Polymerase chain reaction (PCR) remains method of choice for HCV diagnosis as this test becomes positive within days of infection. Although PCR for the detection of virus is the gold standard confirmatory test but it is not used commonly due to its high cost. The common diagnostic tools available for diagnosis of HCV infection are not reliable and those which are accurate are expensive and difficult to perform. There is a need for a test which would be much cheaper than ELISA and PCR, would not consume much time and easier to perform. For this matter, Raman Spectroscope is a device which makes use of changes in chemical structure of every molecule present in patient's serum to determine whether a disease is present in human serum or not.[4,5] This new modality would prove efficient, accurate, fast and cost effective diagnostic tool which is easy to perform and needs not such type of expertise as in PCR[6,7,8].

Light consists of photons which are mostly scattered from a molecule such that the wavelength of scattered photons is same as that of incident photons, it is called elastic scattering. But 1 out of 107 photons is scattered with wavelength lower or higher than the incident photons. This inelastic scattering is Raman effect or Raman scattering. Raman scattering occurs due to change in vibrational level[4]. Difference between wavelength of incident and scattered photon corresponds to the difference between vibrational levels. Energy levels of Raman Scattering Raman spectrum is a graph plotted between the number of scattered photons and the Raman shift which is measured in cm^{1}.[4].

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MATERIALS AND METHODS

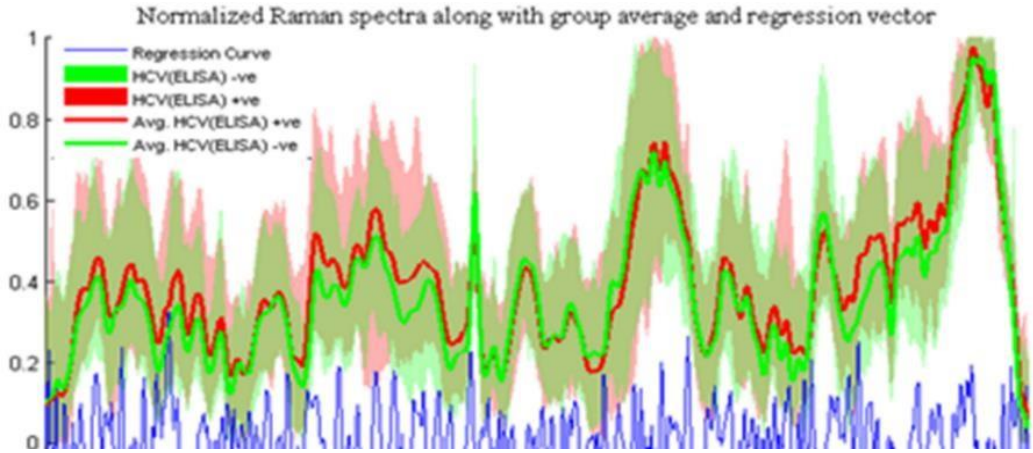
The study was conducted from April 2019 to October 2019 at Pathology Laboratory at Holy Family Hospital / University of Health Sciences Lahore / Biophotonics Laboratory National Institute of Lasers and Optronics (NILOP) after getting approval from Ethical Review Committee and Advance Studies and Research Board. Sample size was calculated to be one hundred and forty. Virus and antibody confirmed HCV positive and negative cases were selected as controls. Raman spectra, from a drop of each serum sample were obtained using a Raman spectrometer (PeakSeeker Pro™ by AGILTRON). The Raman Systems Microscope (RSM) is coupled with Raman system spectrometer in an upright position[4]. From a 10X magnification objective lens a 50mW diode laser of 785nm wavelength is focused on the dry sample surface. To avoid degradation of sample the power of laser is carefully optimized. The exposure time for recording each Raman spectrum is 20 seconds between 200 to 2000 cm^{1}. [4]

Statistical Analysis: Partial least squares regression was used to predict responses (clinical results) from variables (Spectrum). This model described the correlation between the reference clinical results through ELISA and the predicted results from the multivariate model. The correlation between the clinical results and the predicted results was measured by r 2 value. r 2 > 0.9 is accepted clinically[10.]

RESULTS

Multivariate model was developed by teaching the Raman spectra of positive and negative controls to the model. Leave-one-sample-out (LOO) method of cross validation was used to develop the multivariate model. This method of cross validation was repeated in case of all the samples which were used to train the model. Left out sample was then predicted. This process was repeated as many times as there were samples.

Red: HCV Positive
Green: HCV Negative



Comparison of Raman spectra of HCV infected and healthy sera samples have been plotted in overlap manner to compare the differences in the intensities of various Raman bands in both groups. For convenience a shaded region has been displayed to show the variation range of spectra of corresponding groups. A bold solid line is indicative of the average of all the spectra of corresponding group. These averages show a clear indication of the variations of chemical composition of blood in subjects of both groups. To identify the relation of these variations with infection, we have plotted PLS regression coefficients in blue color. Positive coefficients indicate that biological molecules reported at these Raman bands have been found in high concentration in HCV positive subjects as compared to healthy ones. Similarly negative regression coefficients indicate that biological molecules are reported at these Raman bands have lowered their concentration due to HCV infection as compared to healthy ones.In testing process, it was found that out of 14 ELISA based positive samples our model predicted 11 samples as true positive while 3 were predicted false negative. Moreover, it was found that out of 16 ELISA based negative samples our model predicted 15 samples as true negative while 1 sample was predicted false positive. From these calculations, accuracy, specificity and sensitivity of this model were determined to be 86.67%, 93.75% and 78.57% respectively. The value of area under the ROC curve was found to be about 1 which shows that the model is accurate.

DISCUSSION

Raman Spectroscope is a device which makes use of changes in chemical structure of every molecule present in patient's serum to determine whether a disease is present in human serum or not.[6] PLS-based multivariate regression is used in Raman spectroscopy because it includes the whole spectra instead of prominent peaks and hence minute spectral variations can be picked which are important for the prediction of disease process [4,7]. Model was fed Raman spectra of positive and negative controls to develop multivariate model. LOO cross validation method was used for authenticating the model.13 This method was repeated for all the samples used to train the model and then left out sample was predicted. This process was repeated for all the samples.[11,12]. The calibration curve is formed by the multivariate model which fitted with the predicted values of LOO cross validation.[14]

CONCLUSION

This study is unique in its nature which is based on the PLS regressionon the basis of Raman spectra and hence performance of its results cannot be compared to any study inliterature. Detection of chemical and conformational change in molecules in sample with high sensitivityand selectivity has been made possible through it.This approach has beenused first time in Pakistan for HCV detection and our basic purpose is to introduce this technique for clinical practices because it is an ideal technique for studying biological and chemical samples inaqueous solutions.

Conflict of interest: Nil

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