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Discrimination of Malaria and Dengue Infection in Children using Raman Spectroscopy

Palak Patel[1], Gajanand Singh Tanwar[2*]

[1] Resident, Department of Pediatrics, S.P. Medical College, Bikaner, Rajasthan, India.
[2]Associate Professor, Department of Pediatrics, S.P. Medical College, Bikaner, Rajasthan, India.
*Corresponding Author

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ABSTRACT

Background: Dengue virus infection is a global health issue affecting millions of people worldwide each year. The present study was conducted to discriminate malaria and dengue infection in children using Raman spectroscopy. **Methods:** The present study was conducted on 62 children of both genders. A 15 µl of each sample was placed on the glass slide and dried for about 2 hour at room temperature. Raman spectra from all the samples were measured using Raman spectrometer having a spectral resolution of 4 cm−1. Raman spectra were recorded within the spectral range of 600 cm^{-1} to 1750 cm^{-1}. **Results:** Out of 62 patients, males were 34 and females were 28. Normal sera showed major Raman-peaks at 1004, 1156, 1120, 1460, 1525 and 1654 whereas dengue sera showed at 740, 856, 1034, 1308, 1336 and 1358 and malaria sera at 758, 884, 1050, 1352, 1346, 1365. Raman peaks at 740 labelled to Human RBC, 856 to Proline, Tyrosine, 1004 to β-Carotenoids, 1034 to Phenylalanine-IgG, 1120 to Labeled for IgG, 1156 to Carotenoids, 1308 to IgM, 1336 to Phenylalanine, 1358 to Tryptophan-IgG, 1460 to Carotenoids, 1525 to Amide I band and 1654 to Amide I band. At 758 Tryptophan-IgG, 884 C C stretching mode lipid and protein, 1050 C N stretching of Carotenoids, 1346 Phenylalanine-IgG, 1365 C C stretching. **Conclusion:** There are different major Raman-peaks for normal subjects appeared at 1004, 1156, 1120, 1460, 1525 and 1654; for dengue sera at 740, 856, 1034, 1308, 1336 and 1358; and for malaria sera at 758, 884, 1050, 1352, 1346 and 1365.

Keywords: Dengue, Malaria, Raman-peaks.

INTRODUCTION

Dengue virus infection is a universal health problem affecting millions of people. It is a mosquito-borne infectious disease transmitted by the bite of a mosquito from one of the four dengue virus serotypes. The transmission has amplified largely in urban and semi-urban areas and has become a major worldwide public health concern.[1] In some cases, the disease developed into life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) resulting in bleeding, low levels of blood platelets, blood plasma leakage, and dangerously low blood pressure. In Asia, the risk of severe dengue infection is greater in children (≤15 years) than in adults.[2]

Sometimes asymptomatic malaria or that with low-parasitemia levels remains undetected. Rapid diagnostic testing (RDT), polymerase chain reaction (PCR), serological tests, and mass spectrometry (MS) are among various diagnostic tests available in the market. They are thought to be gold standard, but they often fail. WHO recommends malaria RDT (MRDT) diagnosis for all people with suspected malaria before treatment is administered. The MRDT kit detects persisting antigens, such as histidine-rich protein II (HRPII) and lactate dehydrogenase for falciparum and vivax infection, respectively.[3]

Raman spectroscopy is based on the molecular vibration that originates from an inelastic scattering of light by the sample. As a result of interaction of light with intra-molecular bonds, Raman scattered photons comes outside with different energy.[5] This difference in energy (wavelength) between incident and emitted photons, termed as Raman shift, represented as cm^{-1}. There are different chemical bonds inside the body fluids and tissue, each produces its own characteristics spectra. Therefore 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.[5] The present study was conducted to discriminate malaria and dengue infection in children using Raman spectroscopy.

Name & Address of Corresponding Author
Dr. Gajanand Singh Tanwar
Associate Professor,
Department of Pediatric Medicine,
Sardar Patel Medical College, Bikaner, Rajasthan
E mail : drgstanwar@gmail.com

MATERIALS AND METHODS

The present study was conducted in the department of Pediatrics. It comprised of 62 children of both genders. The approval for the study was obtained from institutional ethical committee. All parents were informed regarding the study and written consent was obtained.

Demographic profile of patients was recorded. Blood samples were obtained from all patients and age and gender matched control. All the samples were

centrifuged at 3500 rpm for 10 min using Hittich Centrifuge D-7200. The obtained sera have been aliquoted in different tubes and stored at −80 ◦C till use. A 15 µl of each sample was placed on the glass slide and dried for about 2 hour at room temperature. Raman spectra from all the samples were measured using Raman spectrometer having a spectral resolution of 4 cm^{-1}. A continuous laser beam at 532 nm from a laser diode has been used for the excitation. 100 X magnification was used both for light focusing and collection in backscattering configuration. Raman spectra were recorded within the spectral range of 600 cm^{-1} to 1750 cm^{-1}. Results thus obtained were subjected to statistical analysis. P value less than 0.05 was considered significant.

RESULTS

Table 1: Distribution of patients

Total- 62		
Gender	**Males**	**Females**
Number	34	28

[Table 1] shows that out of 62 patients, males were 34 and females were 28.

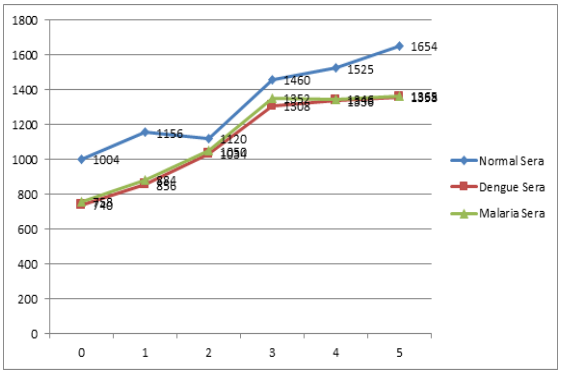

Figure 1: Raman peaks in normal, malaria sera and dengue sera

[Figure 1] shows that normal sera showed major Raman-peaks at 1004, 1156, 1120, 1460, 1525 and 1654 whereas dengue sera showed at 740, 856, 1034, 1308, 1336 and 1358 and malaria sera at 758, 884, 1050, 1352, 1346, 1365.

Table 2: Comparison of Raman peaks of normal and dengue infected blood sera

Raman shift (cm^{-1})		Dengue Sera	Malaria Sera	Normal Sera
740	Human RBC	V	-	-
758	Tryptophan-IgG	-	V	-
856	Proline, Tyrosine	V	-	-
884	C C stretching mode lipid and protein	-	V	-
1004	β-Carotenoids	-	-	V
1034	Phenylalanine-IgG	V	-	-
1050	C N stretching of Carotenoids	-	V	-
1120	Labeled for IgG	-	-	V
1156	Carotenoids	-	-	V
1308	IgM	V	-	-
1336	Phenylalanine	V	-	-
1346	Phenylalanine-IgG	-	V	-
1358	Tryptophan-IgG	V	-	-
1365	C C stretching	-	V	-
1460	Arise from -Carotenoids	-	-	V
1525	Amide I band	-	-	V
1654	Amide I band	-	-	V

[Table 2] shows that raman peaks at 740 labelled to Human RBC, 856 to Proline, Tyrosine, 1004 to β-Carotenoids, 1034 to Phenylalanine-IgG, 1120 to Labeled for IgG, 1156 to Carotenoids, 1308 to IgM, 1336 to Phenylalanine, 1358 to Tryptophan-IgG, 1460 to Carotenoids, 1525 to Amide I band and 1654 to Amide I band. At 758 Tryptophan-IgG, 884 C C stretching mode lipid and protein, 1050 C N stretching of Carotenoids, 1346 Phenylalanine-IgG, 1365 C C stretching.

DISCUSSION

There have been as many as 3.9 billion cases of dengue and malaria in 128 countries, has worsened the situation. The CDC suggests that the earliest diagnosis of acute dengue infection can be established by testing sera during the first 5 days of symptoms and/or early convalescent phase; contrary to the fact, the more rapid and accurate the diagnosis is, the better the patient management and recovery are due to the lack of specific therapy and vaccine against dengue.6 Dengue diagnosis relies on serological tests (primarily ELISA for detection of IgM and IgG antibodies), dengue virus isolation, and molecular methods (detection of viral nucleic acid/ antigen).[7]

Dengue infections manifests as hepato-gastrointestinal, neurological, and cardiac presentation, remain underdiagnosed.[8] CDC and the Angola Ministry of Health is a whistle-blower, where patients in the endemic regions of Africa were confirmed as positive using a dengue RDT kit, including one patient with symptoms consistent with severe dengue that turned-out to be false-positive. A new research highlighted the limitations of current dengue diagnostic tests. Simply relying on detection of a particular antigen or antibody is not sufficient and sometimes misleading for dengue diagnosis, particularly, in the case of concomitant infections

with malaria.[9] The present study was conducted to discriminate malaria and dengue infection in children using Raman spectroscopy.

In this study, out of 62 patients, males were 34 and females were 28. Patel et al,[10] in 130 human sera linear discriminant analysis (PC-LDA) of acquired RS-spectra was used which was done to classify malaria and dengue with a minor overlap of 16.7%. Receiver operating characteristic (ROC) analysis of test samples showed sensitivity/specificity of 0.9529 for malaria vs healthy controls (HC) and 0.9584 for dengue vs HC. The Raman findings were complemented by mass spectroscopy (MS)-based metabolite analysis of 8 individuals, each from malaria, dengue, and HC. Several of the metabolites, including amino acids, cell free DNA, creatinine, and bilirubin, assigned for the predominant RS-bands were also identified by MS and showed similar trends. Results of their study indicated that RS-based serum analysis using a microprobe has immense potential for early, accurate, and automated detection and discrimination of malaria and dengue, and in the future, it could be extrapolated in field-settings combined with hand-held RS. Further, this approach might be extended to diagnose other closely related infections with similar clinical manifestations.

We found that normal sera showed major Raman-peaks at 1004, 1156, 1120, 1460, 1525 and 1654 whereas dengue sera showed at 740, 856, 1034, 1308, 1336 and 1358 and malaria sera at 758, 884, 1050, 1352, 1346, 1365. The raman peaks at 740 labelled to Human RBC, 856 to Proline, Tyrosine, 1004 to β-Carotenoids, 1034 to Phenylalanine-IgG, 1120 to labeled for IgG, 1156 to Carotenoids, 1308 to IgM, 1336 to Phenylalanine, 1358 to Tryptophan-IgG, 1460 to Carotenoids, 1525 to Amide I band and 1654 to Amide I band. At 758 Tryptophan-IgG, 884 C C stretching mode lipid and protein, 1050 C N stretching of Carotenoids, 1346 Phenylalanine-IgG, 1365 C C stretching.

Raman spectroscopy has been widely used for qualitative characterization of biological tissues, for diagnosis of periodontitis and lung cancer from saliva, skin cancers and atherosclerosis along with malaria and dengue fever.

CONCLUSION

Authors found that major Raman-peaks at normal subjects appeared at 1004, 1156, 1120, 1460, 1525 and 1654 whereas dengue sera showed at 740, 856, 1034, 1308, 1336 and 1358 and malaria sera at 758, 884, 1050, 1352, 1346, 1365.

REFERENCES

1. Guzman M G, Vazquez S and Kouri G. Malaysian J. Med. Sci. 2009;16:5-12.
2. Guzman M G and Kour G. Clin. Diagn. Lab. Immunol. 1996;3:621-625.
3. Vorndam V, Kuno G. Laboratory diagnosis of dengue virus infections Dengue and Dengue Hemorrhagic Fever ed D J Gubler and G Kuno (New York: CAB International).1997;313.
4. Rosario D, Alvarez M, V Azquez S, Amin N, Rodrıguez R, Valdes K and Guzman M GBiotecnol. 2001;203:1-10.
5. Kong Y Y, Thay C H, Tin T C and Devi S J. Virol. Methods 2006;138:123-127.
6. Khan S, Ullah R, Saleem M, Bilal M, Rashid R, Khan I, Mahmood A, Nawaz M. Raman spectroscopic analysis of dengue virus infection in human blood sera. Optik. 2016 Feb 1;127(4):2086-2088.
7. Haaland D M and Thomas E V. Anal. Chem. 1988;60: 1193-1197.
8. Thomas E V and Haaland D M 1990 Anal. Chem. 1990; 62: 1091-1095.
9. Rein A J, Saperstein D D, Pines S H and Radlick P C Experientia 1976; 32: 1352-1356.
10. Patel SK, Rajora N, Kumar S, Sahu A, Kochar SK, Krishna CM, Srivastava S. Rapid Discrimination of Malaria-and Dengue-Infected Patients Sera Using Raman Spectroscopy. Analytical chemistry. 2019 Apr 29;91(11):7054-7062.

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