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Efficacy Study of Asthmatic Disease using Single Human Hair Fiber – A Spectroscopic Approach

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Abstract: ***Passing from normal tissue to pathological tissue, cellular biochemistry changes. From a diagnostic and therapeutic point of view, it is fundamental to study the physical and chemical changes occurring in the tissues and cells due to diseases and disorders. During the recent times the use of Fourier Transform Infrared spectroscopy (FTIR) has received quite a lot of attention not only for understanding the biological nature of the disease, but also for diagnosing it. In this study, it has been demonstrated that the study of FTIR-ATR spectra of single human hair fiber is used to differentiate between the healthy and asthmatic individuals. Significant differences are observed in terms of optical density of the absorption bands and a satisfactory analysis has been made using paired t – test*** **[1].**

Keywords: Pathological tissue, cellular biochemistry, FTIR-ATR spectroscopy, asthmatic individuals and Optical density

1. Introduction

Asthma is a chronic inflammatory disorder of the airways characterized by airways hyper-responsiveness (AHR) and reversible air flow obstruction that fluctuates overtime. Airway obstruction and allergic inflammation during the disease occurs due to the release of Immunoglobulin E (IgE) and pro-inflammatory cytokines and because of several substances like histamines, leukotrienes, prostaglandins and eosinophils being released by mast cells during an asthma attack [2&3]. Current diagnosis of asthma is based on the history of wheeze, shortness of breath and cough, which are variable in severity and overtime [4]. Human hair is an inert and chemically homogenous tissue. The bio molecules present in the human hair are primarily composed of a fibrous protein called keratin, lipids, nucleic acid, glycogen(Carbohydrates), trace elements and some amount of water [5&6] and makes it as a probe in the investigation of various disorders and diseases in human. The hair of asthmatic individuals shows high level of proteins, lipids and amino acids. The bio-molecular changes in the hair could be well scrutinized using molecular spectroscopic technique, and one such is the FTIR-ATR technique. Eventually, the FTIR spectral analysis on human hair provides a rapid, non-invasive and effective method in diagnosing diseases such as asthma and detecting what molecular and chemical changes have developed for the disease. Several IR approaches to the study of hair are reported in the literature [7&8].

2. Materials and Methods

2.1 Materials

Samples of human scalp hair fiber from 10 asthmatic patients were collected from each subject before and about half an hour after the intake of the asthmatic drug Doxofylline from Chest and Thorax clinic at Chennai, India; together with hair samples from 10 healthy individuals, after getting their written consent. The single hair sample was pluck from the hair root anagen (actively growing) phase, with an intact bulb was carefully selected and stored separately in non-reactive plastic envelopes. Full-length single hair specimens with the root "club" intact were subjected to no prior chemical treatments and only basic grooming, i.e., brushing, combing and shampooing. Before analysis, all the full-length hair specimens were washed by soaking it in acetone and then in distilled water for a minute to eliminate any surface contaminations and the specimens were given laminar air flow to remove the water thoroughly.

2.2.1. Experimental Method

The measurement of hair samples with FTIR-ATR spectroscopy were carried out at Saif - SPU, Chennai-54, using Perkin-Elmer Spectrum Two FTIR/ATR spectrophotometer. Each spectrum was measured in 4000 – 450cm^{-1} range with a 4cm^{-1} resolution and with 16 scans. All the samples were investigated by placing it on the crystal of 2mm surface area with single bounce reflection has 350 cm^{-1} as its cut-off wave number; suitable pressure of about 140N was given to the hair sample to make good optical contact between the sample and the internal reflectance element (IRE) the diamond. These spectra were subtracted against the background of air spectrum. After every scan, the crystal is cleaned with isopropyl alcohol or methanol soaked tissue and a background of new reference air was taken to ensure the crystal cleanliness. The spectra were constructed using the software „Spectrum", provided with FTIR Spectrum Two Spectrophotometer.

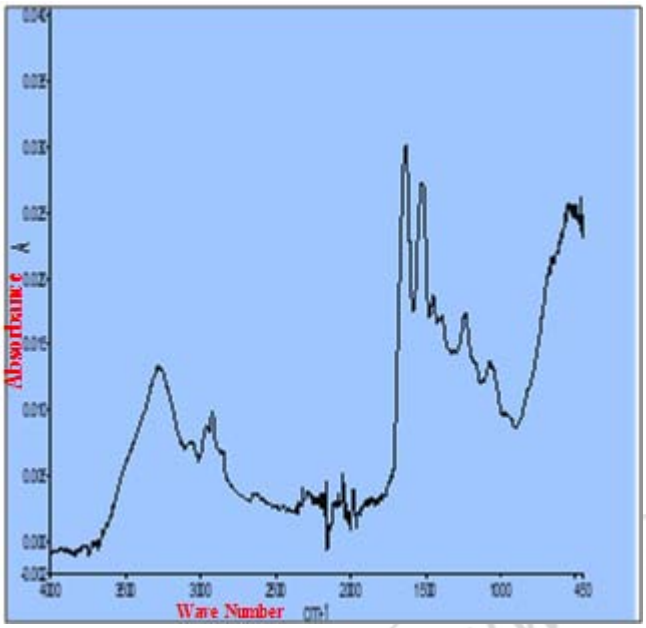


Figure 1: FTIR spectra of Healthy Hair Fiber

2.2.2 Statistical Analysis

Analysis of variance was implemented to identify the spectral variations that were statistically significant. The t-test is one of the most rapid procedures for classification of biological data. In current study, the t-test was used to differentiate certain regions of the FTIR spectra of the examined healthy hair and diseased hair samples of the Asthmatic patients. For entire spectrum from 4000 to 450c m^{-1}, statistical analysis was performed by t-test and it shows the full successful classification to distinguish pre-treated from post-treated hair samples. In t-test analysis, considering the mean difference variance of the analysis, the t-test was carried out. The absorbance values observed gives a macroscopic value difference as compared to the minute variance observed in the hair analysis using FTIR –ATR spectroscopic technique.

The following are the intensity ratio parameters of Healthy, pre and post-treated hair samples; R_1, R_2, R_3, R_4, R_5 and R_6. Using FTIR-ATR, hair samples were analyzed and the results were compared and statistically analyzed using the t-test. The mean variance and standard deviation were observed and showed that there is a significant difference between healthy, pre and post-treated hair samples values. This test is used for correlating the means of two samples, even if they have unequal numbers of replicates.

In simple terms, comparing the actual difference between two means in relation to the variation in the data expressed as the standard deviation of the difference between the means using the t-test. Statistical tests allow for making statements with a higher degree of precision, but cannot actually proving or disproving anything. Significant results at the 95% probability level make perfect data, which is good enough to support a conclusion with 95% confidence (but there is a 1 in 20 chance of being wrong) in biological work, to maintain and accepts this level of significance as being reasonable [9].

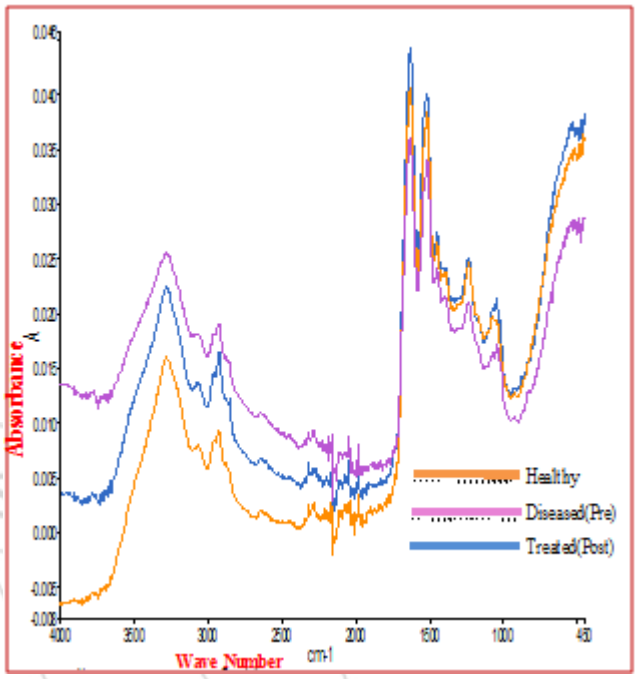


Figure 2: Overlaid spectra of pre and post drug therapy hair fibers of asthmatic individual with healthy hair fibers

3. Results and Discussion

The spectral analysis undertaken in this work primarily focuses upon qualitative and quantitative study of efficacy of asthmatic drug using single hair fiber from the asthmatic patients. The FTIR spectrum exhibits vibrational band characteristics of the various group frequencies, of the spectrum of a control hair sample with that of an asthmatic hair sample, which are the same with respect to the position of the peaks but different in the absorption level of the peaks [10&11]. It is clear that, the absorption peaks of proteins, lipids, glucose, phospholipids and etc, for asthmatic patient is more than that of the healthy individuals as because several substances like histamines, leukotrienes, prostaglandins, eosinophils and cytokines are being released by mast cells during an asthma attack as mentioned earlier. FTIR absorption spectrum of healthy hair sample is shown in Figure1. Figure 2 represents the overlaid FTIR spectra of healthy hair samples with pre and post drug therapy asthmatic diseased hair samples. Since hair root has a good blood supply, substances from the blood are incorporated into the hair during its formation. Because of this, a hair can provide a great deal of information about its “owner” and without exaggerating, we really can say that hair has become a real “informer” [12]. There are about 23 essential amino acids in Human body and are incorporated into proteins by distinct post-translational biosynthetic mechanisms. Among the 23 essential amino acids only 9 show a significant absorbance in the IR region and have been thoroughly investigated by Venyaminov & Kalnin and they have

provided the contribution of the amino acid side chain vibrations in the region between 1800 and 1400 c m^{-1}[13].

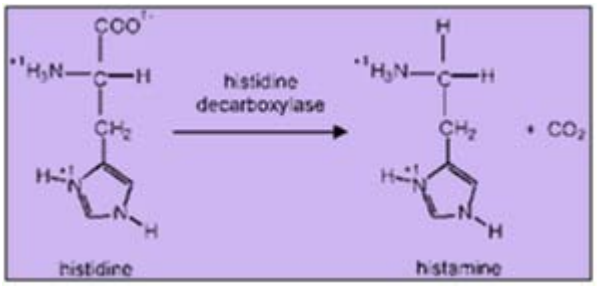


Figure 3: Conversion of Histidine into Histamine by the enzyme Histidine Decarboxylase

One among the 9 amino acids is the Histidine ($C_6H_9N_3O_2$) an essential amino acid found in most animal proteins, essential for tissue growth and repair, also present in eukeratin of the fibrous keratin substance available in the human hair [14]. This histidine is also a precursor to Histamine, a vital inflammatory agent in the immune responses or an amine produced in the body necessary for inflammation and its occurrence in human proteins is only about 1 – 2.3 %. Histamine is a biogenic amine that, in mammals, including humans, is produced primarily by the enzyme L-histidine decarboxylase on the amino acid histidine [15]. Histidine decarboxylase is present in large quantities in leukocytes known as granulocytes, especially in tissue mast cells and basophiles. In these cells it converts histidine to histamines as shown in the chemical reaction represented in Fig.3. The newly formed histamine is then stored in structures within the cells (intercellular granules) in readiness for release in response to signals from a variety of body systems; in inflammation, whether produced in defending the body from injury or infection, or as a result of an allergic reaction, these signals come from lymphocytes, cytokines and antibodies. Histamine ($C_5H_9N_3$) is a neurotransmitter, an amine that causes dilation of capillaries, contraction of smooth muscles and it is released during allergic reactions. The investigation undertook by Venyaminov & Kalnin on the contribution of the amino acid side chain vibrations in the region between 1800 and 1400cm^{-1} has clearly showed the band at 1596cm^{-1} corresponds to Histidine with ring vibration, [16&17] which on the biosynthesis get converted to histamine that is released during an asthma attack. In this study the variation in the optical density is observed, due to the FTIR absorption of each hair sample. Hence, no differences in the spectral signatures of the hair fibers were noticed. It is learnt that asthmatic hair fiber doesn't bring any foreign functional groups; instead it affects the existing functional groups of bio molecules viz., proteins (keratin), lipids and glycogen. In order to get exact deviations and the intensity of absorption in the discrimination of asthmatic from healthy hair fiber, internal ratio parameters were calculated. This deals with the ratio of the intensity of Infrared absorption of specific Infrared bands. The Infrared bands are chosen with respect to its sensitivity. The band at 1596cm^{-1} is due to Histidine and it is because of the ring breathing mode. The prominent absorption peak at 1538cm^{-1} is due to Amide II bands which arises from N-H inplane bending vibration strongly coupled to C-N stretching vibration of protein. The absorption peaks in the region of 1400-1300cm^{-1} arises due to the asymmetric C-H scissoring of methyl and methylene group of proteins. The absorption peak at 1240cm^{-1} is due to symmetric stretching vibration of phosphate groups in phospholipids. The glucose or sugar moieties are found in the region 1180-950cm^{-1} and the peak at 1080cm^{-1} is due to glucose. The sensitivity exhibited by the FTIR spectral bands of protein, lipids and glycogen bands due to the IR absorption of asthmatic hair fiber clearly indicates that these are the key biomarkers in the investigation of asthma.

Table 1: Intensity ratio parameters for Healthy individual single Hair Fiber

Sample	R_1	R_2	R_3	R_4	R_5	R_6
1	1.401	1.903	1.351	1.198	1.059	1.248
2	1.351	1.827	1.367	1.211	1.076	1.312
3	1.313	1.811	1.292	1.183	1.071	0.125
4	1.292	1.801	1.404	1.231	1.096	1.293
5	1.428	1.991	1.084	1.237	1.093	1.316
6	1.449	1.914	1.405	1.317	1.154	1.295
7	1.421	1.806	1.397	1.221	1.098	1.304
8	1.561	1.709	1.329	1.228	1.092	1.128
9	1.333	1.826	1.196	1.167	1.079	1.215
10	1.438	1.845	1.314	1.221	1.091	1.137
Mean	1.399	1.843	1.314	1.221	1.091	1.137
Standard Deviation	0.080	0.077	0.103	0.040	0.025	0.362
Standard Error Mean	0.025	0.024	0.032	0.012	0.008	0.114

Internal ratio parameter is calculated to fortify the results obtained from the FTIR intensity of absorptions. In order to quantify the spectral differences, six intensity ratio parameters **R_1** Histidine (1596cm^{-1}) / Glucose (1080cm^{-1}), **R_2** Amide II (1538cm^{-1}) / Glucose (1080cm^{-1}), **R_3** LDL (1450cm^{-1}) / Glucose (1080cm^{-1}), **R_4** Amino acid (1395cm^{-1}) / Glucose (1080cm^{-1}), **R_5** Amino acid (1303cm^{-1}) / Glucose (1080cm^{-1}), **R_6** Phospholipids (1240cm^{-1}) / Glucose (1080cm^{-1}) have been introduced and calculated for control and pre, post asthmatic hair samples [18] which are tabulated in Tables 1 & 2. Figures 4, 5, 6, 7, 8 & 9 shows the histograms of Mean intensity ratios for healthy and pre, post drug therapy of Asthmatic individuals by analyzing with help of their hair fibers. Figure 10 is a histogram which shows the comparison of mean intensity ratio for Healthy and pre, post drug therapy of asthmatic individuals. The ratio values of integrated areas of bands at 1596cm^{-1}/1080 cm^{-1}, 1538cm^{-1} /1080c m^{-1} , 1450cm^{-1} /1080cm^{-1} , 1395 cm^{-1} /1080c m^{-1} , 1303cm^{-1} /1080c m^{-1} , 1240cm^{-1} / 1080 cm^{-1} showed remarkable differences in the bulb region

Table 2: Intensity ratio parameters of Pre and post drug therapy for Asthmatic individual using single Hair Fiber

Sample	R_1 Histidine (1596cm^{-1})/ Glucose (1080cm^{-1})		R_2 Amide II (1538cm^{-1}) /Glucose (1080cm^{-1})		R_3 LDL (1450cm^{-1}) /Glucose (1080cm^{-1})		R_4 AminoAcid (1395cm^{-1}) / Glucose (1080cm^{-1})		R_5 AminoAcid (1303cm^{-1}) / Glucose (1080cm^{-1})		R_6 Phospholipids (1240cm^{-1})/ Glucose (1080cm^{-1})	
	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
1	1.481	1.447	1.927	1.904	1.451	1.383	1.325	1.255	1.146	1.011	1.306	1.254
2	1.411	1.368	1.851	1.789	1.376	1.355	1.241	1.211	1.085	1.066	1.250	1.227
3	1.444	1.441	1.865	1.835	1.426	1.406	1.278	1.224	1.182	1.152	1.318	1.318
4	1.522	1.352	1.932	1.811	1.491	1.337	1.335	1.219	1.161	1.067	1.304	1.245
5	1.608	1.545	2.063	2.016	1.551	1.482	1.386	1.361	1.189	1.177	1.356	1.348
6	1.538	1.497	1.919	1.918	1.456	1.435	1.333	1.323	1.155	1.146	1.263	1.261
7	1.475	1.442	2.078	1.880	1.572	1.455	1.394	1.300	1.211	1.165	1.411	1.315
8	1.628	1.581	1.807	1.694	1.498	1.349	1.347	1.263	1.157	1.125	1.245	1.216
9	1.408	1.374	1.976	1.856	1.483	1.329	1.352	1.261	1.170	1.075	1.329	1.269
10	1.501	1.459	1.915	1.892	1.435	1.367	1.307	1.270	1.150	1.067	1.321	1.270
Mean	1.501	1.451	1.933	1.860	1.474	1.390	1.330	1.269	1.161	1.105	1.310	1.272
Standard Deviation	0.075	0.075	0.087	0.086	0.058	0.053	0.046	0.048	0.033	0.055	0.051	0.042
Standard Error Mean	0.024	0.024	0.027	0.027	0.018	0.017	0.015	0.015	0.011	0.017	0.016	0.013

Table 3: Mean Intensity ratio parameters of Pre and post drug therapy for Asthmatic individuals using single Hair Fiber by t-test Analysis

Ratio of Component Groups	Pre Drug Therapy				Post Drug Therapy		
	N	Mean	Standard Deviation	Standard Error Mean	Mean	Standard Deviation	Standard Error Mean
Histidine(1596cm^{-1})/ Glucose (1080cm^{-1})	10	1.501	0.075	0.024	1.451	0.075	0.024
Amide II(1538cm^{-1})/ Glucose (1080cm^{-1})	10	1.933	0.087	0.027	1.860	0.086	0.027
LDL (1450cm^{-1})/ Glucose (1080cm^{-1})	10	1.474	0.058	0.018	1.390	0.053	0.017
AminoAcid(1395cm^{-1})/ Glucose (1080cm^{-1})	10	1.330	0.046	0.015	1.269	0.048	0.015
AminoAcid(1303cm^{-1})/ Glucose (1080cm^{-1})	10	1.161	0.033	0.011	1.105	0.055	0.017
Phospholipids (1240cm^{-1})/ Glucose(1080cm^{-1})	10	1.310	0.051	0.016	1.272	0.042	0.013

Table 4: t-test results of intensity ratio parameters of single hair fiber from pre and post drug therapy for asthmatic individuals

Ratio of Component Groups	Degrees of Freedom	t Stat	P(T<=t) one-tail	P(T<=t) two-tail	Pearson Correlation
Histidine(1596cm^{-1})/ Glucose (1080cm^{-1})	9	3.6297	0.0027	0.0055	0.8241
Amide II(1538cm^{-1})/ Glucose (1080cm^{-1})	9	3.7635	0.0022	0.0045	0.7422
LDL (1450cm^{-1})/ Glucose (1080cm^{-1})	9	4.7925	0.0005	0.0010	0.5033
AminoAcid(1395cm^{-1})/ Glucose(1080cm^{-1})	9	5.5101	0.0002	0.0004	0.7215
AminoAcid(1303cm^{-1})/ Glucose (1080cm^{-1})	9	4.0574	0.0014	0.0029	0.6210
Phospholipids (1240cm^{-1})/ Glucose(1080cm^{-1})	9	3.9108	0.0018	0.0036	0.7941

near the root, probably due to the major DNA content with respect to proteinic components. These findings offer a better insight into the structural changes of hair.

For entire spectrum from 4000 to 450 cm^{-1}, statistical analysis was performed by t-test, considering the mean difference variance of the analysis and it shows the full successful classification to distinguish Healthy and Pre, Post drug therapy of asthmatic individuals as shown in Tables 3 & 4. The low p-value (< 0.05) indicates that there is a significant difference between the means of the intensity ratio parameter calculated for pre and post drug hair samples and hence stands as an evidence for the method adopted [18&19]. A Pearson correlation has been done and shown in Table 4, which gave a set of values greater than 0 and this indicates a positive association; that is, as the value of one variable (here pre-treated hair sample) increases, so does the value of the other variable (post-treated hair sample). The results obtained in this study using single hair fiber by FTIR spectra and from internal ratio parameter with that of t-test analysis are well in agreement with the results obtained using blood sera from asthmatic individuals [20].

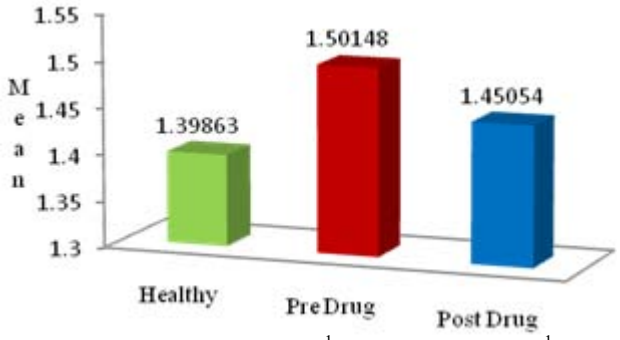


Figure 4: Histidine (1596cm^{-1})/ Glucose(1080cm^{-1})(R_1)

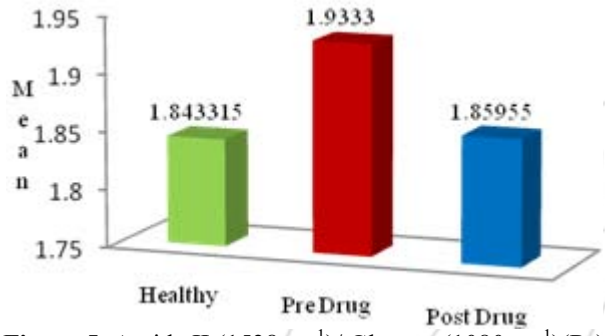


Figure 5: Amide II (1538cm^{-1})/ Glucose (1080cm^{-1})(R_2)

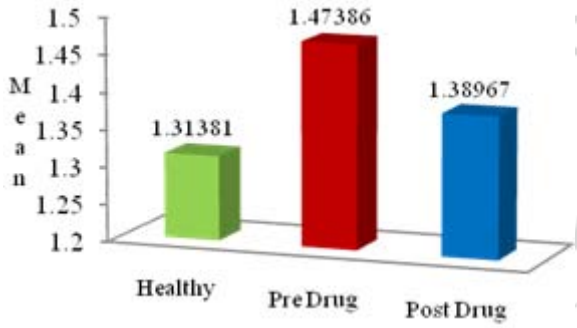


Figure 6: LDL (1450cm^{-1})/ Glucose (1080cm^{-1})(R_3)

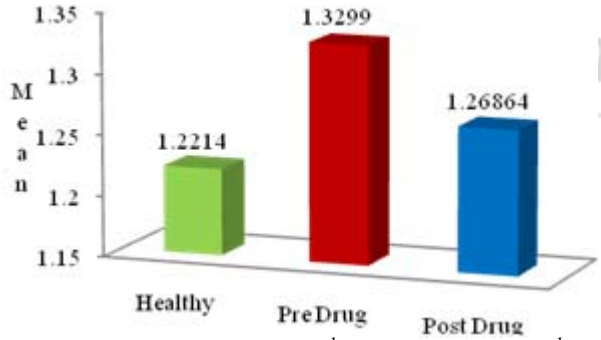


Figure 7: Amino Acid (1395cm^{-1})/ Glucose (1080cm^{-1})(R_4)

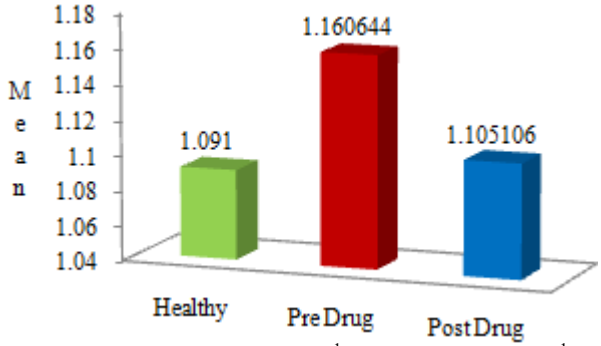


Figure 8: Amino Acid (1303cm^{-1})/ Glucose (1080cm^{-1})(R_5)

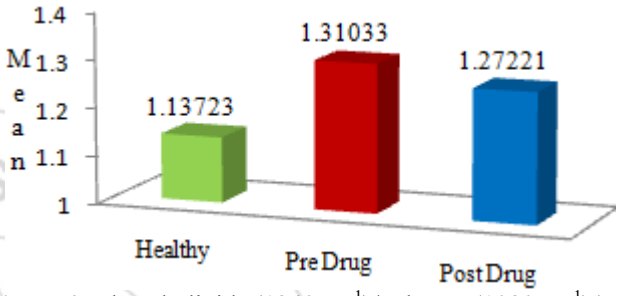
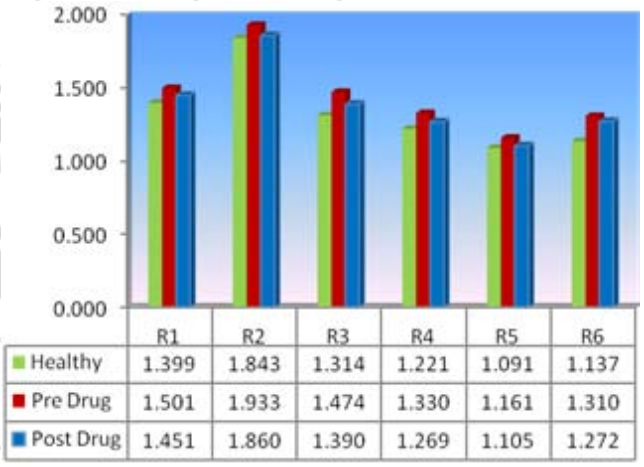


Figure 9: Phospholipids (1240cm^{-1})/ Glucose(1080cm^{-1})(R_6)



	R1	R2	R3	R4	R5	R6
Healthy	1.399	1.843	1.314	1.221	1.091	1.137
Pre Drug	1.501	1.933	1.474	1.330	1.161	1.310
Post Drug	1.451	1.860	1.390	1.269	1.105	1.272

Figure 10: Comparison of intensity Ratio Parameters of Healthy, Pre, Post drug Asthmatics

4. Conclusion

This study has demonstrated the utility of Attenuated Total Reflectance spectroscopy to analyze and compare the human-scalp hair with healthy and asthmatic (pre & post drug therapy) individuals. Hair can possibly be a biological sample that reveals the chemical deposition records in human, which may result in the formation of a diagnostic chemical fingerprint in hair samples [21]. FTIR spectroscopy of body tissues has shown potential in a multitude of biomedical applications, including pharmacological and forensic applications [22]. Thus, the FTIR spectra of body tissues could monitor metabolic changes occurring with the disease. The study was carried out to explore the potential applicability of FTIR-ATR spectroscopy in detecting asthmatic disease and to find the efficacy of the asthmatic

drug by a non-invasive sampling technique. Based on the spectral variations in the hair samples, intensity ratio parameters were used for successful differentiation between pre-treatmental, post-treatmental & healthy subjects. The result of this study further revealed the power & sensitivity of FTIR – ATR spectroscopy in the diagnosis of Asthma. Although the results obtained in this study could be considered only as a preliminary one, it forms a promising basis for the future study.

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