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Analysis on renal failure patients blood samples: characterization and efficacy study

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Abstract: This study attempts to evaluate the spectral difference between healthy and renal failure blood sera and also to find the efficacy of peritoneal dialysis in renal failure patients using FTIR spectroscopic technique. Six chronic renal failure patients were enrolled in the study. FTIR blood spectra were recorded over the region 4000 – 400 cm^{-1} on a Perkin Elmer spectrum one FTIR spectrometer. The internal standard ratios among the absorption peaks were calculated and it is found to be an indicator to differentiate the diseased subjects from the healthy one. In order to find the efficacy of peritoneal dialysis the absorption values of the specific bands of both pre and post treatment spectra were noted and the internal ratio parameter were calculated. The comparison of these values showed that the spectra are not similar when compared down to finer details.

Keywords: Blood, renal failure, peritoneal dialysis, FTIR spectroscopy.

Introduction

Renal failure occurs where there is damage to the kidneys that impairs their ability to filter and remove waste products from the blood. Diagnosis of renal failure is made by collection of blood and urine samples for analysis. Two substances in blood namely urea and creatinine are commonly analyzed, as these are the products of metabolism that are normally excreted by the kidneys. Shaw *et al*. evaluated the sensitivity and accuracy of mid – IR spectroscopy in the determination of urea, creatinine and total protein from the IR spectra of dried urine films and compared the results provided by standard clinical chemistry assay(Shaw *et al*.,1998). Infrared spectroscopy offers an approach to clinical analysis that is conceptually very appealing. Whereas countless assays rely on the use of chemical agents to "recognize" the analyte of interest and to react with the analyse to produce specific colour changes. IR- based analysis is found on the rich IR absorption patterns that characterize the analytes themselves. These absorption patterns provide the basis to distinguish among the constituents and to separately quantify them. The most obvious distinguishing feature is that no reagents are required. In addition, IR- based analytical methods require very small sample volumes (typically microlitres), show good precision over the entire physiological range, and are well suited for automation.

There are several approaches to IR-based analysis with the first choice being whether to use the near- IR or mid- IR spectral range. Near – IR spectroscopy has gained notoriety within the clinical chemistry community through the many efforts to develop a noninvasive blood glucose monitor based on this technology and in that vein it has been shown that glucose concentrations can be recovered from the near – IR spectrum of native serum (Heise, 1996; Budinova *et al*., 1997; Khalil, 1999). The main reason for the focus on near-IR spectroscopy is that tissue is quite transparent to near-IR light, hence the attraction for *in vivo* work. However, this is obviously not a factor for *in vitro* analysis. The mid-IR spectrum offers some potential advantages. Near-IR spectroscopy typically requires a sample volume of at least 0.1 – 0.2 mL, whereas a mid-IR assay can be carried out with ≤ 10µL. Although water contributes enormous absorption bands in the mid – IR, these can be eliminated by simply drying the sample to a film and using the spectrum of the dry film as the basis for analysis. This film may then be archived for subsequent reanalysis.

The IR-based quantification methods were calibrated by comparison with the results provided by standard clinical chemistry assays. Several previous studies have illustrated the potential roles for IR spectroscopy in the clinical laboratory. For example, six serum analytes have been shown to be suitable for IR-based analysis, namely, albumin, total protein, glucose, triglycerides, urea and cholesterol, no work has been carried out in the quantification of urea and creatinine present in renal failure blood using mid – IR spectroscopy (Hall & Pollard,1993; Heise *et al*., 1997; Liu *et al*., 1998; Khalil, 1999). The aim of the present study is to find the spectral difference between healthy and renal failure blood sera and also to study the efficacy of peritoneal dialysis using FTIR spectroscopic technique.

Experimental Description

Healthy blood samples were collected from six healthy volunteers of the age group 30-45 years. Renal failure blood samples before and after dialysis were collected from Government Royapettah, Hospital, Chennai. Blood samples were obtained from renal failure patients after overnight fasting. After centrifugation of blood in refrigerated centrifuge, the plasma aliquots were transported to the laboratory in a portable freezer and kept at 20^0 C until analyzed. Using the conventional method, the sample could be prepared by spreading a small volume of serum on an IR- transparent material, allowing to dry and measuring the absorption spectrum of the film. The accuracy of the method may be compromised by any variation in the amount of serum successfully deposited on the KBr

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Fig.1. FTIR spectrum of a healthy human serum sample

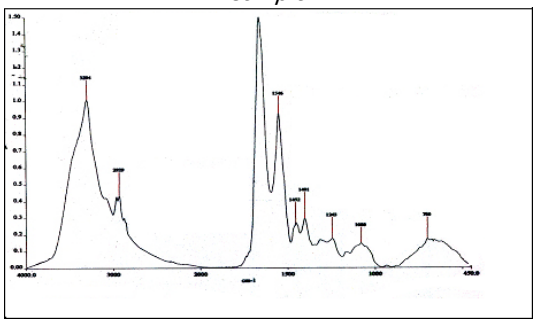


Fig.2. FTIR spectrum of a renal failure patient blood serum sample

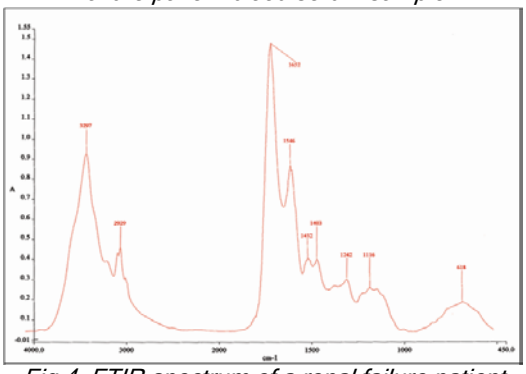


Fig.3. FTIR spectrum of a renal failure patient blood serum - before peritoneal dialysis

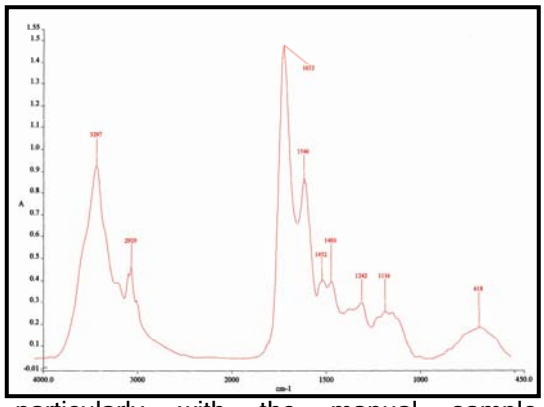
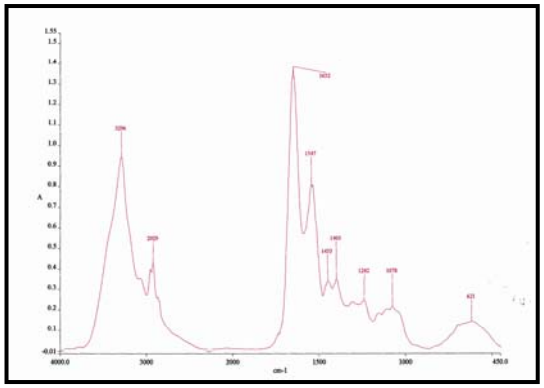


Fig.4. FTIR spectrum of a renal failure patient blood serum - after peritoneal dialysis



window, particularly with the manual sample preparation. In order to make up fort this variation and to assess its impact on the overall accuracy of the method, a standard solution is added to each serum sample. The solution is chosen in such a way that it responds to IR radiation at the point where serum sample contains no absorption peak. Shaw *et al.* reported that the IR absorption spectrum of the thicyanate ion (SCN) includes absorption at 2060 cm^{-1} in a spectral region where serum sample and subsequently normalizing all of the spectra to equal intensities therefore compensated for the imprecision in the film preparation (Shaw *et al.*,1998).

A volume of 1 ml of serum was diluted with an equal volume of 4 mg/ L aqueous potassium thiocyanate (KSCN) solution. 20 µl of each diluted sample was spread evenly over the surface of a circular KBr window (9mm diameter and 2mm thickness). All the specimens were air dried for 30 minutes prior to measuring the IR spectra (Heise *et al.*, 1994,1997; Shaw *et al.*, 1998). Infrared spectra in the region 400-4000 cm^{-1} were recorded on a PERKIN - ELMER Spectrum - One FTIR spectrometer equipped with an air-cooled DTGS (Deuterated Triglycine Sulphate) detector at IIT, Chennai. It is known that the strong absorption band of water in the mid IR region is hindered and to eliminate the same, the serum samples are air dried to form a thin uniform film on the KBr pellet (Heise *et al.*,1994). Infrared transparent KBr material without the sample was scanned as background for each spectrum and 16 scans were co added at a spectra resolution of ±1 cm^{-1}. The collected signal was transferred to the PC. The data were processed by windows based data program- Spectrum software. The spectra were base line corrected and they were normalized to acquire identical area under the curves and the maximum absorbance values of the corresponding characteristic bands were noted. The spectral differences between healthy and renal failure blood sera are identified.

Results and discussions

The infrared spectrum is an essence of reflection of the infrared color pattern characteristic of the sample. The basis of quantization is that each constituent contributes a unique absorption pattern to the overall spectrum governed by the unique set of molecular vibrational characteristic of each distinct molecular species. The quantitative information is carried by the relative intensities of vibrational frequencies of the various constituents contributing to the unique absorption profile of each serum specimen.

The IR spectrum of serum includes spectral contribution from protein, cholesterol, triglycerides, urea, glucose and other more dilute contributes a complex set of several absorptions falling within the

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mid-IR spectral. It is impossible to find any single any single component; coincident absorptions from other species would degrade or completely sabotage the effort (Heise *et al*., 1994, 1997).

A vibrational band assignment of the absorption bands of the spectra is done with the idea of the group frequency of various constituents of the serum samples. A satisfactory assignment of the vibrational bands has been carried out by interpreting the well established infrared spectra of blood and serum (Jantsch *et al*.,1989; Shaw *et al*., 1998). A build up of the toxins namely, urea and creatinine in the blood occurs in the case of renal failure subjects and hence the present work aims to quantify these two blood constituents to characterize the blood samples of healthy and renal failure subjects and to study the significance of peritoneal dialysis of renal failure patients. Fig.1 & 2 represents the FTIR spectrum of a healthy and a renal failure patient blood serum sample. Some specific absorption peaks were identified and the internal ratio parameter were calculated and summarized in Table 1 & 2.

Dialysis may be used for very sick patients who have suddenly but temporarily lost their kidney function. There are two types of dialysis namely, hemodialysis and peritoneal dialysis. The Peritoneal dialysis removes wastes such as urea from the blood as well as excess fluid when the kidneys are incapable of this (i.e., renal failure). Peritoneal dialysis filters waste using the peritoneal membrane inside the abdomen. Dialysis fluid is instilled via a peritoneal dialysis catheter which is placed in the patient's abdomen running from the peritoneum out to the surface of the anterior abdominal wall near the navel. The abdomen is filled with the special solutions called dialysate for a specific period of time and then the fluid is drained out through the tube and discarded. This cycle or exchange is repeated 4-5 times per day in course of a peritoneal dialysis, three transport processes- diffusion, ultra filtration and absorption occur simultaneously. The dialysis solution contains sodium, chloride, lactate and is rendered hyper osmolar by the inclusion of a high concentration of glucose and the resulting osmotic pressure causes the fluid to move from the blood into the dialysate (Goldman & Ausiello., 2007). As a result, more fluid is drained than was instilled. In the present work, the blood samples from six chronic renal failure patients were taken before and after peritoneal dialysis and the efficacy of dialysis is studied by FTIR spectroscopic technique. The FTIR spectrum of pre and post dialysis blood serum samples is presented in Fig.3 & 4 respectively. The internal ratio parameter among some predominant peaks before and after peritoneal dialysis were calculated and summarized in Table 3.

Table 1. Internal Standard Ratio parameters calculation for healthy volunteer blood sera

Intensity Ratio	Samples					
	I	II	III	IV	V	VI
I_{591}/I_{1716}	0.593	0.507	0.666	0.640	0.692	0.615
I_{716}/I_{1055}	0.750	0.796	0.750	0.781	0.787	0.720
I_{1055}/I_{1242}	0.580	0.591	0.511	0.586	0.584	0.592
I_{1242}/I_{1403}	0.794	0.698	0.573	0.743	0.773	0.675
I_{1403}/I_{1546}	0.520	0.568	0.554	0.419	0.584	0.503
I_{2929}/I_{3020}	0.981	0.878	0.990	0.942	0.967	0.984

Table 2. Internal Standard Ratio parameters calculation for renal failure patients blood sera

I_{591}/I_{1716}	1.560	1.483	1.084	1.049	1.423	1.320
I_{716}/I_{1055}	1.136	1.115	1.353	1.206	1.040	1.050
I_{1055}/I_{1242}	0.758	0.765	0.714	0.755	0.714	0.750
I_{1242}/I_{1403}	0.725	0.773	0.712	0.726	0.729	0.826
I_{1403}/I_{1546}	0.714	0.772	0.719	0.756	0.774	0.732
I_{2929}/I_{3020}	1.439	1.484	1.643	1.471	1.719	1.142

Clinical Analysis

Urea is the major end product of protein metabolism in human. It constitutes the largest fraction of the non- protein nitrogen component of blood. Urea is produced in the liver and excreted through the kidneys in the urine. Consequently the circulating levels of urea depend upon protein intake, protein catabolism and Kidney function. Elevated serum urea concentrations are observed in impaired kidney function, liver disease, congestive cardiac failure, diabetes, infections and diseases which impair kidney function. The estimation of urea in serum involves the enzyme catalyzed reactions. The reagent and Aqua-4 are allowed to attain room temperature. Equal amount of reagent and Aqua- 4 are added and mixed gently and aspire standard followed by samples (Levey et al., 1998). The absorption change ΔA for the standard and unknown samples are determined by using the formula

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Table 3. Internal Standard Ratios among some predominant peaks before and after peritoneal dialysis

Intensity Ratio		R_1 I_{591}/I_{716}	R_2 I_{716}/I_{1055}	R_3 I_{1055}/I_{1242}	R_4 I_{1242}/I_{1403}	R_5 I_{1403}/I_{1546}	R_6 I_{2929}/I_{3020}
Sample I	Pre	1.560	1.136	0.758	0.725	0.714	1.439
	Post	0.708	0.857	0.609	0.697	0.605	1.051
Sample II	Pre	1.483	1.115	0.765	0.773	0.772	1.484
	Post	0.757	0.881	0.600	0.693	0.666	1.082
Sample III	Pre	1.084	1.353	0.714	0.712	0.719	1.643
	Post	0.710	0.861	0.692	0.666	0.629	1.154
Sample IV	Pre	1.049	1.206	0.755	0.726	0.756	1.471
	Post	0.756	0.843	0.604	0.661	0.630	1.090
Sample V	Pre	1.423	1.040	0.714	0.729	0.774	1.719
	Post	0.727	0.733	0.612	0.645	0.655	1.346
Sample VI	Pre	1.320	1.050	0.750	0.826	0.732	1.258
	Post	0.773	0.785	0.729	0.698	0.670	1.190

ΔA=A1-A2. The concentration of urea is calculated as: Urea (mg/dl) = ΔA of test/ΔA of standard conc. of standard (mg/dl).

Creatinine is a waste product formed in muscle from the high energy storage compound, creatinine phosphate. The amount of creatinine is fairly constant and is primarily a function of muscle mass. It is removed from plasma by glomerular filtration and then excreted in urine without any appreciable re-absorption by the tubules. Creatinine is a useful indicator of renal function. Elevated creatinine level in serum is usually associated with various renal diseases. In the earlier stage of renal disease, creatinine clearance test is a sensitive index of impaired renal function (Hall & Pollard,1992). Creatinine reacts with alkaline picorate to produce an orange – yellow colour (Jaffe's reaction). Specificity of the assays has been improved by introduction of an initial rate method. However, Cephalosporin ibiotics are still major interferants. The absorbance of orange- yellow colour formed is directly proportional to creatinine concentration and is measured photometrically at 500-520 nm (Anthony Shaw *et al*., 2000). The two reagents (picric acid reagent and sodium hydroxide reagent) are mixed and kept 15 minutes before use. The serum is mixed with the reagents and the initial absorbance A1 (20secs after mixing) and final absorbance A2 (80 secs after mixing) were noted (ΔA = A2-A1). The creatinine concentration is calculated by the formula: creatinine (mg/dl) = ΔA of test/ ΔA of standard Conc of standard (mg/dl). Table 4 & 5 summarize the clinical data of blood urea and creatinine for pre- and post- dialysis blood sample.

In the case of analysis, it is clear that IR spectroscopy can meet the standards of accuracy that are required for a number of standard clinical serum tests. In serum analysis, no reagents are required. There is generally no need to dilute very concentrated specimens (as may be required for certain other methods). Several analysis are available simultaneously from a single IR spectrum, and at least in the method using dried films – very little sample (microlitre) is required. Time will tell whether these advantages will be exploited through the development of dedicated, IR based analyzers posteriori to the "Gold standard" diagnosis.

Conclusion

A systematic approach has been made using FTIR spectroscopic technique to study the spectral difference between healthy and renal failure patients' blood samples and also to find the efficacy of peritoneal dialysis on renal failures. The internal standard ratio was calculated among some specific absorption peaks selected. The comparison of these values shows that the spectra are not similar when compared down to finer details. The spectral results are well supported by the clinical values.

References

1. Anthony Shaw R, Sarah Low Yig, Micgeal Leroux and Henry H.Mantsch (2000) Toward reagent free clinical analysis, Quantization of urine urea, creatinine and total protein from the mid- infrared spectra of dried urine films. *Clin. Chem*. 46, 1493-95.
2. Budinova G, Salva J and Volka K (1997) Applications of molecular spectroscopy in the mid-infrared region to the determination of glucose and cholesterol in whole blood and in blood serum. *Applied Spectroscopy.* 51, 631-35.
3. Goldman L and Ausiello D (2007) Cecil Textbook of Medicine, 23rd ed, Philadelphia.
4. Hall JW and Pollard A (1992) Near- infrared spectrophotometry: a new dimension in clinical chemistry. *Clin. Chem.* 38,1623-1631.
5. Hall JW and Pollard A (1993) Near- infrared Spectroscopic determination of serum total proteins, albumin globulins and urea. *Chin. Biochem*. 26, 483-490.
6. Heise HM (1996) Non invasive monitoring of metabolites using near infrared spectroscopy, state of the art. *Horm. Mctab. Res.* 28, 527-534.
7. Heise HM, Bitter Manbach R, Koschinsky TH and Gries FA (1997) *Ex Vivo* determination of blood glucose by microdialysis in combination with

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infrared attenuated total reflection spectroscopy. *Frensenuis J. Anal. Chim.* 359, 83-87.

8. Heise HM, Morbach R, Koschinsky T and Gries FA (1994) Multicomponent assay for blood substrates in human plasma by mid-infrared Spectroscopy and its evaluation of clinical analysis. *Appl. Spectrosc.* 48, 85-89.
9. Janatsch G, Kruse-Jarres JP, Marbach R and Heise HM (1989) Multivariate calibration for assays in clinical chemistry using attenuated total reflection infrared spectra of human blood plasma. *Anal. Chem.* 61, 2016-2023.
10. Khalil OS (1999) Spectroscopic and clinical aspects of non invasive glucose measurements. *Clin. Chem.* 45,165-177.
11. Liu KX, Dembinski TC and Mantsch HH (1998) Rapid determination of the fetel lung maturity from infrared spectra of amniotic fluid. *Am.J. Obset. Gynecol.*178, 234-241.
12. Shaw RA and Mantsch HH (1998) Multianalyte serum analysis using mid-infrared spectroscopy. *Ann Clin Biochem.* 35, 624-632.
13. Shaw RA and Mantsch HH (1998) Multianalyte serum analysis using mid-infrared spectroscopy. *Ann. Clin. Biochem.* 35, 624-632.
14. Tietz N (1976) Fundamentals of Clinical Chemistry, W.B. Saunders Co., Philadelphia PA.
15. Levey A, Beto JA, Corondo BE *et al.* (1998) Controlling the epidemic of cardiovascular disease in chronic renal disease, What do we know? What do we need to learn? Where do we go from here? *Am. J. Kidney Dis*. 32, 853-906.