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Utility of FTIR spectroscopic analysis of saliva of diabetic pregnant women in each trimester

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Abstract

FTIR spectroscopy has been successfully employed to compare the saliva of the diseased and normal pregnant women. The absorption values of the specific bands of the spectra in each trimester compared, and the internal ratio parameter are calculated. The study shows that there is a change in the salivary pattern of normal and diseased women in each trimester.

Keywords: Saliva, normal pregnant women, diabetic pregnant women, trimester, FTIR spectroscopic analysis.

Introduction

There is an ongoing need for improvements in non-invasive, point-of-care tools for the diagnosis and prognosis of diabetes. Diabetes is a remarkably complex disease. The diagnosis is determined by measurements of a single biomarker (glucose). The specific salivary biomarkers such as glucose, α-amylase, and ghrelin appetite hormone exhibit strong diagnostic potential for diabetes (Belazi *et al.,* 1998; Borg Andersson *et al.,*1998; Aydin, 2007).

Clinical significance

Saliva, like blood, contains an abundance of protein and nucleic acid molecules that reflects physiological status. However, unlike other bodily fluids, salivary diagnostics offer an easy, inexpensive, safe, and noninvasive approach for disease detection, and possess a high potential to revolutionize the next generation of diagnostics. A salivary test is safer than using serum, which is more likely to expose operators to blood-borne diseases. The noninvasive collection approach could dramatically reduce anxiety, discomfort, and increase their willingness to undergo health inspections. This will greatly increase the opportunity to monitor their general health over time and to diagnose morbidities in the early stage. With the current rate of progression, salivary diagnostics becomes a key player in routine health monitoring the early detection of disease using a simple and effective assay.

Pregnancy is a complex metabolic state that involves dramatic alterations in the hormonal milieu, increases in estrogen, progesterone, prolactin, cortisol, human chorionic gonadotropin, placental growth hormone and human placental lactogen (Barbour *et al.,* 2007). The aim of diabetic management is to normalize blood glucose level. Therefore measurements of glucose are made more frequently to regulate the treatment of the patient more accurately. Early in pregnancy, increase in estrogen and progesterone level which lead to pancreatic beta cell hypertrophy and insulin excretion alters maternal carbohydrate metabolism. Plasma cellulose responses to similar carbohydrate loads are higher in pregnant women than non pregnant women. Salivary estrogen level has been suggested as a screening test to detect the risk potential for preterm labor. Salivary estrogen also increases the proliferation and desquamation of the oral mucosa and an increase in subgingival crevicular fluid levels (Laine *et al.,* 1988).

Changes in salivary pattern in normal and diabetic pregnant women in each trimester have been compared using FTIR spectroscopy both qualitatively and quantitatively (Raziya Sultana *et al.,* 2011). The type of spectral signatures differentiates the progesterone levels in pregnancy in each trimester qualitatively whereas the intensity ratio among the absorption bands characterizes quantitatively. The FTIR analysis of saliva in normal and diabetic pregnant women in each trimester and the changes in salivary pattern during pregnancy in mid-IR spectroscopy is made.

Materials and methods

The saliva samples were collected from normal and diabetic pregnant women in each trimester from the Hospital, Chennai. The FTIR Spectral measurements of all the samples were carried out at Sophisticated Analytical Instrumentation Facility IIT, Madras, Chennai-36, using Spectrum-One Perkin- Elmer FTIR Spectrophotometer. The spectra were recorded in the range 4000 - 400 cm^{-1} in the absorption mode. All the spectra were baseline corrected and normalized to acquire identical area under the curve.

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Fig.1. Comparison of normal and diabetic pregnant women in the 1st trimester

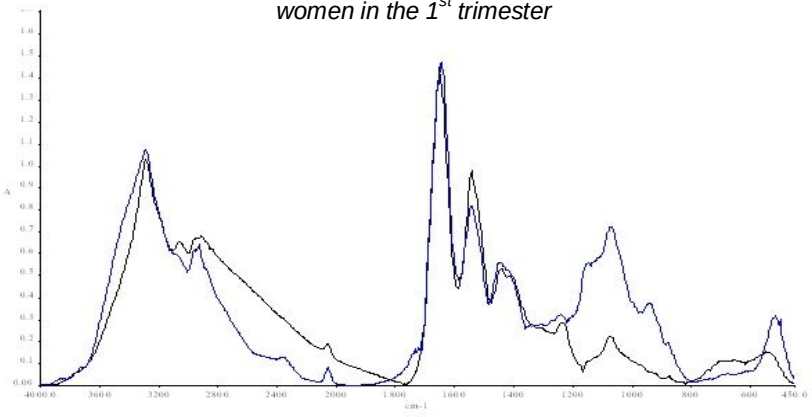


Fig.2. Comparison of normal and diabetic pregnant women in the 2nd trimester

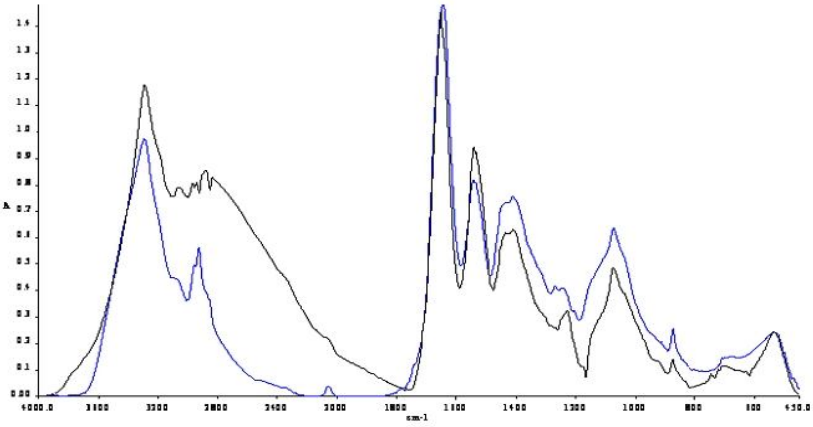
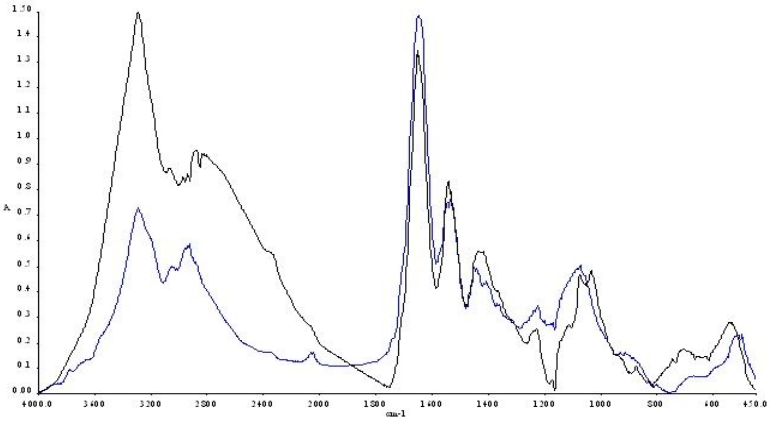


Fig.3. Comparison of normal and diabetic pregnant women in the 3rd trimester



Results and discussion

FT-IR spectroscopy has been employed to monitor some specific molecules that also represent diabetes-related signals such as early glycation products (Kiefer *et al.,* 2009) and glucose (Liu *et al.,* 2003). The quantitative information is carried out by the relative intensities of the various contributing spectra to the unique absorption profile of each specimen (Low-Ying *et al.,* 2002).

Constructing a comprehensive catalogue of saliva proteins using proteomic approaches is a necessary first step to identify potential protein biomarkers of disease (Xie *et al.,* 2005). All disease processes must be accompanied by changes in the relative content of biomolecules, the changes in the structure and functions. It is mainly due to the perturbations of the intermolecular or intramolecular interactions. The chief organic materials in saliva have been made up of mucin glycoprotein, carbonic anhydrase, free amino acids, urea, uric acid. The spectra of normal and diabetic pregnant women in each trimester are illustrated in Figs.1-3. The characteristic vibrational modes are mainly dominated by the of the protein constituents of the sample (Deleris & Petibois, 2003; Petibois *et al*., 2001). Vibrational band assignments are done with the idea of the group frequencies of the various analytes present in the sample. The spectral region 3600 - 3000 cm^{-1} comprises of C-H, O-H and N-H stretching vibrations of the proteins. The asymmetric and symmetric stretching C-H vibrations of methyl and methylene group have been found to be present around 2930 - 2875 cm^{-1} and 2992 cm^{-1} is due to C-H stretching. The peak at 2936 cm^{-1} is due to C-H stretching. The absorption peak at 2882 cm^{-1} is due to C-H lipid region, CH_3 CH_2-lipid and protein. The absorbance band at 1600-1800 cm^{-1} is due to C=O stretching. The prominent absorption peak at 1646 cm^{-1} is due to amide-I C_5 methylated cytosine C=O stretching C=C uracyl, NH_2 guanine. The band at 1620 cm^{-1} is due to progesterone (Rabolt John *et al.,* 2005) and it is the peak of nucleic acids due to the base carbonyl stretching and ring breathing mode. The absorption peak at 1646cm-1 is due to C_5 methylated cytosine C=O stretching C=C uracyl, NH_2 guanine.(Dovbeshko *et al.,* 1997). The peak at 1520 cm^{-1} is due to stretching C=N, C=C, C=N of adenine and cytosine.(Dovbeshko *et al.,* 2002). The peak at 1544 cm^{-1} is due to Amide II bands (arises from C-N stretching & CHN bending vibrations (Huleihel & Salman, 2002) The absorption peaks in the region 1400 - 1200 cm^{-1} arise due to the C-H deformation of methyl and methylene group of the proteins.

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The prominent absorption peak is at 1224 cm^{-1} is due to symmetric stretching of phosphate groups in phospholipids (Fabian *et al.,* 1995).The spectral region 1250-925 cm^{-1} is predominantly occupied by C-O-C asymmetric and symmetric vibrations of phospholipids of proteins (Randhawa, 2003). The glucose or sugar moieties are found in the region 950-1180 cm^{-1}, the peak at 940 cm^{-1} and 1075 cm^{-1} due to glucose. The band at 1075 cm^{-1} is due to C-N stretching absorption of aliphatic amines is weak and it is the (PO_2^-) symmetric phosphate stretching modes originate from the phosphodiester groups in nucleic acids and suggest an increase in the nucleic acids (Fujioka *et al.,* 2004) and at 940 cm^{-1} is due to carotenoid (Hanlon *et al.,* 2000). The peak at 545cm^{-1} gives an estimate carbohydrate concentrations (Mordechai, 2001; Huleihel & Salman, 2002).

FTIR spectrum of normal pregnant women of 1st, 2nd and 3rd trimester and diabetic pregnant women in each trimester are illustrated in Figs.4-5. The spectral feature are the same for normal and diabetic pregnant women in each trimester but the amount of absorption is decreased in 2nd trimester than that of 1st trimester and further decreased in the third trimester than that of the 2nd trimester. This decrease in absorbance is due to the salivary changes occur during pregnancy i.e., an increase in the secretion of progesterone in the 1st trimester and decreases in the 2nd trimester and further decreases in the third trimester. In order to quantify the results further internal standard ratio of six characteristic modes R_1 (I_{1224}/I_{1075}), R_2 (I_{1544}/I_{1075}), R_3(I_{1544}/I_{3292}), R_4 (I_{2936}/I_{545}), R_5(I_{1646}/I_{1075}), R_6(I_{1646}/I_{1544}), have been calculated and the results are compiled in Table 1.

Fig.4. Comparison of normal pregnant women in each trimester

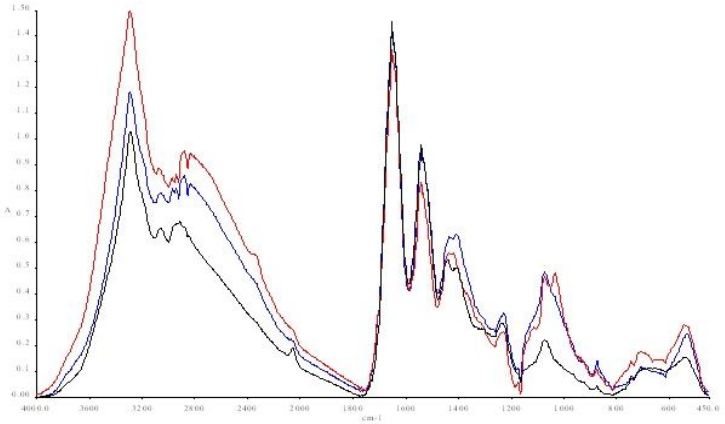

Fig.5. Comparison of diabetic pregnant women in each trimester

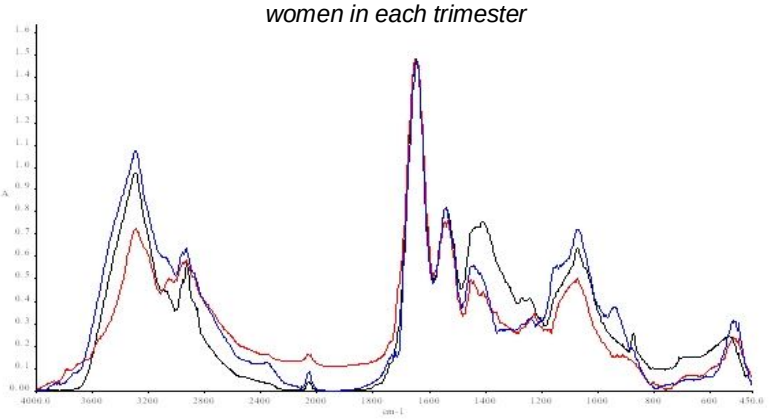

Table1.Comparison of intensity ratio parameters of normal and diabetic pregnant women in each trimester

Intensity Ratio	Pregnant women					
	Normal			Diabetes		
	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
$R_1(I_{1224}/I_{1075})$	1.54	1.5	1.44	2.21	1.59	1.5
$R_2(I_{1544}/I_{1075})$	0.56	0.51	0.5	0.87	0.77	0.68
$R_3(I_{1544}/I_{3292})$	1.22	1.04	0.9	1.32	1.20	0.97
$R_4(I_{2936}/I_{545})$	1.42	1.17	0.96	1.5	1.42	1.28
$R_5(I_{1646}/I_{1075})$	0.35	0.33	0.32	0.48	0.42	0.34
$R_6(I_{1646}/I_{1544})$	0.49	0.48	0.47	0.55	0.54	0.51

Saliva as a body fluid expresses variations in diabetic pregnant women due to increase in the glucose level and also the changes in the salivary pattern due to progesterone and estrogen in 1st , 2nd and 3rd trimester. It is observed there is an increase in the absorbance value of diabetic pregnant women.

Conclusion

The role of FTIR spectroscopy in the analysis of saliva of normal and diabetic pregnant women of 1st , 2nd and 3rd trimester are clearly demonstrated both qualitatively and quantitatively. Among the three trimester the progesterone levels are compared, the absorption of vibrational peaks were highest in the 1st trimester and lower in 2nd trimester and lowest in the 3rd trimester for both normal and diabetic pregnant women which is verified using Internal Standard. The intensity ratio parameter has been calculated and found that the spectra of the diseased saliva shows a greater absorption value than the spectra of the normal saliva due to glucose or sugar moieties present in the diseased saliva of the pregnant women in each trimester.

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