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A Vibrational Spectroscopic Method for Detection of Rheumatoid Arthritis using Bodily Fluids

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Rheumatoid arthritis (RA) has a significant level of clinical variability and is also diagnosed based on a variety of clinical factors. The absence of bio-marker prediction approach makes prompt identification and therapeutic diagnosis of these individuals difficult. With the advent of targeted medicines, it has become increasingly crucial to diagnose RA early in order to provide safe and timely disease management that can minimize long-term consequences that include joint tissue damage. Raman spectroscopy is currently gaining clinical acceptability as a label-free, non-invasive tool for obtaining a thorough biochemical signature of biological sample composition. The purpose of this research was to look at using confocal Raman spectroscopy in conjunction with statistical data analysis as an auxiliary or supplementary tool for completing the diagnostic process of RA using peripheral blood serum. Raman intensities and Functional group frequencies are assigned to amide chains in proteins and other intermediate structural components such as lipids of a normal individual and a RA patient were distinguished, and biochemical alterations were found. Experimental results shows that spectral shift in region I and region II in Figure 1 identifies the concentration of nucleic acids and TNF-α increases respectively, which causes inflammation in our body that initiates RA disease.

Keywords: Rheumatoid arthritis; Raman spectroscopy; Nucleic acids; TNF-α

1 Introduction

Rheumatoid Arthritis (RA) is a common autoimmune disorder characterized by chronic inflammation of unknown origin that primarily impacts the joints including extra articular areas[1-4]. RA affects between 0.5-1.5% of the global population between the ages of 40 and 60, and it affects women more than males. RA is a progressive illness with articular, inflammatory and generalised symptoms. The severity of RA symptoms varies widely amongst people, ranging from extremely minor to severe. RA presents clinically as Synovial inflammation is distinguished by joint discomfort and swelling, as well as persistent early morning stiffness[1-5]. RA is also known as auto- immune disease that affects directly the bone marrow of our functioning immune system. The biochemical pathways that carry inflammatory signals from tissue that is infected enhance monocyte migration from the bone marrow. Various monocytes develop into phagocytic macrophages and entered the lymph nodes with dendritic cells (DCs). Now interaction of antigen presentation cells (APCs) like DCs and naive T-cells improves the reactive immune response to pathogens[6,7].

The challenges in the clinical identification of RA are mostly attributable to the disease's complexity. Unfortunately, most instances of RA are only discovered in their later stages. The absence of a single biomarker prediction approach makes prompt identification and treatment of these individuals difficult. With the introduction of targeted medicines, it has become more crucial to appropriately diagnose RA at the beginning stages of disease crucial to appropriately diagnose RA. Sensitive and precise diagnostic procedures that allow for simple differentiation between the disease and healthy individuals, as well as distinguishing RA from illnesses with overlapping symptoms, are thus important for reliable diagnosis and long-term therapeutic success[8]. The grading of joint swelling and pain examinations, as well as blood tests for evaluating inflammatory levels, are constituted for determination of biomarker.

Overall, our literature review show that Raman spectroscopy may be a useful diagnostic technique for distinguishing between inflammatory and autoimmune illnesses with similar clinical symptoms. The purpose of this study was to look at the use of Raman spectroscopy for diagnostic tool of RA using blood serum from healthy individuals and RA patients.

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2 Materials and methods

The Blood serum samples of RA-affected individual and healthy persons were collected from Kailash Hospital, Noida in EDTA anti-coagulation coating tube. It prevents the coagulation of the blood sample. The blood sample was centrifuged for 5 minutes at room temperature at 1500 rpm to divide the blood sample in plasma, buffy coat and RBCs. The buffy coat is extracted and a very thin smear of the sample on the glass slide is prepared. In the healthy and affected male of age group 50-60 years without related chronic diseases were sampled for the study.

The Raman spectrum acquired using a confocal system in Via WITec alpha300 RA Raman spectrometer (Oxford instruments, Ulm, Germany) equipped with a charge coupled device (CCD) detector. A laser of wavelength 532 nm with a power output of 0.5- 10 mW was used to excite the serum sample. Each sample has four spectra taken for 3.09 seconds with twenty accumulations. The lateral resolution 200nm and spectral resolution down to 0.1 relative to wavenumbers. Scan range 100×100×200 μm^3 piezo stage. Because the dried sample was not homogeneous, the drop was imaged at numerous spatial locations to capture the whole heterogeneity of the sample.

Total of five spectra recorded for RA individual as well as healthy controls. All spectra were processed in the MATLAB[8]environment.

3 Results and Discussion

Fig. 1 depicts the mean Raman spectrum for a healthy individual (N) and a RA patient (RA). It reveals modest changes in the chemical structure of both groups' sera. Fig. 1 have the spectral region markers separating as follows: I) 775-900 cm^{-1}, II) 901-1000 cm^{-1}, III) 1011 -1090 cm^{-1}, IV) 1100- 1198cm^{-1}, V) 1200-1390 cm^{-1}, VI) 1401 - 1500cm^{-1}; VII) 1501-1751 cm^{-1}. We have observed various changes in biochemical composition in RA and N individuals. These changes are associated with different groups.

The changes in Regions II, III, and V are displayed in Fig. 1.

A covariance matrix was used to do the main components analysis in a range of 775 cm^{-1} to 1751 cm^{-1}. Here fig. 2(a) shows principal component analysis of our total data. This signifies the differentiation between RA affected individual and healthy control. Fig. 2(b) shows an execution plot for the initial three PCs, which represent PC variation as a function of Raman shift. It is showing the comparison of both PC of RA and N. PC1, PC2 and PC3 shows the variability between two categories of samples RA and N. Changes in Regions I, IV, VI, and VII are of statistical significance and are connected to RA in a unique way.

3.1 Region I (775 -900 cm^{-1})

This category includes compounds such as lipids, glucose, triglycerides, tryptophan, creatinine, cholesterol, albumin, and tyrosine[9,10]. Tyrosine is involved in the pathogenesis of RA, and tyrosine inhibitors help to reduce symptoms and progression of the disease[11]. A comparison shows that the strength of the Raman band in the area (775-900 cm^{-1}) increases which corresponds to nucleic acids and lipids. The increase in intensities of lines corresponding to nucleic acids is most likely due to the inflammation-induced denaturation of DNA initiated by the cytokines[12].

3.2 Region IV (1100 - 1198 cm^{-1})

This region has amide III bands consisting of ν(Cβ–methyl), ν (pyr half-ring) asym, ν (pyr half-ring) asym. Studies about deoxygenated blood show that it involves in the pathophysiology of RA[12].

3.3 Region VI (1401 - 1500 cm^{-1})

This region has the contributions of the albumin mode[13]. Albumin is involved on the transport process of metal ions, fatty acids, bilirubin and drugs in the blood. Changes in vibrational modes of region VI may be due to medications used by patients with RA.

3.4 Region VII (1501 -1751 cm^{-1})

This region has the contribution of amide I vibrational modes. The amide I usually reflects hydrogen bonding in the various secondary structural elements in proteins. Corresponding to the amide I

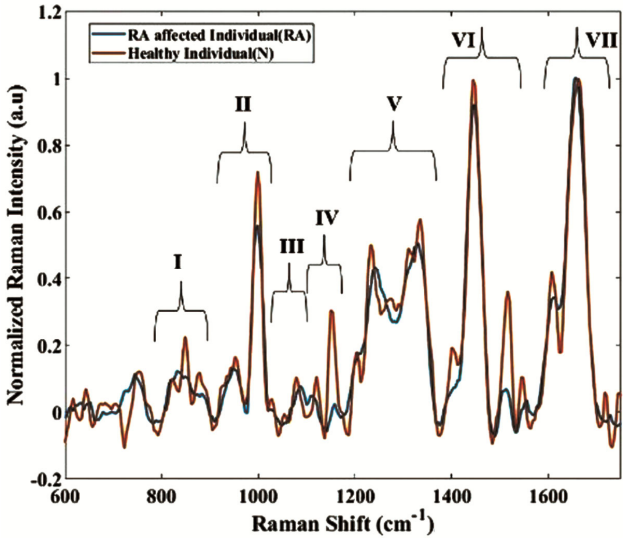

Fig. 1 — Raman spectra averages of Normal and RA affected individuals.

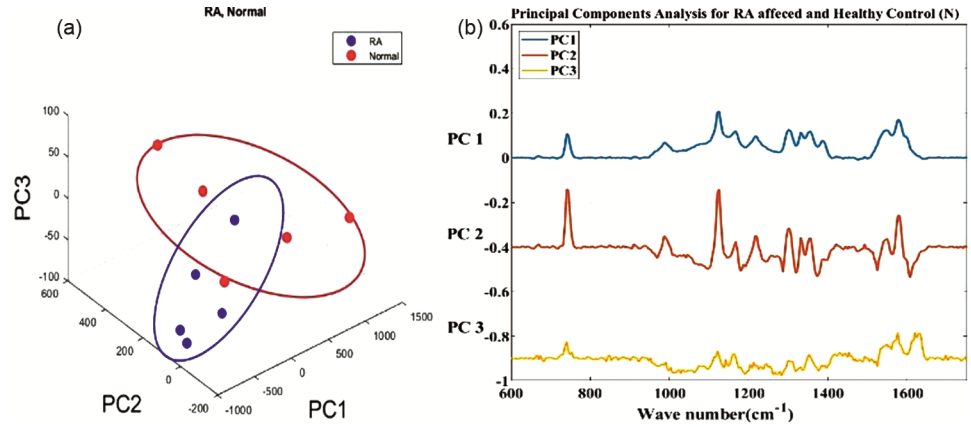

Fig. 2 — (a) The initial loading 3D plot the entire data (b) PCA plot for the first three major principal components of the entire data for each group.

region, PC 2 shows there is a change due to biochemical compounds by change of derivative of the initial graph on the addition of lipids to albumin which reflect not only changes in the hydrogen bonding but also of the presence of changes in the secondary structural elements.

4 Conclusion

This study has demonstrated that Raman spectroscopy can differentiate RA from healthy individual by bodily fluid analysis. Experimental results shows that hyperactive immune response and auto antibody synthesis connected with pro inflammatory cytokines such as TNF-α[14-16], is prominent (Region I) in Raman spectrum of RA person. Due to changes in the molecular and structural composition and chemical entities, the principal component analysis findings demonstrated that this approach is particularly promising for application in the diagnosis of rheumatic illness. We need further optimization processes to identify and presence of TNF-α concentration in our body. The presence of biomarker can be identified using nanoprobe-based bio-sensors in future.

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