Indian Journal of Science and Technology Vol. 4 No. 5 (May 2011) ISSN: 0974- 6846

Saliva signature of normal pregnant women in each trimester as analyzed by FTIR spectroscopy

R. Raziya Sultana[1], S. N. Zafarullah[2] and N. Hephzibah Kirubamani[3]

[1]*Department of Physics, Justice Basheer Ahmed Sayeed College for Women, Chennai-600018*
[2]*Department of Physics, Easwari Engineering College, Chennai 600089,*
[3]*Obstetrics & Gyneacology, Stanley Medical College & Govt. RSRM Lying. In Hospital, Chennai-600013, India*
saalim_raziya@yahoo.com, saalimraziya@gmail.com

Abstract

We report the changes in salivary pattern of normal pregnant women in each trimester qualitatively and quantitatively. FTIR spectroscopic technique has been successfully applied to analyze the saliva samples of various age groups from18 to 25 and 25 to 35. The role of FTIR spectroscopy in the analysis of saliva of normal pregnant women of 1st, 2nd and 3rd trimester of various age groups are clearly demonstrated both qualitatively and quantitatively. Among the three trimesters, the vibrational peaks of the progesterone increased in the 1st trimester: whereas, in the 2nd trimester there was a decrease: In 3rd trimester there was a further decrease. Similarly, in the case of normal pregnant women of age between 25 to 35 has a greater absorption value than the normal pregnant women of age 18 to 25. This may be attributed to the salivary IgA and IgM concentrations higher in ageing people. It is concluded that FTIR salivary signature may be applied to indicate different stage of pregnancy of different age group.

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

Introduction

The use of infrared spectroscopy for biomedical applications has increased tremendously in the recent years. Fourier Transform Infrared (FTIR) Spectroscopy is a non-invasive, reagent free diagnostic tool in the analysis of biological fluids. The results can be best employed in the qualitative and quantitative investigation of biological fluids like saliva, blood, serum, urine etc. In addition, these techniques also provide molecular-level information allowing investigation of functional groups, bonding types and molecular conformations. Spectral bands in vibrational spectra provide direct information about the biochemical composition (Movasaghi *et al.,* 2008). The promise of IR based analysis is that it can rapidly and simultaneously quantify several components without any specific reagents (Deleris & Petibois, 2003).

Human saliva is composed of 98% water, while the other 2% consists of other compounds such as electrolytes, mucus, antibacterial compounds and various enzymes. As part of the initial process of food digestion, the enzymes in the saliva break down some of the starch and fat in the food at the molecular level. Pregnancy involves complex hormonal interactions, which cause profound physiologic changes. Some changes are more evident than others. The changes that occur are the result of increasing maternal and fetal requirements for the growth of the fetus and the preparation of the mother for delivery. An increase in the secretion of female sex hormones, estrogen by 10-fold and progesterone by 30-fold is important for the normal progression of pregnancy (Sies, 1991). Increased hormonal secretion and fetal growth induce several systemic, as well as local physiologic and physical changes in a pregnant woman. The main systemic changes occur in the cardiovascular, hematologic,respiratory, renal, gastrointestinal, endocrine and genitourinary systems. Local physical changes occur in different parts of the body, including the oral cavity. The present work reports the changes that occur during normal pregnancy in each trimester of various age groups i.e., age between 18 to 25 and 25 to 35. IR absorption patterns provide the basis to distinguish among the constituents and to separately quantify them. In addition, IR- based analytical methods require very small sample volumes (typically microlitres), show good precision over the entire physiological range, and are well suited for automation.

Oral changes seen in pregnancy include gingivitis, gingival hyperplasia, pyogenic granuloma and salivary changes. Increased facial pigmentation is also seen. Elevated circulating estrogen which causes increased capillary permeability, predisposes pregnant women to gingivitis and gingival hyperplasia (Hugoson, 1971; Soory, 2000). Pregnancy gingivitis usually affects marginal and interdental papilla and is related to preexisting gingivitis (Neville *et al.,* 2002). Good oral hygiene can help to prevent or reduce the severity of the hormone-mediated inflammatory oral changes. Pregnancy does not cause periodontal disease but does worsen an existing condition (Tilakaratne *et al*., 2000). Female sex hormones (estrogen, progesterone & human gonadotrophin) are secreted primarily by the placenta. These hormones are responsible for most of the physiologic changes during pregnancy. The main salivary changes in pregnancy involve its flow, composition, pH and hormone levels. Cross-sectional studies show a reduced whole stimulated salivary flow rate in pregnant women, but longitudinal studies show that there is no change in the whole stimulated salivary flow rate (Laine *et al.,* 1988). The change in composition of the saliva

Indian Journal of Science and Technology Vol. 4 No. 5 (May 2011) ISSN: 0974- 6846

includes a decrease in sodium and pH and an increase in potassium, protein and estrogen levels (Laine *et al.,* 1988; Salvolini *et al.,* 1998). 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 sub-gingival crevicular fluid levels. The present work is a new approach in the analysis of saliva in normal pregnant women in each trimester of various age group and the changes in salivary pattern during pregnancy using mid-IR spectroscopy. The present work is also attempted in the study of changes in salivary pattern in normal pregnant women in each trimester using FTIR spectroscopy both qualitatively and quantitatively. The type of spectral signatures qualitatively differentiates the progesterone levels in pregnancy in each trimester: whereas, the intensity ratio among the absorption bands quantitatively characterizes it.

Materials and methods

Saliva samples of normal pregnant women were used for spectral analysis with written informed consent obtained from all subjects as approved by local ethics committee. The samples were collected from 2 sets of study group each consists of 10 volunteers. The first set consists of age group 18 to 25 and the second set consists of age group 25 to 35. The salivary samples were collected from both sets at 1st, 2nd and 3rd trimester of pregnancy. All the sampling procedures were performed between 12 a.m to 1 p.m. 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 mid infrared region of 4000-400 cm^{-1} in the absorption mode. 50 µL of each solution was spread evenly on the thallium bromide crystals window. The samples were air dried for water evaporation to eliminate the stray absorption bands due to water. The spectrometer is equipped with a globar source and DTGS cooled detector. The sampling window is scanned as the background and 32 scans are co-added with a spectral resolution of 1 cm^{-1}. All the spectra were baseline corrected and normalized to acquire identical area under the curve.

Results and discussion

The infrared spectrum is the essence of reflection of the infrared colour pattern characteristics of the sample (Liu *et al.,* 2002). A representative FTIR absorption spectrum of saliva sample of 1st trimester of age from 18 to 25 of normal pregnant women is shown in Fig. 1. A vibration band assignment is done with the idea of the group frequencies of the various analytes present in the sample. FTIR spectroscopy as a bio-diagonostic tool to predict changes occur during pregnancy of 1st, 2nd, 3rd trimester. An increase in the secretion of female sex hormones, estrogen by 10-fold and progesterone by 30-fold, is important for the normal progression of pregnancy. The characteristic vibrational peaks are mainly dominated by the protein constituents of the sample (Petibois *et al.,* 2001; Deleris & Petibois, 2003). The spectral region 3600-3000 cm^{-1} comprises of C-H, O-H and N-H stretching vibrations of the proteins. The prominent absorption peak at 3295 cm^{-1} is due to amide N-H stretching. The asymmetric and symmetric stretching C-H vibrations of methyl and methylene group are found to be present around 2930-2875 cm^{-1}. The observed peak at 2882 cm^{-1} is attributed to C-H lipid region of CH_3CH_2-lipid and protein and 2992 cm^{-1} is assigned to C-H stretching of the vibrational spectra of estriol (C^4 -H) stretching vibration (Minaeva *et al*., 2008), and the band at 2994 cm^{-1} is due to $CH_{\alpha\alpha'}$ stretch. The peak available at 2936 cm^{-1} is due to C-H stretching. The absorption band in the region 1600-1800 cm^{-1} is mainly occupied by C=O stretching. The band at 1653 cm^{-1} is due to C=O, C=N, N-H of adenine, thymine, guanine, cytosine. Absorption at 1620 cm^{-1} is due to progesterone (John *et al.,* 2005) and it is the peak of nucleic acids due to the base carbonyl stretching and ring breathing mode (Chiriboga *et al.,* 1998). The prominent absorption peak at 1646 cm^{-1} is assigned to C_5 methylated cytosine C=O stretching C=C uracyl, NH_2 guanine (Dovbeshko *et al.,* 1997). The observed peak at 1520 cm^{-1} is mainly due to stretching C=N, C=C, C=N of adenine and cytosine (Dovbeshko *et al*., 2002). The band at 1544 cm^{-1} is due to amide II bands (arises from C-N stretching & CHN bending vibrations (Huleihel *et al.,* 2002).The spectral region 1400-1200 cm^{-1} attributed to the C-H deformation of methyl and methylene group of the proteins.

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 peak is at 1075 cm^{-1} is due to symmetric phosphate stretching modes or stretching vibration (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). The peak at 545 cm^{-1} gives an estimate of carbohydrate concentrations (Mordechai *et al.,* 2001; Huleihel *et al.,* 2002). As the IR spectrum exhibits vibration band characteristics of the various group frequencies, the spectrum of a normal pregnant women of 1st trimester and that of 2nd trimester and 3rd trimester saliva samples are the same with respect to the positions of the peaks but different in terms of the absorption levels of the peaks. Fig. 2, 3 shows the FTIR spectrum of normal pregnant women of 2nd trimester and 3rd trimester respectively. It is observed that the spectral features are the same as expected, but the amount of absorption increases in 1st trimester; thereafter

Indian Journal of Science and Technology Vol. 4 No. 5 (May 2011) ISSN: 0974- 6846

Fig. 1. FTIR spectrum of normal pregnant of 1st trimester of age from 18 to 25.

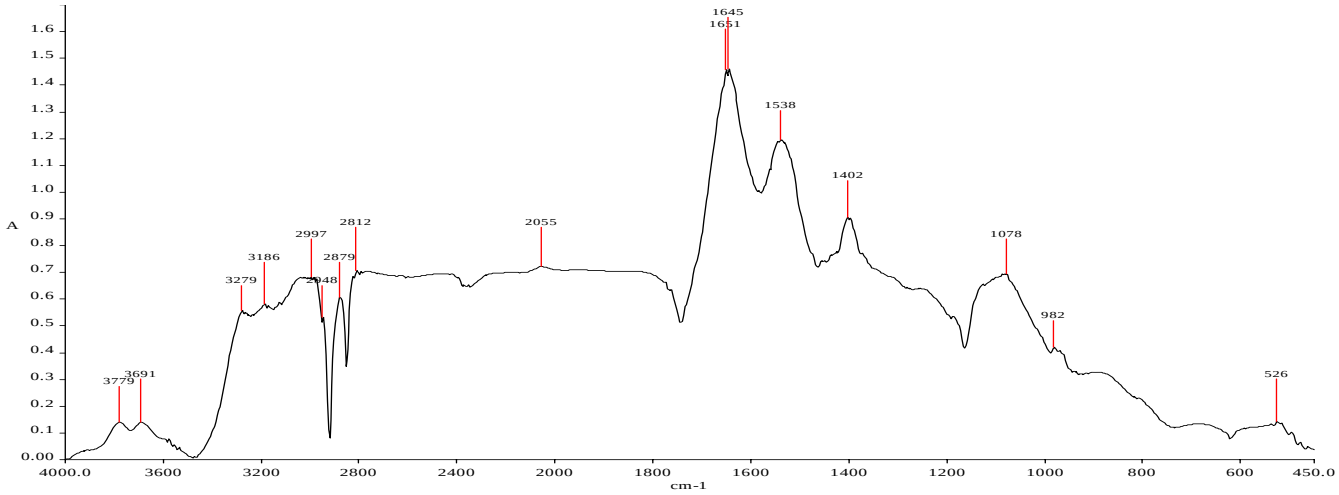


Fig. 2. FTIR spectrum of normal pregnant women of 2nd trimester of age from 18 to 25.

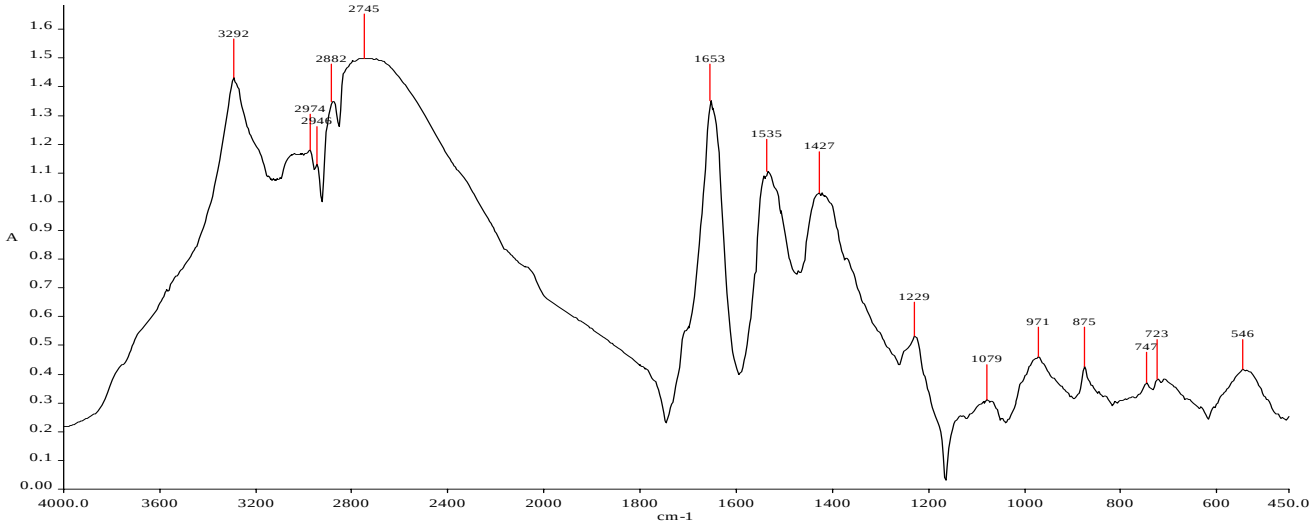
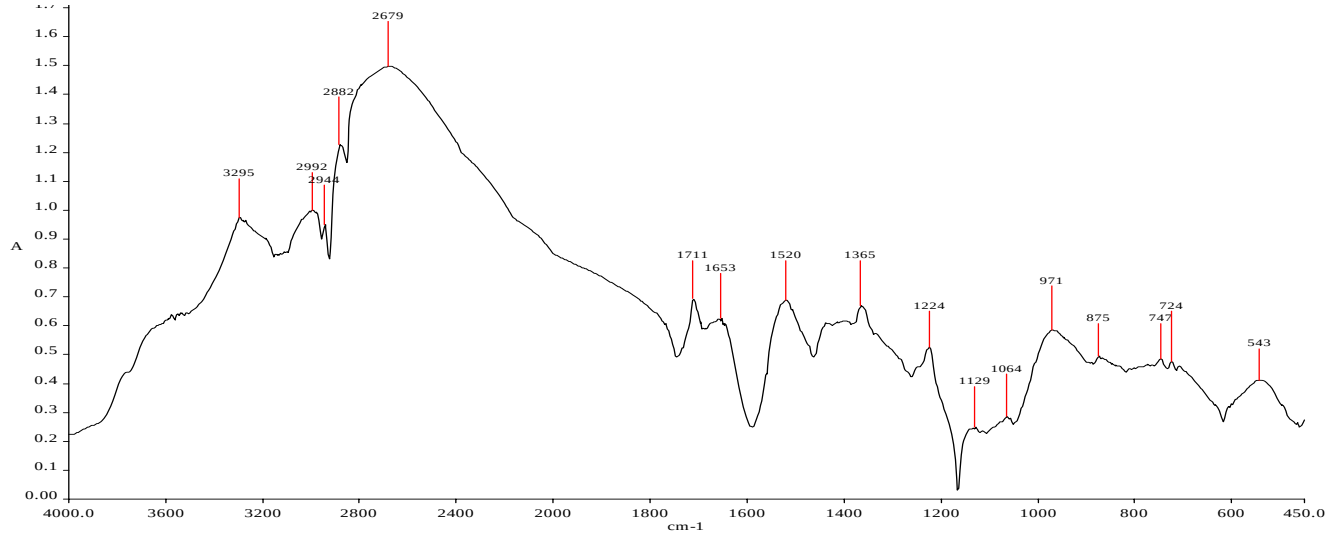


Fig. 3. FTIR spectrum of normal pregnant women of 3rd trimester of age from 18 to 25.



Indian Journal of Science and Technology Vol. 4 No. 5 (May 2011) ISSN: 0974- 6846

Fig. 4. FTIR spectrum of normal pregnant women of 1st trimester of age 25 to 35.

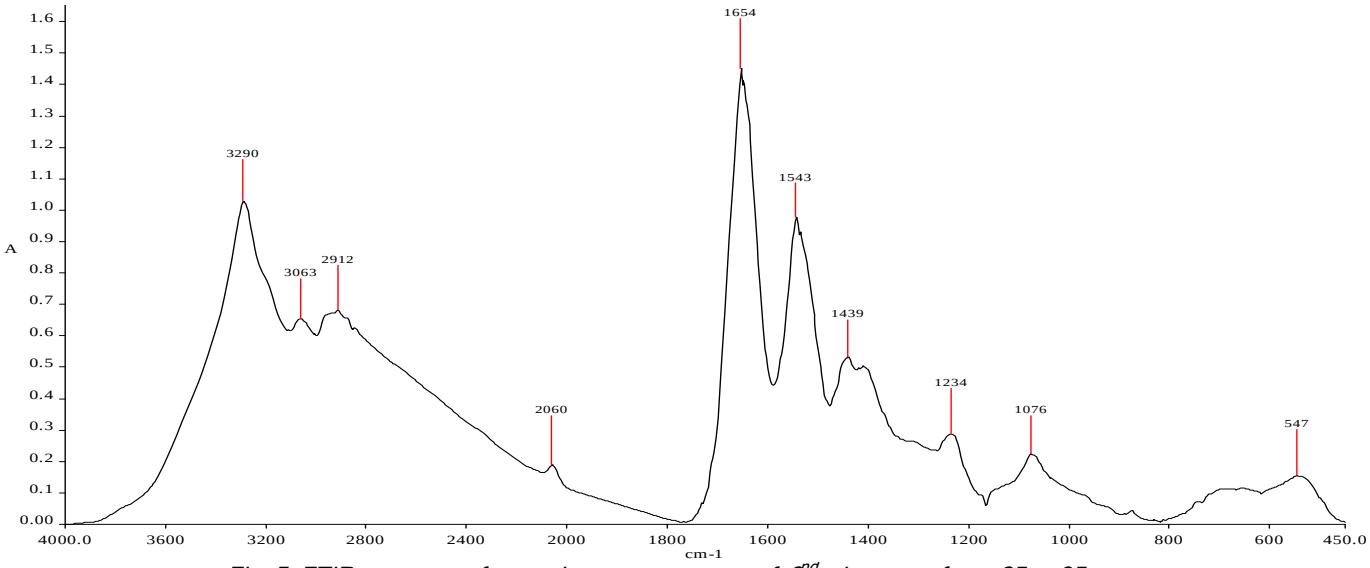


Fig. 5. FTIR spectrum of normal pregnant women of 2nd trimester of age 25 to 35.

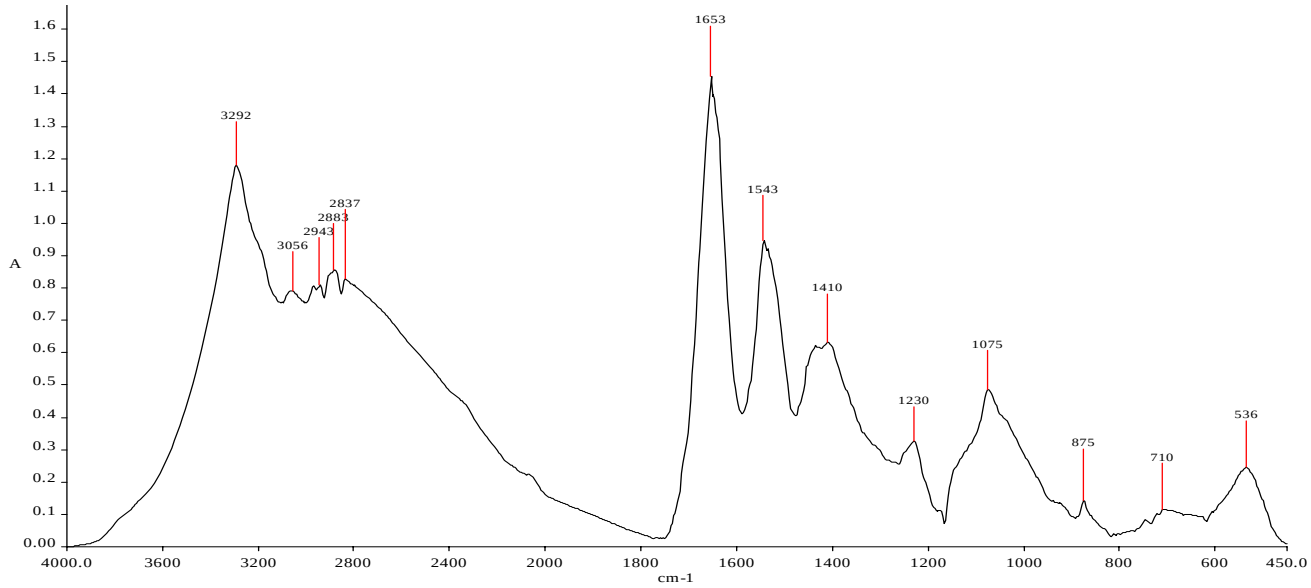
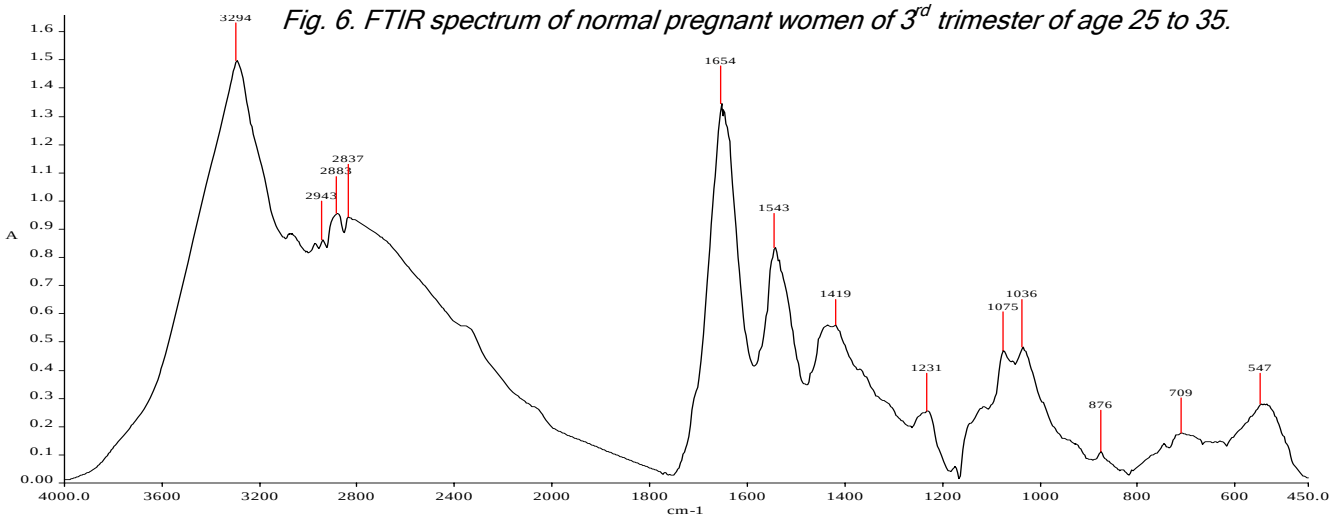


Fig. 6. FTIR spectrum of normal pregnant women of 3rd trimester of age 25 to 35.



Indian Journal of Science and Technology Vol. 4 No. 5 (May 2011) ISSN: 0974- 6846

Table 1. Intensity ratio of normal pregnant women (age 18-25).

Intensity ratio	Age between (18 to 25)		
	1st trimester	2nd trimester	3rd trimester
$R_1=I_{2936}/I_{1646}$	2.07	1.19	0.65
$R_2=I_{2936}/I_{545}$	1.3	0.97	0.72
$R_3=I_{1224}/_{1075}$	1.06	0.58	0.53
$R_4=I_{1646}I_{1544}$	0.54	0.51	0.5
$R_5=I_{1544}/I_{1075}$	0.53	0.44	0.41
$R_6=I_{1646}/I_{1075}$	0.44	0.22	0.2

it decreases in 2nd trimester and further decreased in the 3rd trimester. This decrease in absorption is due to the salivary changes that 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 3rd trimester. Similar pattern is also observed with the normal pregnant women of age group from 25 to 35. In order to quantify the results further, intensity ratio parameters of 6 characteristics absorption bands of saliva R_1 (I_{2936}/I_{1646}), R_2 (I_{2936}/I_{545}), R_3(I_{1224}/I_{1075}), R_4(I_{1646}/I_{1544}), R_5(I_{1544}/I_{1075}), R_6(I_{1646}/I_{1075}), are calculated. It is observed that these values increase in 1st trimester, decreases in 2nd trimester and further decreases in 3rd trimester. Table 1 verifies the same absorption by intensity ratio calculation. The analysis is repeated for the normal pregnant women of age group from 25 to 35. The normal pregnant women of age 25 to 35 has a greater absorption value than the normal pregnant women of age 18 to 25. It may be due to salivary IgA and IgM concentrations higher in ageing people (Pajukoshi *et al.*, 1997). IgA in salivary secretion plays a crucial role in mucosal immunal function and is the first line of defense for the human body against pathogenic microbial invasion. Thus, saliva as a body fluid expresses variations even in the ageing people which can be quantified by FTIR analysis. Fig. 4, 5 and 6 show the absorption levels of the various peaks of normal pregnant women of age between 25 to 35. In order to quantify the results further, 6 intensity ratio parameters R_1(I_{1224}/I_{1075}), R_2(I_{2936}/I_{1646}), R_3(I_{2936}/I_{545}), R_4(I_{1544}/I_{1075}), R_5(I_{1646}/I_{1544}), R_6(I_{1646}/I_{1075}), are calculated. The absorption levels of the peaks increases in 1st trimester and decreases in the 2nd trimester than that of the 1st trimester and further decreases in the 3rd trimester than that of the 2nd trimester. Table 2 verifies the same absorption by intensity ratio calculation.

Table 2. Intensity ratio of normal pregnant women (age 25-35).

Intensity ratio	Age between (25 to 35)		
	1st trimester	2nd trimester	3rd trimester
$R_1=I_{1224}/_{1075}$	2.21	1.59	1.5
$R_2=I_{2936}/I_{1646}$	2.13	1.78	1.55
$R_3=I_{2936}/I_{545}$	1.5	1.42	1.28
$R_4=I_{1544}/I_{1075}$	0.87	0.77	0.68
$R_5=I_{1646}/I_{1544}$	0.55	0.54	0.51
$R_6=I_{1646}/I_{1075}$	0.48	0.42	0.34

Conclusions

FTIR spectra of saliva of normal pregnant women of 1st, 2nd and 3rd trimester differed qualitatively and quantitatively. Among the three trimesters, the vibrational peaks of the progesterone were highest in the 1st trimester and lower in 2nd trimester and lowest in the 3rd trimester. The normal pregnant women of age between 25 to 35 showed a greater absorption value than the normal pregnant women of age 18 to 25 which may be attributed to the higher concentrations of salivary IgA and IgM in ageing people.

References

1. Chiriboga L, Xie P, Yee H, Vigorita V, Zarou D, Zakim D and Diem M (1998) Infrared spectroscopy of human tissue. I. Differentiation and maturation of epithelial cells in the human cervix. *Biospec.* 4, 47-53.
2. Deleris G (2001) Plasma protein contents determined by Fourier-Transform infrared spectrometry. *Clinical Chem.* 47, 730-738.
3. Deleris G and Petibois C (2003) Applications of FT-IR spectrometry to plasma contents analysis and monitoring. *Vibrational Spec.* 32, 129.
4. Dovbeshko GI, Chegel VI, Gridina NY, Repnytska OP, Shirshov YM, Tryndiak VP, Todor IM and Solyanik GI (2002) Surface enhanced IR absorption of nucleic acids from tumor cells: FTIR reflectance study. *Biopolymer (Biospec.).* 67, 470-486.
5. Dovbeshko GI, Gridina NY, Kruglova EB and Pashchuk OP (1997) FTIR spectroscopy studies of nucleic acid damage. *Talanta.* 53, 233-246.
6. Fabian H, Jackson M, Murphy L, Watson PH, Fichtner I and Mantsch HH (1995) A comparative infrared spectroscopic study of human breast tumors and breast tumor cell xenografts. *Biospec.* 1(1), 37-45.
7. Fujioka N, Morimoto Y, Arai T and Kikuchi M (2004) Discrimination between normal and malignant human gastric tissues by Fourier transform infrared spectroscopy. *Cancer Detection Prevention.* 28,32-36.
8. Hugoson A (1971) Gingivitis in women. A longitudinal clinical study. *Odontol Revy.* 22, 65-84.
9. Huleihel M, Salman A, Erukhimovich V, Ramesh J, Hammody Z and Mordechai S (2002) Novel optical method for study of viral carcinogenesis in vitro. *J. Biochem. Biophy. Methods.* 50. 111-121.
10. John FR (Greenville DE, US) and Mei-wei T (Wilmington, DE, US) (2005) IR spectrographic apparatus and method for diagnosis of disease, United States patent application 20090118601.
11. Laine M, Tenovuo J, Lehtonen OP, Ojanatko-Harri A, Vilja P and Tuohimaa P (1988) Pregnancy related changes in human whole saliva. *Arch. Oral Biol.* 33, 913-917.
12. Liu KZ, Shaw RA, Man A, Dembinski TC and Mantsch HH (2002) Reagent-free, simultaneous determination of serum cholesterol in HDL and LDL by infrared spectroscopy. *Clinical Chem.* 48, 499-506.

Indian Journal of Science and Technology Vol. 4 No. 5 (May 2011) ISSN: 0974- 6846

13. Minaeva VA, Minaev BF and Hovorun DM (2008) Vibrational spectra of steroid hormones, estradiol and estriol calculated by density functional theory. The role of low-frequency vibrations (ISSN 0201-8470, 80). Ukr. Biochim. J. 4, 84.
14. Mordechai S, Mordechai J, Ramesh J, Levi C, Huleihel MV, Moser A and Kapelushnik J (2001) Application of FTIR microspectroscopy for the follow-up of childhood leukaemia chemotherapy. Proc. of SPIE subsurface & surface sensing technologies & applications III. 4491, 243-250.
15. Movasaghi Z, Rehman S and Ihtesham ur Rehman (2008) Fourier Transform Infrared (FTIR) Spectroscopy of biological tissues. *Appl. Spec. Rev.* 43, 134-179.
16. Neville BW, Damm DD, Allen CM and Bouquot JE (2002) Oral and maxillofacial pathology. 3rd ed. Philadelphia: WB Saunders. pp:329-330.
17. Pajukoshi H, Jukka H Meurman, Satu Snellman Grohn, Sirpa Keinanen and Raimo Sulkova (1997) Salivary flow and composition in elderly patients referred to an acute care geriatric ward. *Oral Surg. Oral Med. Oral Path. Oral Radiol. Endod.* 84, 265-271.
18. Petibois C, Cazorla G, Cassaigne A, Perromat A and Deleris G (2001) Plasma protein contents determined by Fourier-Transform infrared spectrometry. *Clinical Chem.* 47, 730-738.
19. Randhawa HS (2003) Modern molecular spectroscopy. Macmillan India Ltd. pp:584.
20. Salvolini E, Di Giorgio R, Curatola A, Mazzanti L and Fratto G (1998) Biochemical modifications of human whole saliva induced by pregnancy. *Br. J. Obstet Gynaec.* 105, 656-660.
21. Shaw RA, Low-Ying S, Leroux M and Mantsch HH (2000) Towards reagent-free clinical analysis: Quantitation of urine urea, creatinine, and total protein from the mid-infrared spectra of dried urine films. *Clinical Chem.* 46, 1493-1495.
22. Sies H (1991) Oxidative stress: Oxidants and antioxidants. NY, Academic Press. pp:333-353.
23. Soory M (2000) Hormonal factors in periodontal disease. *Dent. Update.* 27(8), 380-383.
24. Stander HJ (1945) Textbook of obstetrics. Ed. 3, D. Appleton-Century Co., NY.pp:520.
25. Tilakaratne A, Soory M, Ranasinghe AW, Corea SM, Ekanayake SL and de Silva M (2000) Periodontal disease status during pregnancy and 3 months post-partum in rural population of Sri-Lankan women. *J. Clin. Periodontol.* 27, 787-792.