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Analysis of Plasma Homocysteine Levels Among Patients with Chronic Renal Failure-A Spectroscopic Approach

A. BRIGHT, T.S. RENUGA DEVI* and S. GUNASEKARAN†
Department of Physics, Women's Christian College, Chennai-600 006, India
E-mail: devi_renuga@yahoo.com

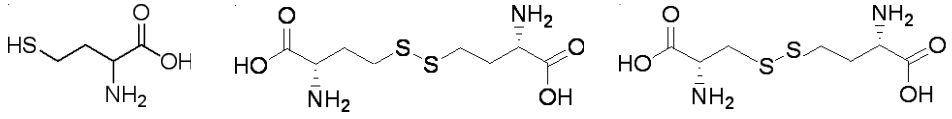
Plasma homocysteine concentration exhibits a strong relationship with renal function. Individuals with chronic renal failure were among the first in which homocysteine could be detected in blood. Recent population based observations demonstrate that plasma total homocysteine concentration are elevated in patients with renal insufficiency and that a lower glomerular filtration rate (GRF) is associated with a higher plasma homocysteine concentration. Hyperhomocystenemia is an independent risk factor for atherothrombotic disease in patients with pre-dialysis and end-stage renal disease. The present study aims at employing FTIR spectroscopy for analyzing the blood plasma of renal failure patients with elevated homocysteine levels to detect spectral parameters which might serve as biomarker for identifying and detecting homocysteine levels. The FTIR spectrum of blood plasma of the renal failure patients were recorded and analyzed. The analysis let to the identification of specific modes of vibration pertaining to homocysteine in blood plasma. The internal ratio parameter was calculated. The absorbance values at these specific modes of vibration varied significantly from that of healthy volunteers. These parameters could be used as a basis for deriving a spectral method for determining and measuring plasma homocysteine spectroscopically.

Key Words: FTIR spectroscopy, Plasma, Homocysteine, Chronic renal failure.

INTRODUCTION

Homocysteine, a sulphur-containing amino acid derived from the metabolism of methionine or cysteine, is rapidly oxidized to homocystine and cysteine-homocysteine, creating a special class of uremic toxins. Homocysteine is found in the plasma and in erythrocytes and cells of the liver and other organs. The plasma of patients with chronic renal insufficiency has a higher concentration of homocysteine[1]. Accumulation of homocysteine is a potentially severe complication because of the association between homocysteine levels and cardiovascular disease[2]. By convention, plasma or serum total homocysteine refers to the sum of homocysteine and its disulfide derivatives whether they exist in the bound or the free state. The structure of homocysteine, homocystine, cysteine-homocysteine is shown in Figs. 1(a-c) below:

†Periyar University, Salem-636 011, India.



(a) (b) (c)

Fig. 1. (a) Homocysteine (b) Homocystine (c) Cysteine-homocysteine

About 70 % of homocysteine is bound to proteins and hence is not removed by dialysis[3]. Studies in rats and human suggest that the kidney eliminates about 70 % of the daily homocysteine burden and this indicates why homocysteinemia levels are high in predialysis as well as renal transplant patients[4,5]. Also among dialysis patients the homocysteine levels are high because of the water soluble vitamins like folic acid, vitamins B6, vitamin B12 are all removed with dialysis. These vitamins act as the cofactors and substrates in the metabolism of methionine and homocysteine[6].

FTIR spectroscopy has been used by chemists as a powerful tool to characterize inorganic and organic compounds[7]. It has been applied in biology for studying the structure and conformation of molecules like proteins, nucleic acids and lipids[8-10]. The mid-IR region has been shown to be useful in the identification of disease patterns using the FTIR spectrum of human sera[11]. Precise quantification of serum components, such as glucose and total protein, cholesterol and urea has been achieved using mid IR spectroscopy[12,13].

Though different biomolecules in the blood plasma has been analyzed by many, not much work has been done in spectroscopically analyzing the plasma homocysteine. In the present study we have examined the FTIR spectra of plasma samples obtained from healthy individuals and chronic renal failure patients who have elevated levels of plasma homocysteine. The modes of vibration pertaining to homocysteine biomolecule were assigned. The result presented in this study show several spectral peaks which might serve as useful biomarkers for detecting the homocysteine levels.

EXPERIMENTAL

Clinical analysis: Subjects included in the study were 10 middle aged men who were suffering with chronic renal insufficiency. Also 10 healthy individuals in the same age group were chosen. 2 mL of blood of each individual were collected in EDTA vacutainers. The blood was centrifuged immediately and the plasma was separated. It was subjected to a clinical test (immunoassay-chemiluminescence) and the homocysteine levels were measured clinically[14,15] in the reference range of 10-12 μmol/L[16]. Among the 10 patients with renal insufficiency, two had homocysteine levels greater than 30 μmol/L.

FT-IR spectra acquisition: The capillary blood samples (approximately 2 mL) of the patients and healthy individuals were collected. The blood was immediately centrifuged for 3 min to separate plasma from erythrocytes. The samples were then

stored at -20 ºC before analyses. After the samples returned to room temperature (25-30 ºC) a volume of 1 mL of serum was spread evenly over the surface of a thallium chromide pellet. All the specimens were air dried for 0.5 h prior to measuring the spectra[17]. The strong absorption band of water in the mid IR-region poses hindrance and hence to eliminate this, the serum samples were air dried. The dried serum forms a thin uniform film on the pellet[18]. Infrared transparent thallium chromide 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 spectra were baseline corrected and they were normalized to acquire identical area under the curves. The spectra were recorded in the wave number range of 4000-400 cm^{-1}.

RESULTS AND DISCUSSION

Assignment of absorption bands of plasma homocysteine: By careful inspection of the obtained spectra (Fig. 2), several spectral parameters can be identified as possible future biomarkers for the detection of elevated levels of plasma homocysteine.

The wide multiple band between 3300 and 2300 cm^{-1} corresponds to the anti-symmetric and symmetric stretching frequencies of N-H[19]. An absorbance peak was noticed at 3295 cm^{-1} due to N-H stretching vibrations. The spectra were dominated by absorbance bands at 1542 and 1656 cm^{-1}, *i.e.*, the amino acid and amide I bands, respectively[20]. The peak at 1542 cm^{-1} was due the bending vibration of NH_2. The amide I band showing a peak at 1656 cm^{-1} was due to stretching vibrations of C=O. The absorbance at 2930 and 1456 cm^{-1} were due to the asymmetric bending and asymmetric stretching vibrations of the CH_2 molecule. The bands in 2996-2819 cm^{-1} region were assigned to symmetric and asymmetric stretching vibrations of CH_2. The absorbance peak at 1480-1360 cm^{-1} was attributed to stretching vibrations characteristic of amino acids (COO^-)[21]. The C-S vibrations resulted in a band at 710-570 cm^{-1} with a maximum absorption at 698 cm^{-1}. No significant peaks could be detected for the weak vibrations corresponding to the disulphides (S-S) at 540-500 cm^{-1}[21,22]. The absorption bands corresponding to the weak stretching vibrations of thiols (S-H) were also insignificant due to its dimeric nature[19].

Calculation of internal ratio parameter: Among the various mathematical methods applied for classification in biology and medicine internal standard parameter calculation is one of the simplest procedures. In present study this technique was used to classify the patients with renal insufficiency with elevated homocysteine level from that of healthy individuals with the help of the FTIR spectra of corresponding groups. The internal standards for the specific modes of vibration of a healthy volunteer and those renal patients with elevated plasma homocysteine levels (> 30 µmol/L) are shown in Table-1. The internal standards for the specific modes of vibration of renal patients and healthy volunteers were also calculated and the results obtained are shown in Tables 2 and 3, respectively.

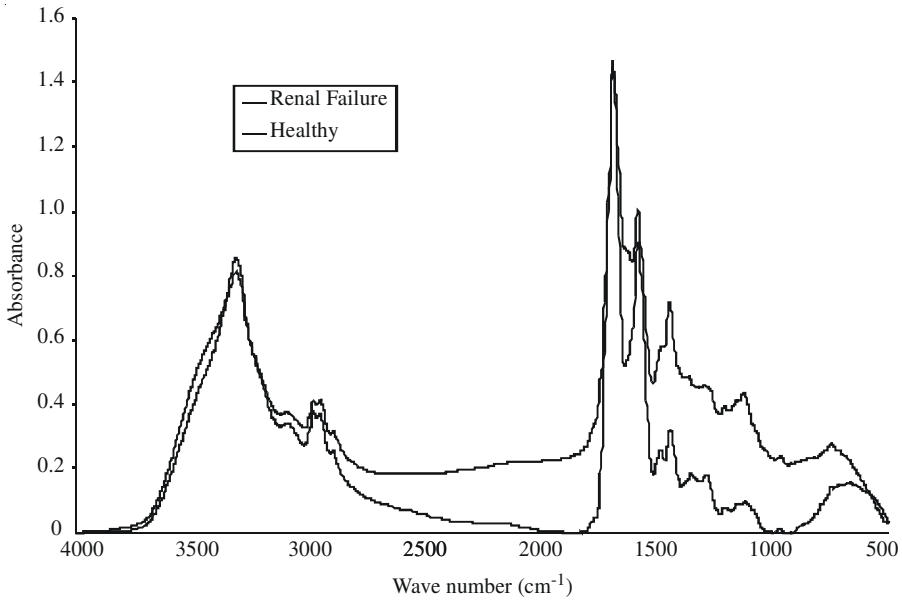


Fig. 2. Overlaid spectra of a renal failure patient and a healthy volunteer

TABLE-1
INTERNAL STANDARD EVALUATION OF PLASMA HOMOCYSTEINE FOR A HEALTHY VOLUNTEER AND RENAL FAILURE PATIENTS WITH ELEVATED HOMOCYSTEINE LEVELS (> 30 μmol/L)

Sample	Internal standard of specific modes of vibration at 3295 (cm^{-1})							
	$A_{3295/3295}$	$A_{2930/3295}$	$A_{2848/3295}$	$A_{1656/3295}$	$A_{1542/3295}$	$A_{1456/3295}$	$A_{1402/3295}$	$A_{698/3295}$
Healthy	1.0000	0.5479	0.3851	1.7949	0.0567	0.7350	0.8929	0.3249
Patient 1	1.0000	0.4235	0.2263	1.4948	0.8761	0.2696	0.3404	0.1736
Patient 2	1.0000	0.4314	0.2331	1.6906	1.0558	0.2970	0.3768	0.1653
Internal standard of specific modes of vibration at 2930 (cm^{-1})								
	$A_{3295/2930}$	$A_{2930/2930}$	$A_{2848/2930}$	$A_{1656/2930}$	$A_{1542/2930}$	$A_{1456/2930}$	$A_{1402/2930}$	$A_{698/2930}$
Healthy	1.8245	1.0000	0.6969	3.2757	2.3239	1.3414	1.6296	0.5929
Patient 1	2.3585	1.0000	0.5344	1.1004	2.0690	0.6368	0.8040	0.4099
Patient 2	2.3117	1.0000	0.5389	1.1056	2.4346	0.6866	0.8710	0.3820
Internal standard of specific modes of vibration at 2848 (cm^{-1})								
	$A_{3295/2848}$	$A_{2930/2848}$	$A_{2848/2848}$	$A_{1656/2848}$	$A_{1542/2848}$	$A_{1456/2848}$	$A_{1402/2848}$	$A_{698/2848}$
Healthy	2.6188	1.4358	1.0000	4.7305	3.3551	1.9249	2.3750	0.8511
Patient 1	4.4185	1.8711	1.0000	6.6288	3.9002	1.2117	1.5085	0.7669
Patient 2	4.2894	1.8600	1.0000	7.2520	4.5297	1.2970	1.61699	0.7114
Internal standard of specific modes of vibration at 1656 (cm^{-1})								
	$A_{3295/1656}$	$A_{2930/1656}$	$A_{2848/1656}$	$A_{1656/1656}$	$A_{1542/1656}$	$A_{1456/1656}$	$A_{1402/1656}$	$A_{698/1656}$
Healthy	0.5536	0.3035	0.21139	1.0000	0.7093	0.4069	0.5021	0.1799
Patient 1	0.6666	0.2823	0.1508	1.0000	0.5884	0.1828	0.2276	0.1157
Patient 2	0.5915	0.2565	0.1379	1.0000	0.6246	0.1788	0.2230	0.0981

Internal standard of specific modes of vibration at 1542 (cm^{-1})								
	$A_{3295/1542}$	$A_{2930/1542}$	$A_{2848/1542}$	$A_{1656/1542}$	$A_{1542/1542}$	$A_{1456/1542}$	$A_{1402/1542}$	$A_{698/1542}$
Healthy	0.7805	0.4279	0.2981	1.4099	1.0000	0.5737	0.7079	0.2520
Patient 1	1.1329	0.4797	0.2564	1.6996	1.0000	0.3107	0.3868	0.1966
Patient 2	0.9469	0.4106	0.2208	1.6010	1.0000	0.2863	0.2570	0.1570
Internal standard of specific modes of vibration at 1456 (cm^{-1})								
	$A_{3295/1456}$	$A_{2930/1456}$	$A_{2848/1456}$	$A_{1656/1456}$	$A_{1542/1456}$	$A_{1456/1456}$	$A_{1402/1456}$	$A_{698/1456}$
Healthy	1.3605	0.7459	0.5195	2.4576	1.7430	1.0000	1.2338	0.4421
Patient 1	3.6465	1.5441	0.8253	5.4658	3.2187	1.0000	1.2449	0.6329
Patient 2	3.3071	1.4341	0.7717	5.5913	3.4924	1.0000	1.2467	0.5484
Internal standard of specific modes of vibration at 1402 (cm^{-1})								
	$A_{3295/1402}$	$A_{2930/1402}$	$A_{2848/1402}$	$A_{1656/1402}$	$A_{1542/1402}$	$A_{1456/1402}$	$A_{1402/1402}$	$A_{698/1402}$
Healthy	1.1027	0.6045	0.4211	1.9918	1.4127	0.8105	1.0000	0.3584
Patient 1	2.9292	1.2404	0.6629	4.3945	2.5855	0.8033	1.0000	0.5084
Patient 2	2.6526	1.1503	0.6184	4.4849	2.8013	0.8021	1.0000	0.4399
Internal standard of specific modes of vibration at 698 (cm^{-1})								
	$A_{3295/698}$	$A_{2930/698}$	$A_{2848/698}$	$A_{1656/698}$	$A_{1542/698}$	$A_{1456/698}$	$A_{1402/698}$	$A_{698/698}$
Healthy	3.0770	1.6870	1.1750	5.5583	3.9422	2.2617	2.7906	1.0000
Patient 1	5.7612	2.4395	1.3039	8.6432	5.0853	1.5799	1.9668	1.0000
Patient 2	6.0296	2.6147	1.4057	10.1943	6.36755	1.8233	2.2731	1.0000

TABLE-2
INTERNAL RATIO PARAMETER OF THE SPECIFIC MODES OF VIBRATION OF PLASMA HOMOCYSTEINE AMONG RENAL FAILURE PATIENTS

Samples	A_{3295}/A_{2930}	A_{2930}/A_{2848}	A_{1656}/A_{1542}	A_{1542}/A_{1456}	A_{1456}/A_{1402}
1	2.0196	2.3133	1.5060	2.0540	0.8722
2	2.2441	1.8047	1.5784	3.0741	0.8156
3	2.6372	2.4190	1.5557	2.5277	0.8477
4	2.5811	2.1889	1.6279	2.9723	0.8099
5	2.1689	2.0177	1.6862	2.9819	0.8474
6	2.3203	2.5755	1.4815	2.9255	0.8555
7	2.2593	2.1703	1.6212	2.9819	0.8575
8	2.6796	1.6966	1.5790	2.9254	0.8421

TABLE-3
INTERNAL RATIO PARAMETER OF THE SPECIFIC MODES OF VIBRATION OF PLASMA HOMOCYSTEINE AMONG HEALTHY VOLUNTEERS

Samples	A_{3295}/A_{2930}	A_{2930}/A_{2848}	A_{1656}/A_{1542}	A_{1542}/A_{1456}	A_{1456}/A_{1402}
1	1.8239	1.4357	1.4021	1.7430	0.8094
2	1.8323	1.4103	1.4001	1.7229	0.7947
3	1.8976	1.4669	1.4169	1.6464	0.8043
4	1.9536	1.4951	1.4785	1.8129	0.7849
5	1.8552	1.4406	1.4545	1.8688	0.8061
6	1.9831	1.4505	1.4229	2.1368	0.8033
7	1.9374	1.5931	1.5189	2.1900	0.7435
8	2.0820	1.4452	1.5214	2.1536	0.7025

Conclusion

In the present study we examined the potential of FTIR spectroscopy for identifying elevated levels of plasma homocysteine among patients with renal insufficiency by comparing with a healthy volunteer with optimal levels of plasma homocysteine and to identify specific modes of vibrations pertaining to plasma homocysteine. We were able to distinguish between the healthy individual from that of renal patients with elevated homocysteine levels by calculating the internal standards for the specific modes of vibration. Although the results obtained in this study could be considered only as preliminary results, it forms a promising basis for a future study including a large number of samples. Furthermore, for this technique only a small amount of plasma is required and the results can be obtained in a very short time. As it is cheaper when compared to clinical tests it is worthwhile to continue developing this technique as an efficient and reliable tool for the diagnosis and identification of plasma homocysteine levels.

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