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Efficacy of vitamin supplementation on plasma homocysteine levels among hyperlipidemic patients – a spectral and clinical analysis

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ABSTRACT

Homocysteine, 2-amino-4-mercaptobutyric acid is a sulphur-containing non-essential amino acid biosynthesized from methionine in blood plasma. It is metabolized through two pathways, remethylation and transulfuration, which use as cofactors folate, vitamin B6 and vitamin B12. Hyperhomocysteinemia has been identified as a risk factor for cerebrovascular disease, dementia, impaired cognitive function and depression. Several drugs may interfere with metabolic pathways of homocysteine, leading to an alteration of plasma homocysteine levels. Lipid lowering drugs used by patients with high levels of plasmatic lipids can increase plasma homocysteine levels.For the present study five hyperlipidemic patients were enrolled. Before the initiation of vitamin supplements along with their regular medication the FTIR spectra of the blood plasma was recorded and their homocysteine levels were clinically tested. They were orally administered a daily dosage of folic acid(5 mg), vitamin B12(250mcg) and vitamin B6(25mg) supplements for a period of two months. Efficacy of these vitamin supplements were analyzed both clinically and spectroscopically. The FTIR spectra were recorded at the end of the first and the second month and also the homocysteine levels were clinically tested. The absorption values of the specific modes of vibration pertaining to homocysteine of both pre and post-treatment spectra were noted and the percentage of efficacy of the multivitamins was calculated. The spectral and clinical investigation showed that the addition of these vitamins can markedly reduce the homocysteine levels in blood plasma.
Keywords: FTIR spectroscopy, plasma homocysteine, vitamin supplementation, hyperlipidaemia.

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INTRODUCTION

Homocysteine is a thiol – (sulfhydryl-) containing non essential amino acid which converts itself to several beneficial compounds required for energy including ATP, cysteine and S-adenosylmethionine (SAM-e). If homocysteine is not completely broken down it begins to cause oxidative damage to the walls of the arteries, oxidation of blood fats and abnormal blood clotting by making the platelets stick together[1]. Homocysteine also enhances the binding of lipoprotein-a to fibrinogen initiating a series of biochemical reactions eventually leading to blockages [2]. Blocking may be followed by heart attacks, strokes and other circulation calamities. The enzyme co-factors especially vitamin B6, vitamin B12 and folic acid play a vital tool in the metabolism of homocysteine [3].

Homocysteine is metabolized via remethylation, resulting in the formation of methionine, or via transulfuration, resulting in the formation of cysteine and finally taurine [4]. The remethylation pathway of homocysteine is strongly dependent on the availability of folic acid in the active form of 5-methyltetrahydorfolate and vitamin B12. The latter is essential for optimal activity of the enzyme methionine synthase, which is responsible for methylation of homocysteine to methionine [5]. In the transulfuration pathway an essential cofactor is the active form of vitamin B6, pyridoxal -5'-phosphate P5P. Deficiency of any of these vitamins is associated with hyperhomocysteinemia [6]. Many drugs increase the level of homocysteine either by interfering with the metabolism of folate or vitamin B6 or vitamin B12 [7]. Certain lipid lowering drugs administered in patients with high levels of plasmatic lipids can increase homocysteine levels [8]. Elevated levels of plasma homocysteine have been documented in epileptic patients after chronic treatment with anti epileptic drugs [9].

There have been a number of clinical reports about the role of vitamin supplementation in normalizing homocysteine levels among hyperlipidimic and epileptic patients [10]. Only a very few researchers have analyzed the efficacy of drugs spectroscopically [11, 12]. The goal of this study is to determine the percentage of efficacy spectroscopically and substantiate it with the clinically obtained results. The former has lot of advantages and hence can be implemented as a prospective tool for the diagnosis and monitoring of plasma homocysteine levels.

MATERIALS AND METHODS

Subjects and Methods

A group of five hyperlipidemic patients of the same age and blood group were enrolled for the study. They were undergoing treatment in the Deepam Hospital, Chennai. Before the administration of vitamin supplements along with their regular medication, the FTIR spectra of the blood plasma were recorded and their homocysteine levels were clinically tested. They were orally administered a daily dosage of folic acid(5 mg), vitamin B12(250mcg) and vitamin B6(25mg) supplements for a period of two months. The FTIR spectra were recorded at the end of the first and the second month and also the homocysteine levels were clinically tested.

Clinical Analysis

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 in the reference range of 10µmol/l to 12µmol/l [13]. Almost all the patients enrolled for the study had homocysteine levels much greater than 12µmol/l before they were administered with vitamin supplements (pre-treatment). There was a marked reduction in the plasma homocysteine levels at the end of the first month (Day 30) and second month (Day 60). The clinical values of the measured homocysteine levels are shown in Table 1.

FT-IR spectra acquisition

The capillary blood samples (approximately 2ml) of the patients before they were administered with vitamin supplements were collected. The blood was immediately centrifuged to separate plasma from erythrocytes. The samples were then stored at -20ºC before analyses. After the samples returned to room temperature (around 25 ºC - 30 ºC) a volume of 1ml of serum was spread evenly over the surface of a thallium chromide pellet. The specimen was air dried for thirty minutes prior to measuring the spectra [14]. 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 [15]. 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 400cm^{-1} – 4000cm^{-1} on a Perkin-Elmer FTIR spectrometer at Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology, Chennai, India. The spectra of the patients were recorded again at the end of the first month (Day 30) and second month (Day 60) after administrating the vitamin supplements.

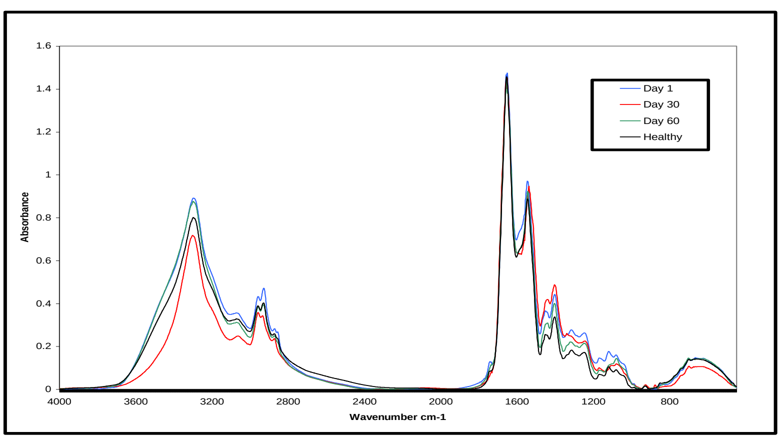

Fig.1. An overlaid spectrum to show the efficacy of vitamin supplements on homocysteine in an hyperlipidemic patient

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RESULTS AND DISCUSSION

Assignment of absorption bands of plasma homocysteine

By careful inspection of the obtained spectra, several spectral parameters can be identified as possible biomarkers for the detection of elevated levels of plasma homocysteine. The wide multiple bands between 3300 and 2300 cm^{-1} corresponds to the anti-symmetric and symmetric stretching frequencies of N-H [15]. 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 [16]. 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 at 2996 – 2819 cm^{-1} 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^-) [17]. The C-S vibrations resulted in a band at 570 – 710 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 500 – 540 cm^{-1} [18-19]. The absorption bands corresponding to the weak stretching vibrations of thiols (S-H) were also insignificant due to its dimeric nature [15].

Calculation of percentage of efficacy

In order to find the efficacy of folic acid, vitamin B6 and vitamin B12 in bringing down the homocysteine levels, the absorption values of the vibrational bands at 3295cm^{-1}, 2930 cm^{-1}, 2848 cm^{-1}, 1656 cm^{-1}, 1542 cm^{-1}, 1456 cm^{-1}, 1402 cm^{-1} and 698 cm^{-1} corresponding to plasma homocysteine of both pre- and post treatment spectra were noted. The percentage of efficacy was calculated using the formula,

$$\% \text{ of efficacy of vitamin supplements} = ((\text{Pre} - \text{Post})/\text{Pre}) * 100$$

The results are shown in table 1

Table 1 **Efficacy of vitamin supplements on homocysteine among hyperlipidemic patients**

Sample	Status	Clinical values µmol/l	Absorbance of specific modes of vibration (cm^{-1})							
			3295	2930	2848	1656	1542	1456	1402	698
1	Day 1	26.82	0.7799	0.3843	0.1825	1.4711	0.9234	0.2963	0.3463	0.1196
	Day 30	20.33	0.7565	0.3654	0.1443	1.4547	0.8785	0.2603	0.3158	0.0944
	% of efficacy	-24.20	-3.00	-4.9	-20.9	-1.10	-4.8	-12.15	-8.8	-10.36
	Day 60	16.75	0.7122	0.3166	0.1342	1.4451	0.8746	0.2520	0.3084	0.0972
	% of efficacy	-37.55	-8.70	-17.6	-26.4	-1.76	-5.2	-14.95	-10.9	-12.35
2	Day 1	16.03	0.8016	0.4807	0.2373	1.4319	0.9653	0.3643	0.3946	0.1103
	Day 30	15.15	0.7925	0.4496	0.2157	1.4244	0.9214	0.3188	0.3705	0.1098
	% of efficacy	-5.49	-1.14	-6.46	-9.14	-0.52	-4.55	-12.49	-6.11	-0.45
	Day 60	14.64	0.7687	0.4579	0.2070	1.4020	0.9190	0.3170	0.3694	0.1095
	% of efficacy	-8.64	-4.10	-4.74	-12.80	-2.09	-4.80	-12.98	-6.39	-0.45
3	Day 1	15.84	0.9835	0.4046	.2314	1.4712	0.9169	0.3072	0.3386	0.1536
	Day 30	12.44	0.8144	0.3447	0.1512	1.4657	0.8851	0.2774	0.3177	0.1437
	% of efficacy	-21.46	-17.19	-14.78	-34.66	-0.37	-3.47	-9.70	-6.17	-6.45
	Day 60	11.26	0.7592	0.3367	0.1497	1.4598	0.8753	0.2782	0.3058	0.1300
	% of efficacy	-28.91	-22.81	-16.76	-35.31	-0.77	-4.54	-9.44	-9.69	-15.43
4	Day 1	17.12	0.8081	0.4439	0.2335	1.4615	0.9108	0.3422	0.3781	0.1181
	Day 30	12.90	0.7952	0.4275	0.2039	1.4414	0.9063	0.2951	0.3385	0.0977
	% of efficacy	-24.65	-1.60	-3.69	-12.68	-1.38	-0.49	-13.76	-10.47	-17.02
	Day 60	11.64	0.7763	0.4007	0.1961	1.4242	0.8858	0.2899	0.3301	0.0900
	% of efficacy	-32.01	-3.94	-9.73	-16.02	-2.55	-2.74	-15.28	-12.70	-31.41
5	Day 1	20.81	0.8569	0.4006	0.2277	1.4737	0.9091	0.2981	0.3487	0.1286
	Day 30	17.81	0.8324	0.3366	0.1501	1.4467	0.8619	0.2767	0.3229	0.0972
	% of efficacy	-14.45	-2.86	-15.98	-34.08	-1.83	-5.19	-7.15	-7.40	-24.41
	Day 60	16.43	0.6826	0.3268	0.1497	1.4051	0.8542	0.2654	0.3158	0.0954
	% of efficacy	-21.05	-20.34	-18.42	-34.26	-4.65	-6.04	-10.97	-9.44	-25.82

CONCLUSION

The present study was undertaken to utilizing the potential of FTIR spectroscopy in analyzing the efficacy of vitamin supplementation on plasma homocysteine levels among hyperlipidemic patients. The specific modes of vibrations pertaining to plasma homocystine were identified. The percentage of efficacy after 30 days and 60 days of initiation of vitamin supplementation were calculated using the absorption values at the specific modes of vibration. The plasma homocysteine levels had decreased with the progress of the treatment. The spectroscopical outcome was substantiated with the clinical results. This study forms a promising basis for employing spectroscopy in the follow-up of patients undergoing treatment for various ailments. This technique requires a small amount of plasma and the results can be obtained in a short duration. It is much cost effective when compared to clinical tests. It is therefore worthwhile to continue developing spectroscopy as an effective and reliable tool for the diagnosis and follow-up of disease pattern.

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