FTIR SPECTRAL STUDY ON NORMAL AND TYPHOID BLOOD SAMPLES

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ABSTRACT

FTIR spectroscopy has been widely used in the field of medicine. FTIR spectroscopy is an effective and non-destructive method to monitoring cellular changes. Diseases of several organs have been identified by analysing the structure and the quality of biomolecules in biological samples such as protein, nucleic acids and lipids obtained from FTIR spectroscopy. Typhoid fever is endemic in developing countries and its real impact is difficult to estimate. The main objectives of the present study are; to analyse normal blood sample using FTIR spectrum, to compare spectral signature of normal and typhoid sample, to measure bio-markers for typhoid blood samples with specific peaks and compare them with normal blood samples. Total 60 subjects were studied, 30 normal individuals and 30 typhoid patients. The spectra are recorded in the mid infrared region of 4000-400 cm^{-1} in the absorption mode on Perkin Elmer One Spectrometer and the spectra were normalized. The measurement were carried out at SAIF, IIT Madras. From this study, typhoid patient are having significantly higher concentration of protein while glucose, lipids and phosphate were significantly lower than those of normal individuals, typhoid patients having low concentration of glucose compared with lipids and phosphates. The level of glucose decreases because *S.typhi* requires glucose in macrophages for replication. The symptoms of high level of protein and low level of lipids glucose and phosphate in blood is similar to the symptoms of typhoid fever. The observed results were matched with the clinical data and theresults were analysed using an independent samples t-test.

Keywords: Typhoid Fever, FTIR Spectroscopy; Biomarkers; t-test

INTRODUCTION

Typhoid (enteric) fever is the systemic infection caused mainly by S.*typhi* characterized by a continuous fever for 3 to 4 weeks. It affect 30 million individuals worldwide each year. Typhoid fever is transmitted by contaminated food and water by feces and urine of patients and carriers [1]. Fourier transform infrared (FTIR) spectroscopy is one such area that as seen rapid development in the past decade with a promise of easier, rapid and objective diagnosis [2]. FTIR spectroscopy is also an effective and non-destructive method to monitor cellular changes [3, 4]. Diseases of several organs have been identified using structure and quantity of bio-molecules in biological samples such as protein [5], nucleic acid [6] and lipids [7]. The literature survey reveals that to the best of our knowledge, no such report to monitor the spectral changes in typhoid and normal blood sample. The main objectives of the present study are; to analyse normal blood sample using FTIR spectrum, to compare spectral signature of normal and typhoid samples, to measure bio-markers for typhoid blood samples with specific peaks and compare them with normal blood samples. The result were analysed by independent samples t-test. Independent samples t-test compare the means of two independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different. The independent samples t-test is a parametric test. Independent t-test only compare the means of two groups.

MATERIALS AND METHODS

Blood samples are collected from 30 normal individuals and 30 suffering due to typhoid fever. The blood samples is allowed to coagulate without adding any anti-coagulant agent for about half an hour the serum was separated from every sample and centrifuge at the speed of 1200 rpm in REMI electric centrifuge. The serum was subjected to FTIR spectroscopy technique. FTIR spectral measurement of all the samples were carried out of Sophisticated Analytical Instrumentation facility (SAIF) IITM, Chennai 36, using spectrum one Perkin-Elmer FTIR spectrophotometer the spectra are recorded in the mid infrared of 4000-400 cm^{-1} in the absorption mode. FTIR spectra obtained by spreading a small volume of serum on a KBr pellet (IR transparent material) and allowed to dry for few minutes to remove the water bands. To minimize problems from avoidable baseline shifts, the spectra were baseline corrected and normalized.

RESULTS AND DISCUSSION

FTIR absorption spectrum of normal blood sample is shown in **Figure 1**. The important

groups of vibration were identified in the FTIR spectrum of normal blood sera presented in **Table 1**. The spectral region 3600 to 3000cm^{-1} comprises of C-H, O-H stretching vibration of the proteins **[8]**. The prominent absorption peak 3293cm^{-1} is due to the N-H stretching mode (amide A) of proteins. The asymmetric and symmetric stretching C-H vibration of methane and methylene groups are found to be present around 2930 – 2873cm^{-1}. The strong absorption band at 1655cm^{-1} corresponds to C=O stretching vibration (amide I) whereas the vibration band at 1541cm^{-1} is attributed at amide II arising of N-H bending vibration strongly coupled with C-N stretching of protein **[9]**. The absorption peak in the region 1400-1200 cm^{-1} arises due to C-H deformation of methyl methylene group of the proteins. The absorption peak in the region 1313cm^{-1} correspond to deformational vibrational mode of methyl groups. The absorption peak in the region 1242 cm^{-1} arises due to asymmetric PO_2 stretching of lipid phosphates. The absorption peak in the region 1168 and 1086 cm^{-1} correspond to C-O stretching vibrations.

Figure 2 represents the comparison of FTIR spectrum of normal and typhoid blood sample. FTIR spectrum exhibits vibrations bands characteristics of the various group frequencies, the spectrum of a normal blood sample and that of a typhoid affected blood samples are the same with respect to the positions of the peaks but different in of the absorption level of the peaks. It is clear that, the absorption peak of lipids, glucose and phosphate for typhoid patient is less than the normal person.

Three intensity ratio parameters R_1 [Protein (1400cm^{-1})/Glucose (1080cm^{-1})], R_2 [Amide II (1541cm^{-1})/Glucose (1035cm^{-1})] and R_3 [Lipid (2873cm^{-1})/Glucose (1035cm^{-1})] for normal (30) and typhoid patients (30) are calculated and tabulated in **Table 2**. The mean intensity ratio of R_1, R_2, and R_3 are determined using t-test and tabulated in **Table 3**. **Figure 3** represents the mean intensity ratio of Protein (1400cm^{-1})/Glucose (1080cm^{-1}) for normal and typhoid individuals. The mean intensity ratio (R_1) level were 2.6762±0.3583 in typhoid patients and 1.4959±0.34708 in normal individuals. **Figure 4** represents the mean intensity ratio of Amide II (1541cm^{-1})/Glucose (1035cm^{-1}) for normal and typhoid individuals. The mean intensity ratio (R_2) level were 8.5404±0.82462 in typhoid patients and 2.8505±1.03264 in normal individuals. **Figure 5** represents the mean intensity ratio of Lipids (2873cm^{-1})/Glucose (1035cm^{-1}) for normal and typhoid individuals. The mean intensity ratio (R_3) level were 2.3406±0.24562 in typhoid

patients and 1.2495 ± 0.41417 in normal individuals.

Results were also analysed using an independent sample t-test and tabulated in **Table 4**. This analysis revealed a significant difference between the normal and typhoid patients **[10-13]**. For R_1: t (58) $=-12.957$; p$=0.000$, R_2: t (58) $=-23.583$; p$=.000$, R_3: t (58) $=-12.411$; p$=0.000$. t is the ratio of mean difference to the standard error difference. The mean difference is determined by subtracting the mean of the second group from the mean of the first group. The negative t-value represent the mean ratio of second group is significantly higher than the first one. Since p$=.000$, the null hypothesis that the mean ratio (R_1, R_2, R_3) of typhoid and normal individuals are the same. Thus the intensity ratio parameters R_1, R_2, R_3 for typhoid patients were associated with statistically significantly higher than the normal patients. Additionally, the homogeneity of variances was tested and satisfied via Levene's test. For R_1: F (58) $=.596$, p$=.443$, R_2: F (58) $=.001$ p$=.974$, R_3: F (58) $=1.383$, p$=.244$.

By clinical data serum glucose level (mg/dl) for normal person is 172.6 ± 7.5 and typhoid patient is 82.92 ± 0.07 (p$=0.0006$). Total protein (g/dl) for normal person is 7.82 ± 0.11 and typhoid patient is 8.65 ± 0.38 p (0.0978).So the observed resultsare matched with the clinical data. *S. typhi* requires glycolysis and glucose for successful infection of macrophages that cause infection **[14]**. By this glucose level is low in typhoid fever patients. Serum total protein was significantly higher in typhoid fever patient irrespective of their gender.
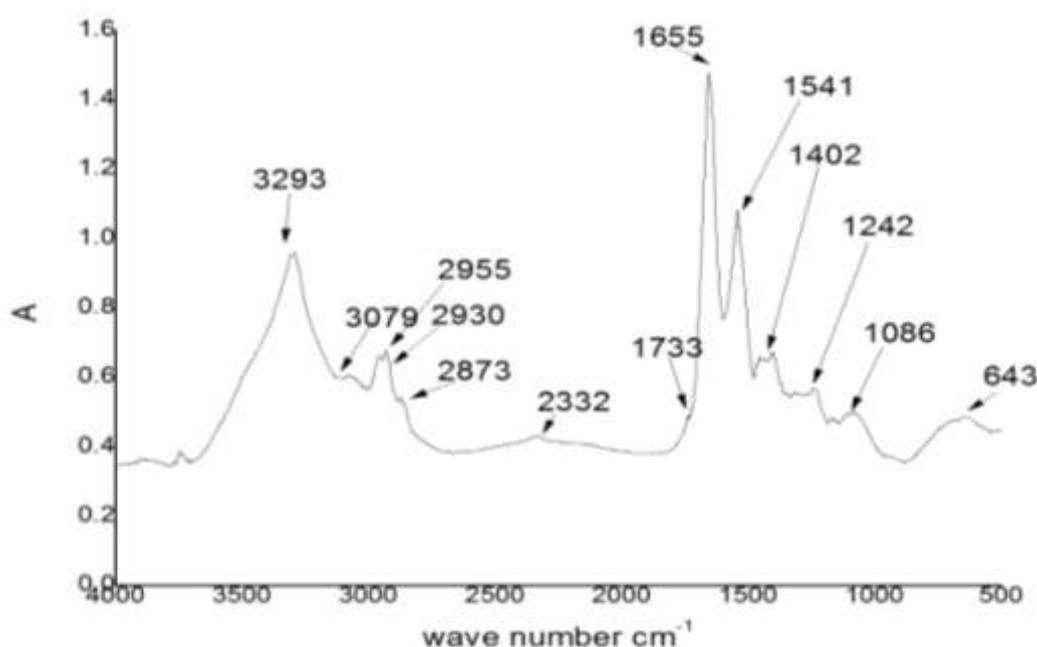

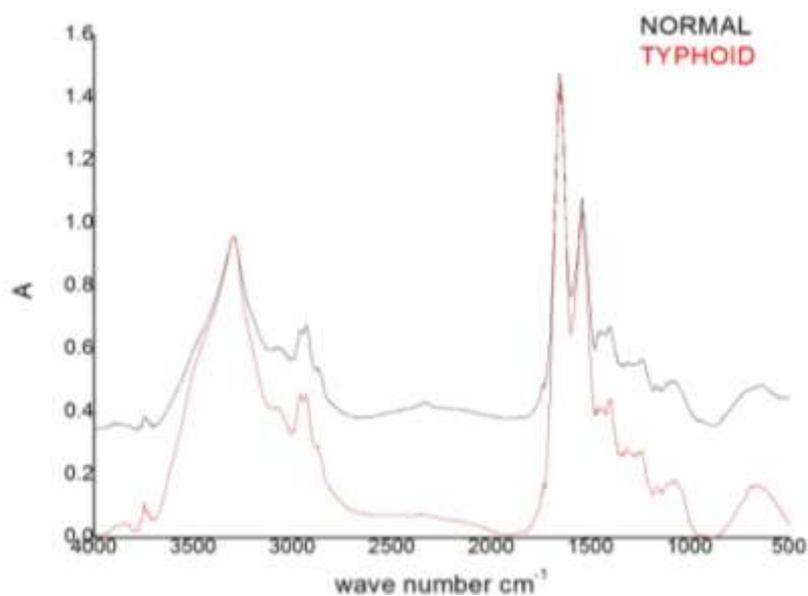**Figure 1: FTIR spectrum of normal blood sample**

Figure 2: FTIR spectrum of normal and typhoid blood sample
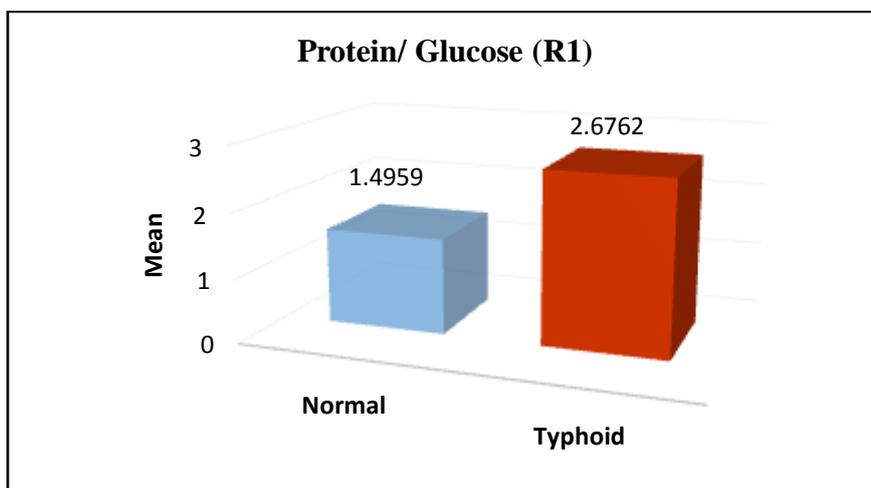

Figure 3: Mean intensity ratio of Protein (1400cm^{-1})/ Glucose (1080cm^{-1}) for normal and typhoid individuals
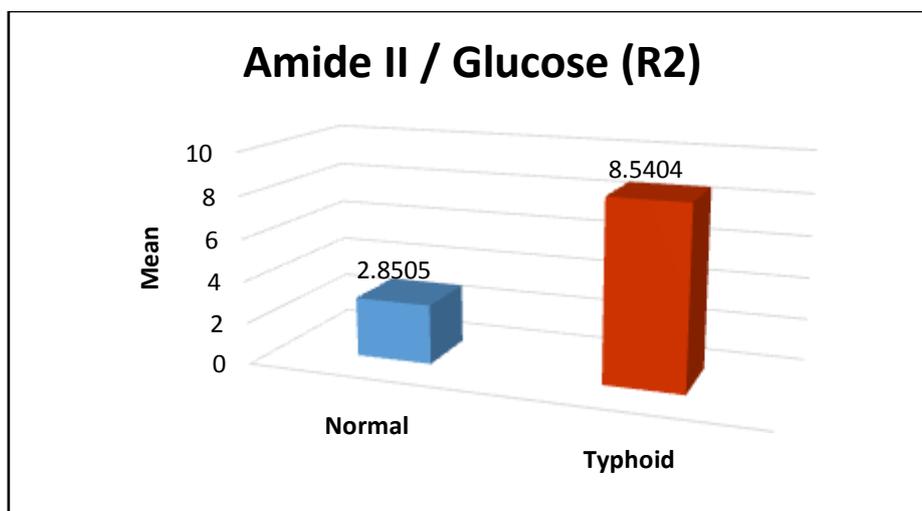

Figure 4: Mean intensity ratio of Amide II (1541cm^{-1})/ Glucose (1035cm^{-1}) for normal and typhoid individuals
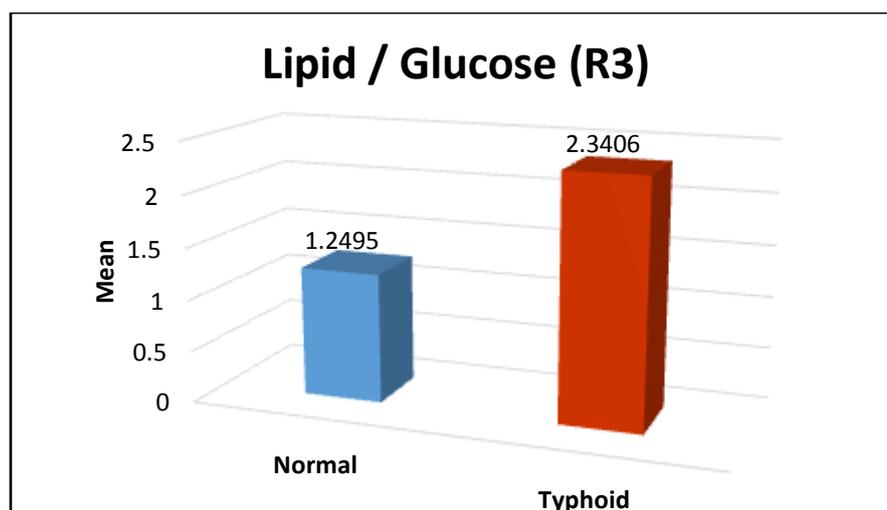
Figure 5: Mean intensity ratio of lipid (2873cm⁻¹)/ Glucose (1035cm-1) for normal and typhoid individuals

Table 1: Vibrational Band Assignment

Wave number (cm⁻¹)	Description
3293	N-H stretching mode (Amide A) of Proteins
3079	Amide –B band due to overtone of Amide I bands
2873	C-H stretching of methane and methylene groups
1655	C=O starching vibration (Amide I)
1402	C-H deformation of methyl methylene group of the Proteins
1313	Deformational vibrational mode methyl groups
1242	Asymmetric PO_2 stretching of lipid phosphate
1168	Ring vibrational mode of C-O-H and C-O-C bands
1086	C-O stretching vibration

Table 2: Intensity ratio parameters of normal and typhoid individuals

Protein (1400cm⁻¹)/Glucose (1080cm⁻¹) [R₁]		Amide II (1541cm⁻¹)/Glucose (1035cm⁻¹) [R₂]		Lipid (2873cm⁻¹) / Glucose (1035cm⁻¹) [R₃]	
Normal	Typhoid	Normal	Typhoid	Normal	Typhoid
1.079	2.4635	1.6566	8.242	1.0499	2.6494
1.17581	4.025	2.3683	10.1985	0.7404	2.7764
1.331	2.9063	2.5367	7.549	1.2154	2.1079
2.2821	2.4305	6.1213	7.5239	2.0029	2.1328
1.5372	2.5616	2.863	9.0337	1.1829	2.1404
1.3403	2.3492	2.362	7.2346	1.1856	2.2011
1.4035	2.7299	2.5643	9.2484	1.118	2.7987
2.0105	2.4078	5.5045	6.3377	1.9485	2.555
1.6614	2.6247	3.7452	8.1408	1.886	2.1798
1.1728	2.7853	2.5314	8.7104	1.2996	2.2322
1.4032	2.4073	2.7382	9.2823	0.9912	2.5108
1.5644	2.4532	2.6518	9.1754	0.1256	2.2511
1.1813	3.2076	1.9873	8.2153	0.8718	2.1598
1.3439	2.7526	2.0319	7.9368	1.221	2.0305
1.074	2.5321	1.9906	7.5528	1.1819	2.1116
1.3278	2.6261	1.7321	7.5624	1.2076	2.0359
1.4153	2.5411	2.2356	9.3251	1.9356	2.6261
2.0011	2.4536	2.7106	9.1001	1.1153	2.2352
2.1855	2.4036	2.551	8.9021	1.1918	2.0351
1.7832	2.707	2.416	8.7333	1.1173	2.1931
1.5549	2.8287	2.1128	9.26	2.0032	2.5132
1.6279	2.7265	2.9813	9.115	1.8359	2.2257
1.5703	2.5512	4.7321	8.9278	0.9351	2.2271
1.7811	2.4153	4.0326	9.1153	0.9391	2.7009
2.0251	2.6612	2.9172	9.3256	1.3756	2.5119
1.5532	2.5716	2.5406	8.8217	1.1126	2.1409
1.0645	2.3432	2.918	7.981	1.0909	2.1715
1.1021	2.7351	2.8883	8.021	1.0815	2.5541
1.0504	2.5648	2.9214	9.1022	1.2529	2.7206
1.27541	3.5214	2.1735	8..53925	1.26879	2.48792

Table 3: Mean intensity ratio parameters of normal and typhoid individuals usingt-test

	Normal				Typhoid			
	N	Mean	Standard Deviation	Standard Error Mean	N	Mean	Standard Deviation	Standard Error Mean
Protein (1400cm^{-1})/Glucose (1080cm^{-1}) [R$_1$]	30	1.4959	0.34708	0.06337	30	2.6762	0.35843	0.06544
Amide II(1541cm^{-1})/Glucose (1035cm^{-1}) [R$_2$]	30	2.8505	1.03264	0.18853	30	8.5404	0.82462	0.15055
Lipid (2873cm^{-1}) / Glucose(1035cm^{-1}) [R$_3$]	30	1.2495	0.41417	0.07562	30	2.3406	0.24562	0.04484

Table 4: Independent sample test

		levene's Test for Equality of Variances	t-test for Equality of Means							
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Protein / Glucose (R$_1$)	Equal variances assumed	0.596	0.443	12.957	58	0.000	-1.1803	0.09109	1.36263	0.99795
	Equal variances not assumed			-12.957	57.94	0.000	-1.1803	0.09109	-1.36264	-0.99795
Amide II/ Glucose (R$_2$)	Equal variances assumed	0.001	0.974	-23.583	58	0.000	-5.6899	0.24127	-6.17286	-5.20695
	Equal variances not assumed			-23.583	55.294	0.000	-5.6899	0.24127	-6.17337	-5.20645
Lipid / Glucose (R$_3$)	Equal variances assumed	1.383	0.244	-12.411	58	0.000	-1.0911	0.08791	-1.26707	-0.91511
	Equal variances not assumed			-12.411	47.153	0.000	-1.0911	0.08791	-1.26794	-0.91425

CONCLUSION

The study of blood by spectroscopic techniques can be used not only for understanding the biological nature of the disease, but also for the diagnosis of the disease.This paper mainly concentrated on the spectral study of typhoid blood samples. FTIR spectrum of normal blood sera compared with typhoid blood sera. The spectrum are same with respect to the position and different in absorption level of specified peaks. The spectral studies and analysis by t-test, significant difference in the intensity ratio parameters for normal and typhoid patients. The observed results matched with clinical data. By this study, it is clear that the typhoid patients having significantly higher concentration of protein and lower concentration of glucose with normal person.

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