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FTIR Spectral Study on Jaundice Blood Samples Before and After Treatment

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Spectroscopy is emerging as a potential diagnostic tool in the medical and pharmacological fields to provide information about the different chemical and morphological structures of healthy and pathological tissues. Blood being the chief circulatory medium of our body, reflects the physiological and pathological changes that take place in the tissues, which leads to the changes in the various plasma and cellular constituents. In the present work, FTIR spectroscopy technique is employed to study the spectral differences in the serum from healthy subjects and patients affected by jaundice, before and after they underwent treatment. Based on the differences in the spectral signatures, four intensity ratio parameters are used and their variation has been studied to understand the spectral changes. Student's t-test has been used to anlayze the set of data obtained from sera of patients pre-treatment and post-treatment.

Key Words: Blood, Serum, Jaundice, Pre-treatment, Post-treatment, FTIR spectrum.

INTRODUCTION

During the normal breakdown of old erythrocytes, haemoglobin is converted into bilirubin. Normally, the bilirubin is removed from the bloodstream by the liver and eliminated from the body in the bile, which passes from the liver into the intestines. There are several conditions that may interrupt the elimination of bilirubin from the blood and cause jaundice. Scientifically speaking, it is the condition associated with abnormally high levels of bilirubin. Fever with jaundice or febrile jaundice therefore, constitutes a challenging situation to be tackled by a good history, a meticulous physical examination and supported by sound laboratory reports initially and in the later stages for monitoring and follow-up. The various causes of febrile jaundice, methods of prevention and different treatments prescribed are discussed in detail by many authors[1-3]. Currently, the researchers all around the world are trying to develop spectroscopy based techniques, where important blood parameters could be determined immediately from the patient in order to allow immediate and adequate treatment and therapy. Many parameters of medical relevance can be determined qualitatively and quantitatively from blood samples by vibrational spectroscopists.

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FTIR spectroscopy is able to detect biochemical changes caused by pathologies[4,5] also at a very early stage of the disease, due to the fact that any biochemical change in the tissue must precede any morphological manifestation of the disease itself. Based on this fact, the authors have characterized the blood serum as normal or jaundice affected with the help of FTIR spectral techniques, while the UV-Visible spectral data has been used to substantiate the results[6]. The present work is an attempt to employ FTIR spectroscopy as a tool to differentiate blood sera of patients before and after treatment for jaundice and hence to understand the spectral signatures as an indicator of jaundice.

EXPERIMENTAL

2 mL of blood sample was collected by vein puncture from 12 healthy volunteers and 40 jaundice patients. After treatment, blood samples were once again collected from those patients who underwent treatment. Each blood sample was left to coagulate naturally without adding any anticoagulant agents for *ca.* 20 to 30 min. The serum was separated from every sample and centrifuged at a speed of 1200 rpm in REMI electric centrifuge. Each clear serum sample that separates after centrifugation was divided into two parts and transferred into sterile vials.

Conventional clinical method: The bilirubin level in each sample stored in one set of vials was determined with the Bilirubin kit from Beacon diagnostics Pvt. Ltd. at Balaji Clinical Laboratory, Chennai. The bilirubin level was determined by adding the reagents with the serum in prescribed quantities and incubated in Biochem thermostat at 35 °C. By using Photochem colorimeter, the absorbance of the serum reagent mixtures at 540 nm was used to quantify the total bilirubin level in both healthy and jaundice samples.

FTIR spectral recording: While recording FTIR spectrum of serum samples, water being abundant, strong water absorptions will hinder the spectral details. In order to remove the water content, the second set of serum was lyophilized using freeze drier at Centralized Instrumentation Laboratory, Madras Veterinary College, Chennai. FTIR spectra of the lyophilized samples were recorded by KBr pellet method in the region 4000-400 cm^{-1} using FTIR ABB Bomem MB series at Dr. CEEAL Analytical lab, Chennai. Each measurement was repeated to ensure the reproducibility of the spectra. All the infrared spectra were baseline corrected and normalized. Absorbance scan on the KBr substrate was done before and immediately after the FTIR recordings on the samples were performed. In this way, absence of any unwanted wet trace due to the hygroscopic character of KBr substrate that eventually could affect the spectra was confirmed.

RESULTS AND DISCUSSION

In recent years, infrared spectroscopy has gained momentum in the usage as a diagnostic tool. The FTIR spectrum of serum provides various useful information on the biomolecules regarding their structure, functional groups, nature of bands

involved and their interaction. Infrared bands are used to derive qualitative information about the serum samples of healthy and various diseases, based on the characteristic spectral features and patterns. Researchers have tapped these differences in order to quantify several metabolites in blood and other biological tissues[7-10]. Based on these studies, various important groups of vibrations were identified in the FTIR spectrum of blood sera (Fig. 1) and presented in Table-1. The interpretation of these bands is discussed in the earlier work[6]. In the current work, the complete spectral region has been divided into three, which corresponds to the glucose region (1300-925 cm^{-1}), protein region (1700-1500 cm^{-1}) and lipids or fat region (3300-2800 cm^{-1}). In order to quantify the spectral differences in these regions, four intensity ratio parameters that show significant difference in Student's t-test have been chosen.

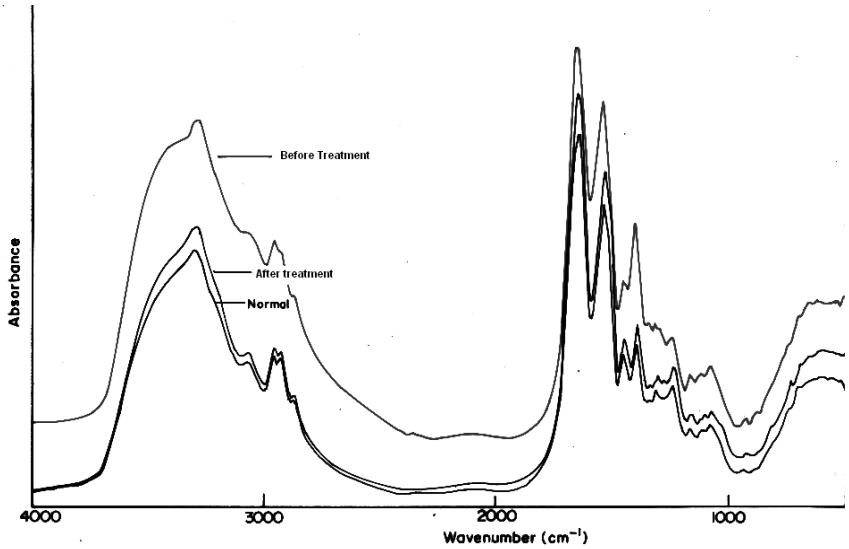

Fig. 1. Overlay of FTIR spectrum of blood serum samples from normal, jaundice subjects-before and after treatment

Data analysis: The data was divided into two categories, from patients who underwent allopathic treatment and from those who underwent unani treatment for jaundice[11]. For various medical and other reasons, sample size of post-treatment category is slightly lesser than pre-treatment category.

A strong band that is observed at about 3300 cm^{-1} and weaker one in the 3068 cm^{-1} region are designated as amide-A and amide-B bands, respectively. In the infrared spectrum usually, N-H stretching region around 3200 cm^{-1} is strongly overlapped by the O-H stretching band of hydrating H_2O molecules. The serum samples have been lyophilized in this study do not have any water component in them and hence the bands in this region could be attributed to amide vibrations themselves. The first intensity ratio parameter value which is the ratio of intensities of amide A and amide B bands of the patients who underwent allopathic treatment and unani

TABLE-1
INFRARED BAND ASSIGNMENT OF HUMAN SERUM

Absorption band (cm^{-1})	Assignment
3303	Amide-A band due to N-H stretching vibration
3068	Amide-B band due to overtone of amide I band
2960	Asymmetric stretching vibrations of CH_3 of proteins and lipids
2873	Symmetric stretching vibrations of methyl group of proteins and lipids
2927	Asymmetric stretching vibrations of methylene group of proteins and lipids
2852	Symmetric stretching vibrations of methylene group of proteins and lipids
1652	Amide-I band mainly due to C=O stretching vibration amide groups
1543	Amide-II band due to the N-H bending vibration strongly coupled to the C-N stretching vibration of protein.
1455	Deformation vibrational modes of methyl groups
1403	Deformational vibrational modes methyl groups
1319	Deformational vibrational modes methyl groups
1240	Asymmetric PO_2 stretching vibration of phospolipids
1170	Ring vibrational mode of C-O-H and C-O-C bonds
1080	C-O stretch of glucose region
1035	C-O stretch of glucose region, a major glucose band
970	P-O symmetric stretching mode due to phospolipids
726	N-H out of plane bending with the contribution of C-N torsional vibration

treatment has an average of 1.5518 and 1.3708 before the treatment commenced. These values fall well within the jaundice range[6]. The mean value of this ratio changes to 1.6591 in the patients who underwent allopathic treatment, whereas this is equal to 1.6345 in case of unani patients. Both these values indicate a shift towards the one for healthy subjects. The second intensity ratio parameter $R_2 = I_{2963}/I_{2873}$, has the mean value of 1.2595 with a standard deviation of 0.0363 in case of allopathic patients, before their treatment started. This is ratio of the asymmetric and symmetric stretching of methyl groups can be seen in the spectrum of bilirubin too, as the latter contains four methyl groups attached to its four pyrrole rings. This value has changed to 1.3581 when the patients came for review. In case of unani patients, this parameter has changed from a value of 1.1708 to 1.3420 after the treatment. A marked difference has been reported by Gunasekaran and Sankari[10] and Manimegalai[12] in the intensity ratio between these methyl bands and used this as a discriminating factor between the healthy subjects and those affected with some diseases like diabetes, cholesterol, thyroid and urea.

The two most intense bands in the spectra of sera samples are amide I and amide II bands that can be located in the spectrum of bilirubin also as very intense bands. With the onset of jaundice, liver loses its capacity to convert bilirubin into readily excretable derivatives. This increases the bilirubin level in the blood, which serves as a primary indicator of jaundice condition. This may be the reason for an increase in the absorbance values of these two amide bands. The intensity ratio

parameters $R_3 = I_{1652}/I_{1543}$, a ratio of absorbance of these to bands can hence be seen as an indicator of jaundice condition.

Zeller *et al.*[13], Kajiwara *et al.*[14] and Back and Polavarapu[15] have identified several characteristic frequencies in the MIR glucose absorption spectra. Specific frequencies identified in blood by Zeller and co-workers[13] are 1365, 1152, 1109, 1080 and 1035 cm^{-1} for the study of glucose component. The intensity ratio parameter $R_4 = I_{1170}/I_{1080}$ shows a conspicuous drift towards the normal values after the patients underwent jaundice treatment. This is an indicator of lowering of serum bilirubin level in blood, due to the medications prescribed. The P-value for R_3 by Student's t-test for observing the difference between pre-treatment and post-treatment samples are 0.002 and 0.001 for allopathic and Unani, respectively. The P-values of R_4 are found to be 0.0009 and 0.001 for the same. These values are less than 0.01 and hence, there is a significant difference between the means of these parameters calculated.

It is clear from the values projected in the Table-2, internal ratio parameter values shift towards the values of healthy sera samples after they undergo treatment. This is evident from the overlay of the FTIR spectra (Fig. 1) of the sera from the same subject obtained pre-treatment and post-treatment, along with that of a healthy volunteer. A considerable difference in the absorbance levels of these bands between the normal sera and sera from jaundice patients before treatment can be observed. After undergoing treatment, the patients showed signs of improvement in their health condition. Moreover, a comparison of intensity ratio parameter values of the pre-treatment category shows a marked deviation from that of healthy samples, which may be considered as an indicator of serum bilirubin level in blood. By clinical data, serum bilirubin value ranged between 2.2 and 12.2 mg/dL in Unani

TABLE-2
INTENSITY RATIO PARAMETERS OF SERA FROM JAUNDICE PATIENTS BEFORE AND AFTER TREATMENT AND t-TEST RESULTS

Intensity ratio parameters	Healthy samples		Allopathy system				P-Value
			Before treatment		After treatment		
	Mean	SD	Mean	SD	Mean	SD	
$R_1 = I_{3303}/I_{3068}$	1.7130	0.0297	1.5518	0.0315	1.6591	0.0439	0.0003
$R_2 = I_{2963}/I_{2873}$	1.3806	0.0279	1.2595	0.0363	1.3581	0.0343	0.0002
$R_3 = I_{1652}/I_{1543}$	1.1565	0.0290	1.2597	0.0241	1.1640	0.0343	0.0003
$R_4 = I_{1170}/I_{1080}$	0.9296	0.0176	0.9861	0.0093	0.9465	0.0154	0.0003
Intensity ratio parameters	Healthy samples		Unani system				P-Value
			Before treatment		After treatment		
	Mean	SD	Mean	SD	Mean	SD	
$R_1 = I_{3303}/I_{3068}$	1.7130	0.0297	1.3708	0.0542	1.6345	0.0385	0.0002
$R_2 = I_{2963}/I_{2873}$	1.3806	0.0279	1.1708	0.0476	1.3420	0.0313	0.0001
$R_3 = I_{1652}/I_{1543}$	1.1565	0.0290	1.3789	0.0458	1.1628	0.0151	0.0002
$R_4 = I_{1170}/I_{1080}$	0.9296	0.0176	1.0283	0.0332	0.9347	0.0131	0.0003

patients and 2.4 to 6.8mg/dL in the allopathic patients who came under the purview of this current work. High bilirubin level has been traced to the fact that most of the patients who opted unani treatment are from lower economic strata of the society and have not sought any medical intervention at early stages due to lack of knowledge on health combined with poverty. Educating people on hygiene factors and the need for good health may improve their health condition. In both the types of treatments, the patients have responded to their respective type of treatments and have shown improvement according to the clinical results too.

Conclusion

FTIR spectroscopy has been employed as a tool to reveal the spectral changes in lyophilized sera of jaundice patients before and after treatment. With the spectral studies and analysis by t-test, very significant difference in the intensity ratio parameters before and after treatment has been obtained in both the types of treatments. The overall interpretation of the spectra appears to provide a direct link to the assessment of the state of health of the healthy person with respect to jaundice patients. Also, it gives an idea how the patients respond to the treatment and hence the course of treatment can be suitably adjusted.

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