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Biological analysis of Fingernails of Healthy and Thyroid disordered subjects by FTIR-ATR spectroscopic technique

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Abstract : Fourier Transform Infrared - Attenuated Total Reflectance (FTIR-ATR) technique is a modern spectroscopic technique used for elemental analysis of biological samples. This technique is based on the principle of total internal reflection. In the present study, FTIR-ATR method is used to investigate the bio-molecules present in the fingernails. As the bio-molecules present in the finger nail can be changed by several pathological, physiological, and environmental factors, we analyze the human fingernails to evaluate the possibility of thyroid disorder. The FTIR-ATR spectrum of human nail has been recorded in the mid-infrared region of 4000-450 cm^{-1}. The FTIR-ATR spectral analysis revealed the differences in some major metabolic components viz., LDL, total cholesterol and triglycerides that clearly demarcated between control and thyroid disordered patient's nail. Measurements were recorded on 30 fingernails belonging to 10 hypothyroid, 10 hyperthyroid and 10 healthy subjects. Hypothyroid patients nail spectra show a remarkable increase from the control persons in LDL, total cholesterol, triglyceridess and glucose whereas nail spectra of hyperthyroid patients show an remarkable decrease in values of LDL, total cholesterol and triglycerides from the control ones. The difference in the values of the disorder are calculated using the internal ratio parameters viz LDL/ glucose R_1 (I_{1460}/I_{1083}), triglycerides/ glucose R_2 (I_{3060}/I_{930}) and total cholesterol/ glucose R_3 (I_{2933}/I_{930}). These parameters could be used as a basis for deriving a spectral method for analyzing thyroid disordered finger nail. It is shown that Fourier Transform Infrared - Attenuated Total Reflectance spectroscopy (FTIR-ATR) could be a possible technique for the analysis of nail and therefore identification of thyroid disorder problems.

Keywords: discriminant analysis; thyroid disorder; hypothyroidism; hyperthyroidism, fingernail.

1. Introduction

Fourier Transform Infrared - Attenuated Total Reflectance (FTIR-ATR) is a modern technique for the analysis of biological samples which is based on total internal reflection[1,2]. The advantages of FTIR- ATR include little or no sample preparation and very easy to use[3], make it very feasible for analysis of

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biological samples such as blood[4], hair[5], skin[6], and nail[7]. Analysis of human tissues using FTIR-ATR technique can be an ideal method for the identification of disease or disorder[8]. The determination of bio-molecules in body fluids and tissues is essential for prevention, diagnosis, and treatment of diseases/ disorder[9]. Among human tissues, finger nail is a favorable biomarker for clinical investigations because it is easy to collect, store, and transport. The component of nail can contain information about metabolic events that occurred during the time of its formation[10,11]. Based on the idea that the biological composition of nail can be changed by several pathological, physiological, and environmental factors[12], it is possible that when some disease/ disorder influences, nails could serve as an accessible biopsy material.

In the present study, Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) spectroscopic method is used for biological analysis of fingernail of healthy and thyroid disordered subjects. Hyperthyroidism, an illness of the thyroid, is associated with an overproduction of the thyroid hormone. The symptoms of hyperthyroidism are palpitations, heat intolerance, nervousness, insomnia, breathlessness, increased bowel movements, light or absent menstrual periods, fatigue and fast heart rate, which are caused by the effects of too much thyroid hormone on tissues of the body[13]. Hypothyroidism is the word used to describe a group of symptoms associated with under-activity of the thyroid gland. The symptoms of hypothyroidism include fatigue, weakness, weight gain or increased difficulty in weight loss, dry hair, dry skin, hair loss, cold intolerance, muscle cramps and frequent muscle aches. Hypothyroidism may be associated with ovulatory dysfunction and adverse pregnancy outcome[14]. Thyroid disorder constitutes to difficulty to conceive or sub-fertility that plays a major psychological burden. The treatment mechanism has consequently increased tremendously on understanding the reproductive failure and opened new perspectives for future interventions, not only to increase the cumulative conceptions, but also to increase spontaneous pregnancy rates[15].

The most common method used to detect hyper and hypothyroidism is a blood test. The tests for T3 and T4 measure circulating hormone levels. The TSH test measures the pituitary thyroid-stimulating hormone, which in turn signals the production of T3 and T4. Elevated tissue calcium stabilizes cell membranes and impairs the transport of thyroid hormone across cell membranes.

Thyroid hormones regulate cholesterol and lipoprotein metabolism, whereas thyroid considerably alter lipid profile and promote cardiovascular disease[16]. Thyroid disorder also has metabolic defects on carbohydrate, protein and glucose and alters the level of mechanism. Usually, hypothyroid patients have elevated lipid profiles, carbohydrate and protein profile when compared to the healthy persons. Also, hyperthyroid patients have low metabolic profiles of carbohydrates, lipids, protein and glucose when compared to the healthy ones[17].

2. Materials and Methods

30 persons are categorized into three groups: 10 healthy persons (certified with no disease/ disorder by the medical practitioner); 10 hypothyroidism patients and 10 hyperthyroidism patients between age group of 21 to 30. Fingernail clippings were taken from free edge of nail plate by nail scissors and kept separately in non-reactive plastic envelopes. Then, all of them were stored at room temperature until analysis. In order to eliminate any surface contaminations, nail samples were washed by soaking in ethanol for 30 seconds followed by distilled water for the same time. Then they were dried at room temperature.

FTIR spectral measurements of human finger nail samples are carried out at Sophisticated Analytical Instrumentation Facility (SAIF), SPIHER, Avadi, Chennai – 600054, India, using Perkin Elmer Spectrum – Two FTIR spectrophotometer with attenuated total reflection accessory (shown in Fig. 1) having highly reliable and single bounce diamond as its internal reflectance element (IRE) (shown in Fig. 2). The FTIR-ATR spectroscopy is based on the optical phenomenon known as total internal reflection (TIR). The IR radiation strikes at the point of incidence between the IRE and the nail sample of a lower refractive index than that of a diamond. The dimension of the diamond crystal is 2 mm cross-section. The IR beam creates an evanescent wave into the sample, in intimate contact with the IRE crystal. Based on the change of dipole moment of vibrations of the bio-molecules of nail tissue sample, energy of the evanescent wave absorbed by the sample and hence the evanescent wave gets attenuated, this is sent back to the reflected wave, the reflected radiation (some now absorbed by the sample) is returned to the detector and hence the spectrum is obtained. This evanescent wave protrudes only a few microns of the order of 0.5 microns to 5 microns beyond the crystal

surface and into the sample. The spectral readings are done at 16 scans of accumulation with resolution ±4 cm^{-1} at absorbance mode.

Before obtaining the spectral profile of the nail sample, the background spectrum of atmospheric air is observed. The effects of the background spectrum are removed from a sample spectrum by ratioing the sample single beam spectrum to the background spectrum[18]. As with FTIR measurements, a background radiation is collected from the clean ATR crystal. The crystal is cleaned by using a solvent soaked piece of lint free tissue. Mostly isopropanol is used to clean ATR crystal. Then the finger nail sample is placed on the diamond pointer and enough force is applied to the nail using the force gauge to hold the sample in place and make proper contact with the diamond pointer.

Fig. 1 Perkin Elmer FTIR-ATR Spectrum Two Spectrophotometer

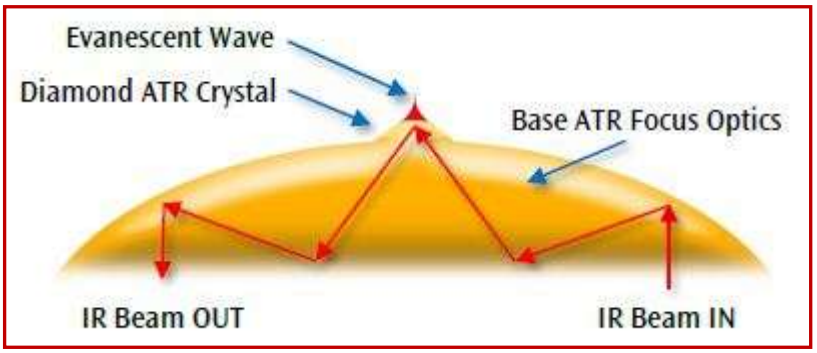

Fig. 2 Diamond ATR Crystal as Single Bounce and evanascent wave Internal Reflectance Element (IRE)

3. Thyroid Disorders: Hypothyroid and Hyperthyroid

Human cells, tissues and body fluids are generally composed of water, lipids, proteins, carbohydrates, glucose and nucleic acids. Thyroid hormones induce the 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase, which is the first step in cholesterol biosynthesis[19]. Moreover, triiodothyronine (T_3) regulates LDL receptors by controlling the LDL receptor gene activation. This T_3-mediated gene activation is done by the direct binding of T_3 to specific thyroid hormone responsive elements (TREs)[20]. Furthermore, T_3 controls the sterol regulatory element-binding protein-2 (SREBP-2), which in turn regulates LDL receptor's gene expression[21]. T_3 has also been associated with protecting LDL from oxidation[22]. Thyroid hormones can influence HDL metabolism by increasing cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL (high density lipoprotein) to the very low density lipoproteins (VLDL) and TGs

(triglycerides) to the opposite direction[20]. Beyond their effect on lipid profile, thyroid hormones can equally affect a number of other metabolic parameters related to cardiovascular disease risk. Indeed, thyroid function can influence adipocyte metabolism and the production of adipokines[23]. Hyperthyroidism has been associated with increased levels of adiponectin, whereas hypothyroidism is not associated with significant changes in adiponectin[24]. Although decreased thyroid function or hypothyroidism is accompanied by reduced activity of HMG-CoA reductase, TC (total cholesterol) and LDL (low density lipoprotein) levels are increased in patients with hypothyroidism[25]. This is due to the decreased LDL-receptors' activity, resulting in decreased catabolism of LDL and HDL[26]. Hypothyroid patients are found with elevated TG levels associated with increased levels of LDL. Hypothyroid patients may also exhibit elevated levels of HDL mainly due to increased concentration of HDL particles. Moreover, decreased activity of the CETP results in reduced transfer of cholesteryl esters from HDL to VLDL, thus increasing HDL levels[27]. Hypothyroid patients have increased lipoprotein levels[28], which are associated with increased cardiovascular disease risk.

The incidence of hyperthyroidism is lower compared with hypothyroidism in the general population. Despite the increased activity of the HMG-CoA reductase, levels of TC, LDL tend to decrease in patients with hyperthyroidism. This is due to increased LDL receptor gene expression resulting in enhanced LDL receptor-mediated catabolism of LDL particles[29]. A decrease in HDL level is also observed in hyperthyroidism, due to increased CETP-mediated transfer of cholesteryl esters from HDL to VLDL and increased catabolism of HDL[30]. On the other hand, no changes in blood pressure[26], has been described in hyperthyroid patients. Therapy of clinical hyperthyroidism results in restoration of those alterations of lipid metabolism[27]. Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels - one diagnostic indiction of hypothyroidism is increased blood cholesterol concentration[31].

Thyroid hormones stimulate almost all aspects of carbohydrate metabolism, including enhancement of insulin-dependent entry of glucose into cells and increased gluconeogenesis and glycogenolysis to generate free glucose[19]. Insulin resistance is also correlated with thyroid function[32]. TSH is positively associated with fasting and postprandial insulin concentration and negatively with insulin sensitivity[33] Hyperthyroidism is typically associated with decreasing blood glucose control and increased insulin requirements. The excessive thyroid hormone causes increased glucose production in the liver, rapid absorption of glucose through the intestines, and increased insulin resistance (a condition in which the body does not use insulin efficiently). It may be important to consider underlying thyroid disorder if a person has unexplained weight loss, deterioration in blood glucose control, or increased insulin requirements. Having diabetes increases a person's risk for heart disease, and many people with diabetes have a heart condition such as coronary heart disease or heart failure. Since hyperthyroidism causes rapid heart rate and increases the risk of abnormal heart rhythm, it may also bring on angina (chest pain), worsen heart failure or interfere with the treatment of heart failure, as well as further increase the risk of other heart problems. Hypothyroidism rarely causes significant changes in blood glucose control, although it can reduce the clearance of insulin from the bloodstream, so the dose of insulin may be reduced which increases the blood glucose control. Moreover, low normal FT4 levels are significantly associated with increased insulin resistance. Protein levels are found to be increased in hypothyroidism whereas the protein levels are decreased in hyperthyroidism[34].

4. Spectral Analysis

The average FTIR-ATR absorption spectrum of finger nail of healthy subjects is shown in Fig. 3. Spectral signatures identified with the idea of the vibrational frequencies of the bio-molecules present in the nail such as protein, lipids, nucleic acid, phospholipdis, carbohydrates and glucose are given in Table 1. IR absorption spectra of nail provide information about these key biological components present in the nail tissue. In IR absorption spectra of nail, three major regions can be distinguished, i.e., lipids (2800–3000 cm^{-1}), protein (1600–1700 cm^{-1}, 1500– 1560 cm^{-1}) and nucleic acid (1000–1250 cm^{-1}) bands as well as additional bands typical of a specific group.

The mid infrared spectra of nail can provide qualitative and quantitative analysis of protein, Low Density Lipids (LDL), Amide and Glucose. A band assignment is done with the idea of the group frequencies of the various bio-molecules present in the sample. The prominent absorption peak 3281 cm^{-1} is due to the N-H stretching mode (Amide A) of protein. The acyl stretching mode of CH_3 at 3060 cm^{-1} is due to the triglycerides

(TGL). The methyl (CH_3) asymmetric and symmetric modes are observed at 2955 cm^{-1} and 2933 cm^{-1} is due to the lipids and total cholesterol (TC). The methylene (CH_2) asymmetric and symmetric modes at 2875 cm^{-1} and 2850 cm^{-1} are due to the lipids[35]. In the IR spectra, very weak evidence of the protonated carboxyl group (COOH) exists, as reflected by the small band of the C=O stretching High Density Lipoprotein (HDL) at around 1736 cm^{-1}[38]. The broad and strong peak at 1649 cm^{-1} is due to C=O stretching coupled with an in- plane bending of the N-H and C-N stretching modes (Amide I band) [34]. While the amide II band centered at around 1550 cm^{-1} is due to C=O stretching coupled with C-N stretching and bending deformation of N-H in the protein backbones[39]. The bands are exemplified as medium, broad absorption at 1460 cm^{-1} (LDL), while the band at 1375 cm^{-1} is due to bending deformation of CH_3 vibration of amino acid[38]. The absorption band at 1253 cm^{-1} is due to contributions of amide III that occurs due to CN stretching and asymmetric stretching modes of nucleic acids and PO^{2-}[40]. The band observed at 1180 cm^{-1} is due to the C-OH groups of amino acid and the C-O groups of carbohydrate[41]. The spectral band at 1118 cm^{-1} is due to the glycogen. The band around 1083 cm^{-1} is due to the contribution of symmetric stretching of CO that corresponds to glucose. The band around 1060 cm^{-1} is due to the COC asymmetric stretching that corresponds to phospholipids and cholesterol. The stretching vibrations of the S-S bonds of cysteine are visible in the 510-545 cm^{-1} region.

The quality of the spectra has been fairly appreciated through the utilization of FTIR-ATR spectroscopy.

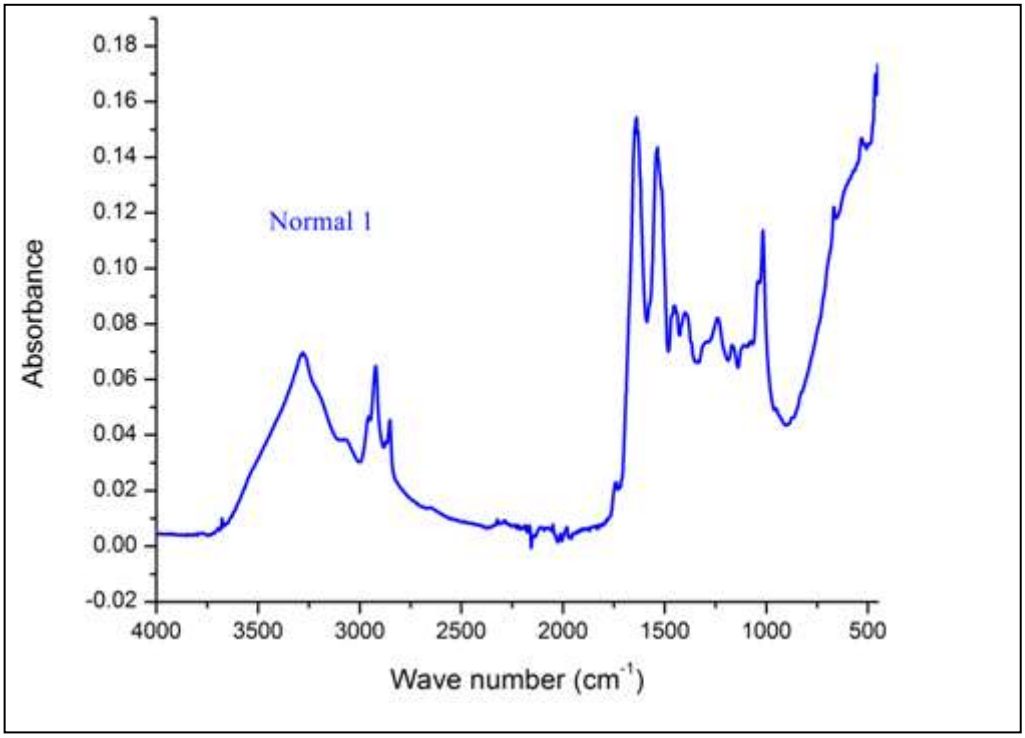

Fig. 3 Characteristic average FTIR-ATR spectrum of healthy human fingernail

Table 1 FTIR-ATR Spectral Vibrational Band Assignments of Human Nail

Vibrational Band (cm^{-1})	**Component Identification**	**Vibrational Mode**
3281	Amide A	Symmetric N-H Stretching
3060	Triglycerides (TGL)	Acyl CH_3 stretching mode
2955	Methyl stretches of lipids in plasma	Asymmetric stretching of CH_3
2933	Methyl stretches of lipids in plasma (Total Cholesterol, TC)	Symmetric stretching of CH_3
2875	Methylene stretches of proteins and lipids	Asymmetric CH_2 Stretching
2850	Methylene stretches of lipids in oral mucosa	Symmetric CH_2 stretching

1736	High Density Lipoprotein (HDL)	C=O groups of cholesterol esters
1649	Amide I (α-helix)	C=O stretching
1550	Amide II	C=O stretching coupled with C-N stretching and bending deformation of N-H
1460	Methylene bending of lipids (LDL)	Bending vibration of CH_2 groups
1447	Methyl bending of lipids (LDL) & proteins	Asymmetric bending of CH_3
1375	Methyl bending of lipids & proteins	Symmetric CH_3 bending
1253	Amide III and Lipid phosphates	C-N stretching and asymmetric PO_2 stretching mode of nucleic acids
1180	Carbohydrates	C-OH groups of amino acids and C-O symmetric stretching of carbohydrate
1118	Glucose	Stretching of Glycogen
1083	Glucose	CO Symmetric stretching of glucose
1060	Phospholipids and cholesterol	COC asymmetric stretching vibration
510	Glucose	S-S symmetric stretching of cysteic acid

Results and Discussion

The overlaid average spectral signature spectra of healthy, hypothyroid and hyperthyroid nail samples are represented in Fig. 4. The overlaid spectra emphasize the difference in the intensity of IR absorption of the bio-molecules exhibited by the nail samples. It is evident from the spectra that thyroid disordered nail sample do not bring any foreign functional groups; instead they affect the existing functional groups of bio-molecules such as protein, lipid and glucose. The variation in the optical density of vibrational bands of biomolecules of each person is observed. The internal ratio parameters are then calculated to get exact deviations in the intensity of absorption in the discrimination of hypothyroid and hyperthyroid from healthy nail samples.

The infrared bands are chosen with respect to their sensitivity exhibited by the FTIR spectral bands of protein, lipid and glucose bands of hypothyroid and hyperthyroid nail samples, clearly indicate that they are the key bio-markers in the observation of thyroid disorder. The sensitiveness witnessed in the IR absorption is due to the increase or decrease in the quantity of the sensitive biomarkers (functional groups) in the protein, lipid and glucose region. Internal ratio parameter is calculated to fortify the results procured from the band absorption. Internal ratio parameter ignores the difference in the amount of sample taken for analysis, nullifies or balances the quantity of the samples and hand over the exact deviation of the nail samples.

The changes occur in the LDL, Triglycerides and total cholesterol bands of finger nail show considerable changes in their intensity, but not in their respective position of the control and thyroid disordered nail spectra. The intensity ratio parameters viz. LDL/Glucose [$R_1(I_{1460}/I_{1083})$], Triglycerides/Glucose [$R_2(I_{3060}/I_{1083})$] and Total cholesterol/ Glucose [$R_3(I_{2933}/I_{1083})$] are calculated among the prominent absorption peaks due to the LDL, triglycerides, total cholesterol and glucose respectively. The absorption values of total cholesterol, triglycerides, LDL and glucose show a significant increase in hypothyroid and decrease in hyperthyroid when compared to the healthy ones.

The deviations in the internal ratio parameters of lipids, protein and glucose of thyroid disorder patients (hypothyroid and hyperthyroid) with healthy (in average) nail samples are provided in Table 2. The changes observed in the LDL and total cholesterol and triglycerides bands of nail samples show considerable changes in their intensity, but not in the respective position of the healthy and thyroid disordered nail samples. The absorption values remarkably increased in the case of hypothyroid and decreased in the case of hyperthyroid from the healthy ones. The small changes in the absorption is also appreciable in the FTIR-ATR spectra as it depends on the short existing effective evanescent wave with 0.5 to 5 micron depth of penetration.

The histograms drawn between the ratios of LDL/glucose [R_1 (I_{1460}/I_{1083})], triglycerides/glucose [R_2 (I_{3060}/I_{1083})], and total cholesterol/glucose [R_3 (I_{2933}/I_{1083})] region are shown in Fig.5. The difference in the altitude of the histogram is important in the discrimination of thyroid disorder nail samples from the healthy subjects. It is evident from the histogram that the intensity of bio-molecules such as total cholesterol, LDL and triglycerides in hypothyroid is more or greater in value than the healthy subjects. Also the intensity of bio-

molecules in hyperthyroid is lesser than the healthy subjects. The histograms support the results obtained from Internal Ratio Parameters.

The clinical values of Triiodothyronine (T3), thyroxine (T4) and Thyroid stimulating harmone (TSH) of the control, hypothyroid and hyperthyroid patients obtained from the clinical laboratory are provided in Table 3. T3 levels are lower whereas T4 and TSH levels are higher than normal range in hypothyroid. Similarly, T3 levels are higher whereas T4 and TSH values are lower than normal range in hyperthyroid[42]. Table 3 is completely satisfactory with the results obtained from the internal ratio parameters and it is evident that the bio-molecules such as LDL, triglycerides and total cholesterol plays a major role in the metabolism of thyroid disordered patients.

The statistical analysis for the independent samples is summarized in Table 4. The statistical table provides useful descriptive statistics for the two variables, including the mean, standard deviation and standard error. It is noticed from Table 4 that the hypothyroid patients have statistically significant higher levels of LDL, total cholesterol, triglycerides and glucose than the healthy subjects. Similarly, the hyperthyroid patients have statistically significant lower levels of LDL, total cholesterol, triglycerides and glucose than the healthy subjects.

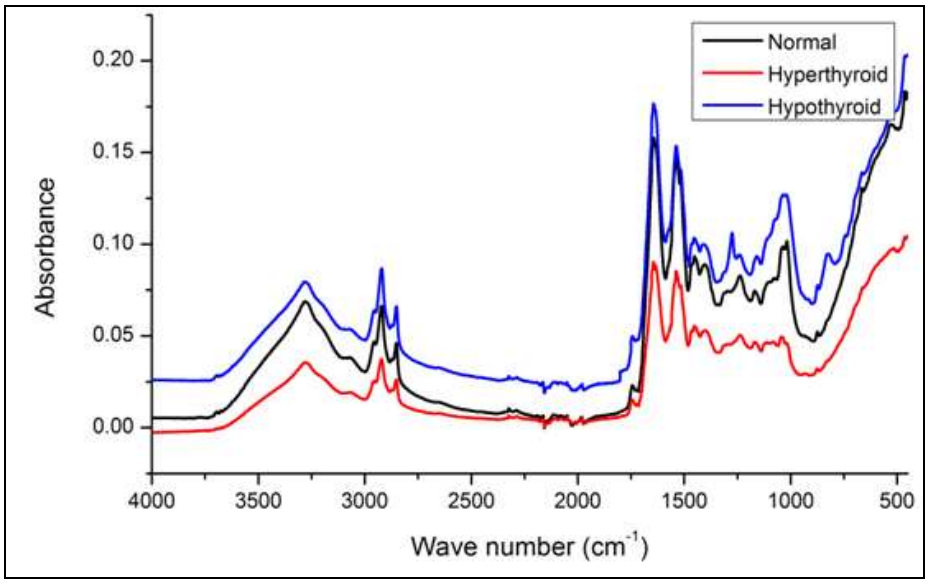

Fig. 4 Overlaid FTIR-ATR average spectral signature spectra of healthy, hypothyroid and hyperthyroid human nail samples

Table 2 Intensity Ratio Parameters and Discrimination of the thyroid disorder nail samples using FTIR-ATR spectroscopic technique

IRP	LDL/ Glucose I_{1460}/I_{1083}			TGL/Glucose I_{3060}/I_{1083}			Total cholesterol/ Glucose I_{2933}/I_{1083}		
Samples	Hypothyroid	Healthy	Hyperthyroid	Hypothyroid	Healthy	Hyperthyroid	Hypothyroid	Healthy	Hyperthyroid
1	1.7261	0.7350	0.2169	2.0862	0.8817	0.3248	1.9256	0.7772	0.2576
2	1.4330	0.6589	0.1460	1.6086	0.8714	0.1811	1.6888	0.8363	0.1830
3	1.7709	0.7380	0.1184	1.8390	1.0079	0.1192	1.8844	0.8725	0.1609
4	1.7413	0.6589	0.0941	1.9144	0.8714	0.1328	1.8769	0.8363	0.1882
5	1.1418	0.7350	0.2505	2.2152	0.8817	0.2874	1.9252	0.7772	0.2180
6	1.9662	0.6589	0.3169	2.0493	0.8714	0.3248	1.9620	0.8363	0.2576
7	1.6986	0.7380	0.1460	1.9619	1.0079	0.1811	1.8580	0.8725	0.1830
8	1.6811	0.6589	0.1184	1.8916	0.8714	0.1192	1.8406	0.8363	0.1609
9	1.6217	0.7350	0.0941	1.9883	0.8817	0.1328	1.8933	0.7772	0.1882
10	1.7261	0.6589	0.2505	2.0862	0.8714	0.2874	1.9256	0.8363	0.2180

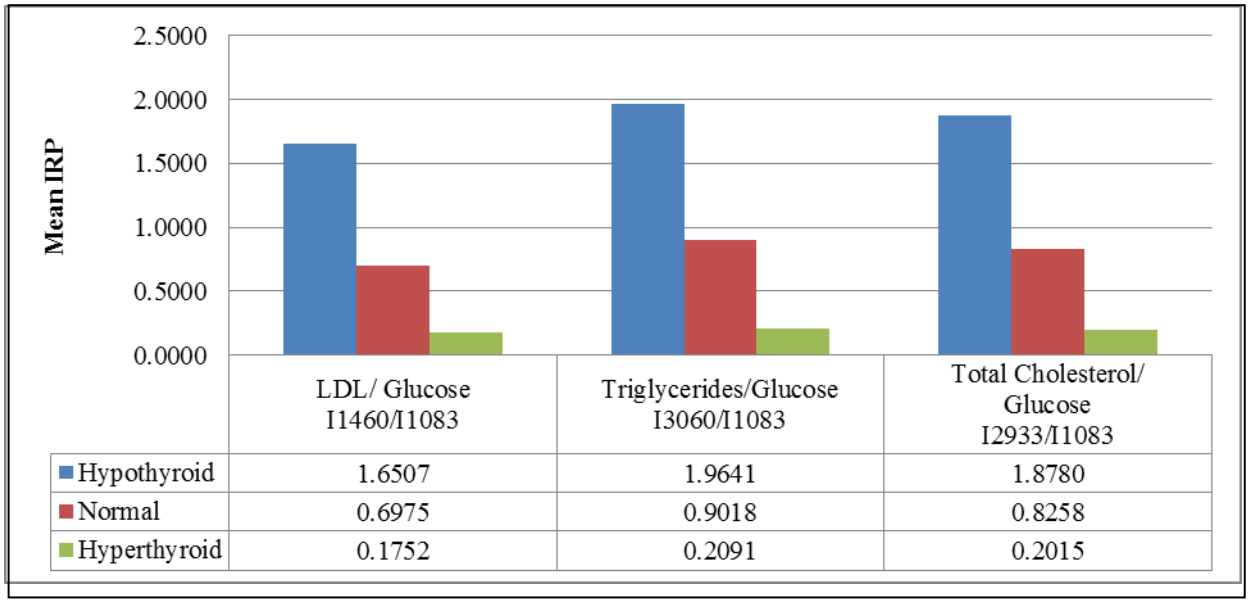

Fig. 5 Histogram – Mean IRP ratio in the discrimination of thyroid disorder human nail samples using FTIR-ATR spectroscopic technique

Table 3 Clinical Values of T3, T4 and TSH of healthy, hypothyroid and hyperthyroid patients

Normal Range: T3 = 80–200 ng/dl, T4 = 4.5–11.7 mcg/dl, TSH = 0.3-5 U/ml

	Healthy			Hypothyroid			Hyperthyroid		
Samples	T3 ng/dl	T4 mcg/ dl	TSH U/ml	T3 ng/dl	T4 mcg/ dl	TSH U/ml	T3 ng/dl	T4 mcg/ dl	TSH U/ml
1	140	5.4	1.2	79	12.3	5.3	230	3.4	0.5
2	143	6.5	1.3	82	14.2	4.9	320	4.5	0.3
3	85	4.7	1.1	65	12.55	6.1	190	2.3	0.8
4	89	7.4	2.8	43	15.1	9.0	208	3.2	0.32
5	114	5.7	2.2	56	13.1	8.3	310	1.4	0.45
6	120	6.3	2.6	67	15.2	6.2	205	4.1	0.12
7	134	6.4	2.3	78	14.5	8.4	211	5.0	0.1
8	124	7.1	2.2	35	15.9	10.2	230	2.7	0.4
9	103	8.3	2.4	80	11.9	4.8	243	3.1	0.22
10	102	4.8	2.4	67	12.5	5.9	253	3.6	0.15

Table 4 Mean, standard deviation and standard error of intensity ratio parameters of normal, hypothyroid and hyperthyroid nail samples using FTIR-ATR spectroscopic technique

IRP	N	Healthy			Hypothyroid			Hyperthyroid		
		Mean	Standard Deviation	Standard Error	Mean	Standard Deviation	Standard Error	Mean	Standard Deviation	Standard Error
LDL/ Glucose I_{1460}/I_{1083}	10	0.6975	0.0408	0.0091	1.6507	0.2221	0.0497	0.1752	0.0077	0.0074
Triglycerides/ Glucose I_{3060}/I_{1083}	10	0.9018	0.0561	0.0125	1.9641	0.1668	0.0373	0.2091	0.0087	0.0075
Total cholesterol/ Glucose I_{2933}/I_{1083}	10	0.8258	0.0365	0.0082	1.8780	0.0758	0.0169	0.2015	0.0081	0.0049

Conclusion

The FTIR-ATR technique could be used as a powerful technique in the clinical laboratory to detect the thyroid disorder. FTIR-ATR spectroscopic technique exhibits qualitative information about bio-molecules present and the method of internal ratio parameter is adopted in characterizing the nail samples quantitatively. Hypothyroid patients' IRP ratio clearly shows an increase in the absorbance values in LDL/Glucose [R_1 (I_{1460}/I_{1083})], Triglycerides/ Glucose [R_2 (I_{3060}/I_{1083})] and Total cholesterol/ Glucose [R_3 (I_{2933}/I_{1083})] when compared to the control ones which indicates that there is a increase in lipid profile in hypothyroid patients, whereas in hyperthyroid patients', it shows an decrease in the absorbance values in those biomarkers when compared to the control ones due to the change in the bio-molecules in particularly lipids and glucose region. The statistical analyses provide useful supportive data that agree with the spectral data and clinical data.

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