

Lactate based optical screening of dengue virus infection in human sera using Raman spectroscopy

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Abstract: This study presents the screening of dengue virus (DENV) infection in human blood sera based on lactate concentration using Raman spectroscopy. A total of 70 samples, 50 from confirmed DENV infected patients and 20 from healthy volunteers have been used in this study. Raman spectra of all these samples have been acquired in the spectral range from 600 cm^{-1} to 1800 cm^{-1} using a 532 nm laser as an excitation source. Spectra of all these samples have been analyzed for assessing the biochemical changes resulting from infection. In DENV infected samples three prominent Raman peaks have been found at 750, 830 and 1450 cm^{-1} . These peaks are most probably attributed to an elevated level of lactate due to an impaired function of different body organs in dengue infected patients. This has been proven by an addition of lactic acid solution to the healthy serum in a controlled manner. By the addition of lactic acid solution, the intense Raman bands at 1003, 1156 and 1516 cm^{-1} found in the spectrum of healthy serum got suppressed when the new peaks appeared around 750, 830, 925, 950, 1123, 1333, 1450, 1580 and 1730 cm^{-1} . The current study predicts that lactate may possibly be a potential biomarker for the diagnosis of DENV infection.

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1. Introduction

DENV infection is the most widely spreading mosquito born disease and become a major public health concern affecting millions of people world wide [1]. Dengue virus is transmitted to human via *Aedes* genus, especially *Aedes aegypti*. It is an enveloped positive single strand virus which belongs to the family of flaviviridae. This family also includes Hepatitis C Virus, West Nile Virus and Yellow Fever Virus. Primary infection by any serotype leads to moderate severity and short immunity against all serotypes whereas lifelong immunity to the specific infecting type. However, subsequent 'secondary' infection by other serotype may lead to life threatening severe disease. In Asia, the risk of severe dengue infection is greater in

children (≤ 15 years) than in adults [2–5]. Common symptoms of dengue infection include high fever, severe muscles/bones pain, headaches, nausea and vomiting. In most cases dengue patients recover from the infection by taking rest and maintaining body fluids. In the advance stage, dengue fever (DF) transfer to its severe life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) [6–10].

Currently there is no specific treatment for dengue, but proper medical care can prevent lives. An early and efficient diagnosis of dengue virus infection is of critical importance for treatment as well as better management of the disease [11–13]. Cells and tissues are highly complex structures made of proteins, nucleic acids, polysaccharides, lipids, vitamins and other components that form the molecular compounds. In almost all diseases, initially changes occurred at cellular level that successively proceed. Different types of laboratory tests such as polymerase chain reaction (PCR) and ELISA are used for viral detection and monitoring of associated biochemical changes respectively. These techniques are quite good but also have some short comings. Since PCR is used for direct detection of virus, so it is effective only in the initial five days after an onset of the disease [14]. Likewise it is expensive, require high level of technical expertise and time consuming (almost a week). The ELISA test which are routinely performed for dengue infection diagnosis is based on immunoglobulin M & G (IgM & IgG) concentration, are comparatively fast and can differentiate between primary and secondary infection. At the same time ELISA have the problem of false positive results which limit its use for monitoring of patients at mass scale.

In recent years, spectroscopic techniques have attracted great interest in the field of biomedical diagnosis because the light that scattered, reflected or passes through the sample provided useful information about the samples. Raman spectroscopy has proved itself an emerging tool for the characterization of biochemical changes in biological samples and achieved significant progress in early detection of different diseases [15–17]. Raman spectroscopy is an inelastic scattering of light by the sample based on their molecular vibrations. Thus, a Raman spectrum provides a fingerprint from which information about molecular composition can be obtained that forms the basis for the diagnosis of the infectious diseases. Our group is standardizing Raman spectroscopic technique for the early diagnosis of dengue infection in recent years [18].

In this article, we are presenting the diagnosis of dengue virus infection in human blood sera based on increase in lactate concentration using Raman spectroscopy. For strengthening our claim, different concentration of lactic acid solution have been added to normal/ healthy sera. The changes occurred in the composition of serum sample have been monitored accordingly. This will be quite helpful in the early diagnosis of DENV infection which is of prime importance for management of the disease.

2. Material and methods

2.1 Sample collection and preparation

In total 70 samples of different age and genders have been used in this study. Among these, 20 samples were from healthy volunteers whereas, 50 were obtained from DENV infected patients. All these samples have been acquired directly from Holy Family Hospital, Rawalpindi, Pakistan in dengue endemic months of September and October 2015. Initially all samples were centrifuged at 3500 rpm for 10 minutes using Hittich Centrifuge D-7200 for serum extraction. The obtained sera were put in micro-centrifuge tube and stored at -16°C till further use. In one part of healthy sera two different concentration 50 mmol/L and 100 mmol/L of lactic acid solution (L 1250, Sigma-Aldrich Chemie GmbH, Germany) were prepared in control manner for observing their effects. The sample collection, preparation and storage procedure is same as mentioned in our previous article [18]. The overall experimental procedure has been carried out after obtaining written approval from the ethical committee of Rawalpindi medical college (RMC). The standard safety rules have been followed during all procedure [19].

2.2 Acquiring Raman spectra

Raman spectra from DENV infected sera, healthy sera, lactic acid solution and lactic acid solution mixed with healthy sera were acquired using Raman spectroscopy. Spectrum acquisition procedure is same as mentioned earlier [20]. About 15 μl of each sample has been put on the glass slide and left for some time at room temperature for water moisture to vaporize. The schematic diagram of experimental procedure is shown in Fig. 1. A Raman spectrometer ($\mu\text{Ramboss DONGWOO OPRTON}$, South Korea) with a spectral resolution of 4 cm^{-1} was used for acquiring Raman spectra from all samples. A laser diode emitting continuous laser beam at 532 nm has been used for the excitation. The measured laser power at the sample surface was 40 mW. A microscope objective having a magnification of 100X has been used both for focusing the light on the sample and collection of backscattering light. An acquisition time of 10 seconds has been used for recording each spectral data. A spectral range from 600 cm^{-1} to 1800 cm^{-1} has been selected for recording Raman spectra as it contained most useful information.

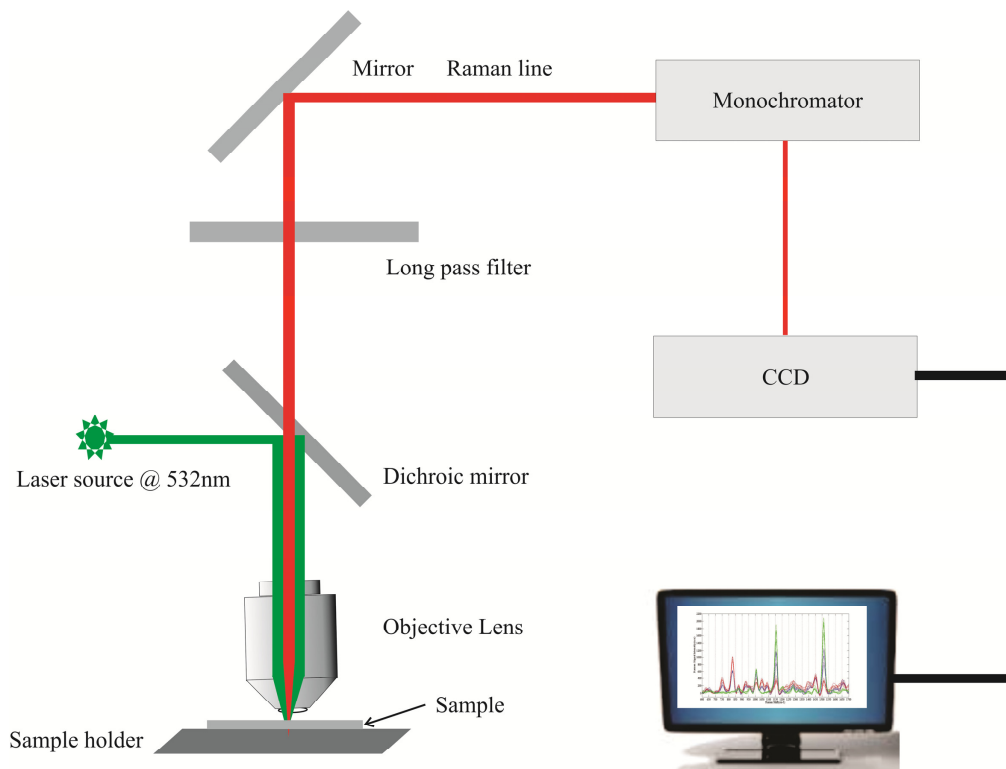


Fig. 1. Sketch of experiment setup.

2.3 Raman spectral analysis

Raman spectra of biological samples always contain background noise. Therefore, all spectra have been processed in Matlab environment to improve signal to noise ratio keeping the integrity of inherently weak Raman peaks in the spectra. Moreover, all Raman spectra have been smoothed using 'Savitzky-Golay' filter with five points and 3rd order polynomial fitting. In general, the preprocessing procedure is similar as mentioned previously [20]. Figure 2 shows the mean vector normalized Raman spectra of healthy and dengue infected sera as well as the mean difference between the normal and infected samples. Normalization has been done to eliminate systematic differences among measurements such as differences in focusing depth, sample volume differences etc. In healthy sera three intense Raman peak

appeared at 1003, 1156 and 1516 cm^{-1} which are always reproducible. In dengue infected samples Raman peaks appeared at 750, 830, 925, 950, 1003, 1123, 1156, 1333, 1450, 1516, 1580, 1680 and 1730 cm^{-1} . Furthermore, obvious differences between the normal and dengue infected samples appeared at 750, 830, 925, 950, 1003, 1123, 1156, 1450, 1516, 1580, and 1730 cm^{-1} as can be seen in the difference plot. The detail assignment of most of these Raman peaks has been given previously [21].

Figure 3 shows the vector normalized Raman spectra of lactic acid solution with two intense Raman peaks at 830 cm^{-1} and 1450 cm^{-1} along with some medium intensity peaks at 750, 875, 925, 1040, 1075 and 1730 cm^{-1} . Furthermore, Fig. 4 is the vector normalized mean Raman spectra of healthy blood sera, dengue infected sera as well as two different concentration of lactic acid solution mixed with healthy sera. For an obvious differentiation, Raman spectra of healthy sera samples are shown in green color, dengue infected in red color, whereas lactic acid solution mixed with healthy sera are shown in blue color (50 mmol/L) low concentration and magenta color (100 mmol/L) high concentration.

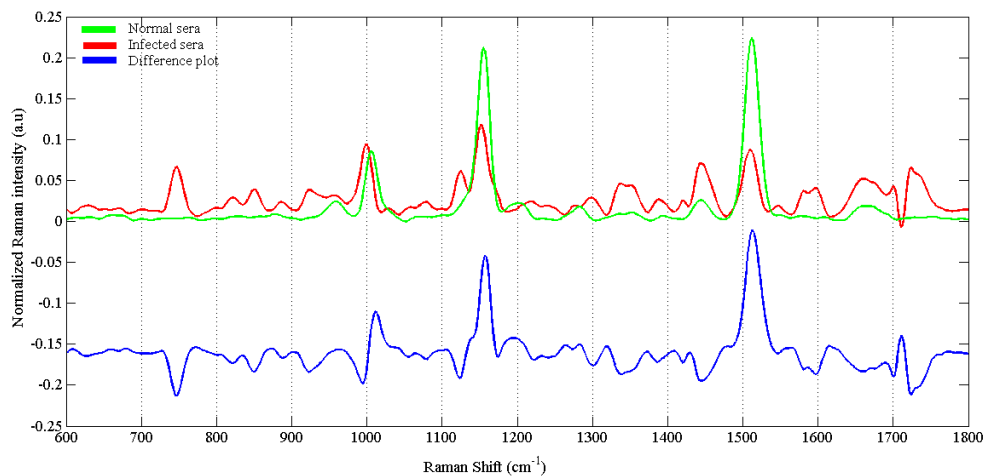


Fig. 2. Vector normalized mean Raman spectra of healthy and dengue infected sera (upper) along with the mean difference between the normal and infected samples (lower).

3. Results and discussion

The Raman peaks appeared in normal human blood sera have been explained previously [18, 21]. Raman peak at 1003 cm^{-1} has been assigned to symmetric ring breathing mode of phenylalanine and β -carotene, whereas the peaks at 1156 and 1516 cm^{-1} have been assigned to β -carotene [17, 22, 23]. These Raman peaks are highly reproducible. In DENV infected sera these three peaks are either suppressed or its intensity decreased whereas new peaks arised at different frequencies. In dengue infected sera, additional Raman peaks appeared at 750, 830, 925, 950, 1123, 1333, 1450, 1580, 1680 and 1730 cm^{-1} as shown in Fig. 2. The main contribution to these peaks are most probably corresponds to high concentration of lactate in DENV infected sera.

In human body, lactate is produced continuously mostly in muscles. It is then transported to different metabolic organs via blood which regulates them [24–26]. Liver is considered to be the key organ that converts blood lactate into pyruvate. Around 50-70% of blood lactate is extracted in liver and converted into pyruvate [23]. Additional amount of lactate is cleared by the kidney and some other organs. In normal conditions, with adequate tissue perfusion, conversion of pyruvate to AcetylCoA is occurred largely, bypassing lactate production. In tissue hypoxia/hypo-perfusion, lactate is produced as an end product of pyruvate in the presence of lactate dehydrogenase enzyme. Lactate exists in two isoforms, L-and D-lactate, such that L-lactate is the primary isomer produced in human body.

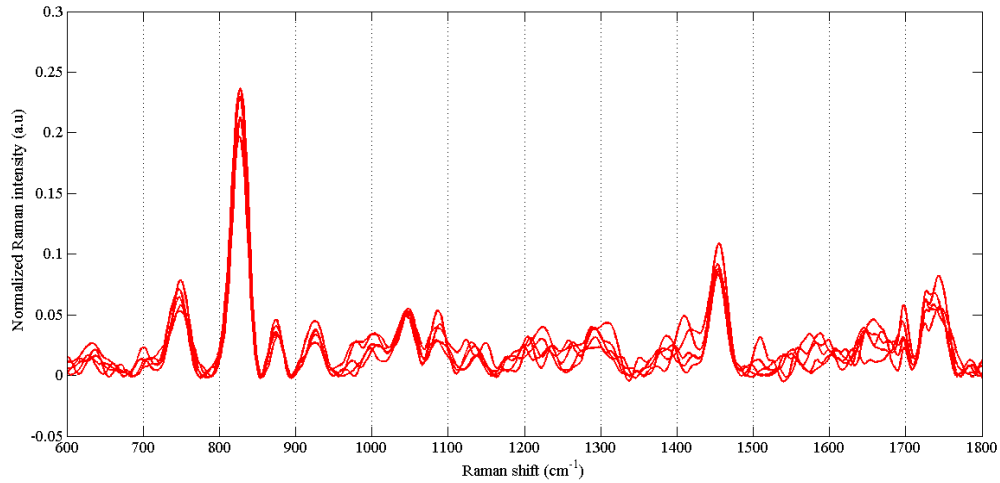


Fig. 3. Vector normalized Raman spectra of lactic acid solution.

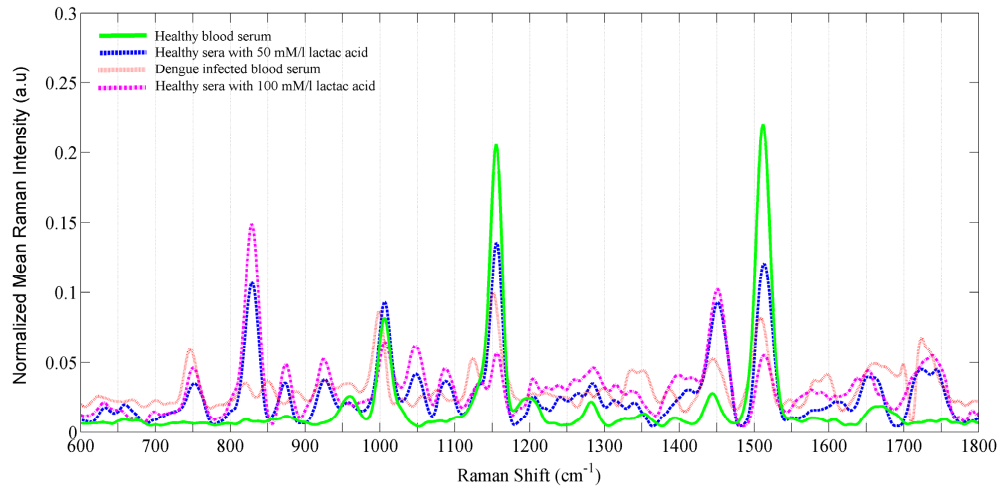


Fig. 4. Vector normalized mean Raman spectra of healthy blood sera (green), dengue infected (red), low and high concentration of lactic acid solution added to healthy sera (blue) and (magenta) respectively.

The biochemical impact of dengue virus infection on the function of various body organs like liver, kidney, lungs, heart etc. as well as elevated level of lactate is well established and reported [27–33]. As stated earlier, liver and kidney are the two main organs in the human body which regulate the lactate level. Changes in hepatic oxygen supply and intrinsic hepatic disorder affect the capacity of the liver to metabolize lactate. In such condition, liver becomes a lactate producing organ rather than using it for gluconeogenesis. Hence, due to dysfunction of these important body organs in dengue infection, blood lactate level increases. A good agreement has been observed by comparing the Raman peaks of lactic acid solution (Fig. 3) and dengue infected sera samples (Fig. 2). More precisely, Raman peaks close to 750, 830, 925, 1123, 1450 and 1730 cm^{-1} are appeared both in lactic acid solution as well as DENV infected samples. Furthermore, a slight blue shift at wave number 1003 cm^{-1} occurs in dengue infected samples. What might cause this shift in dengue is not clear, but it could be due to somewhat different protein composition next to phenylalanine and β -carotene.

For the observation of lactate effects on normal blood sera, two different concentration of lactic acid solution (50 mM/L and 100 mM/L) has been added to healthy sera in controlled manner and their Raman spectra have been recorded as shown in Fig. 4. A gradual decrease in the intensity of the aforementioned three peaks has been observed with increase in the concentration of lactic acid solution in the sera samples as clearly visible in Fig. 4. So we can say that, apart from the carotenoids deficiency in dengue virus infection as described earlier [18], the suppression of Raman peaks at 1003, 1156, and 1516 cm^{-1} in healthy sera may also be attributed to elevated lactate level in the blood. One can use the appearance of lactate in the blood sera as valuable indicator for the presence of disease. In order to evaluate lactate as a potential biomarker for dengue diagnosis, in depth studies will be necessary.

4. Conclusion

This article presents lactate based detection of dengue virus infection in human blood sera using Raman spectroscopy. In dengue infected samples Raman peaks appeared at 750, 830, 925, 950, 1123, 1333, 1450, 1580, 1680 and 1730 cm^{-1} . In dengue infected samples, the Raman peaks close to 750, 830, 925, 950, 1123 and 1450 cm^{-1} are most probably showing elevated lactate level which arises due to the impaired function of important body organs like liver, kidney, lungs etc. Furthermore, it has also been shown that in dengue virus infection the Raman peaks at 1003, 1156, and 1516 cm^{-1} suppress most probably due to elevated lactate level in the blood. The research work in our laboratory is still in progress and efforts are underway to provide alternate and an efficient tool that might help in early detection of different diseases.

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