Preliminary Study in diagnosis and early prediction of Preeclampsia by Using FTIR Spectroscopy Technique

Gehan A. Raouf[1]*, Abdel-Rahman L. Al-Malki[2], Nesma Mansouri[3], Rogaia M. Mahmoudi[4]

[1]Medical Biophysics Lab., King Fahd Medical Research Centre; Biochemistry Dep., Faculty of Science, King Abdulaziz University, 21551 Jeddah –KSA B.O.Box:42805
[2,4]Biochemistry Dept., Faculty of Science, King Abdulaziz University, Jeddah –KSA
[3] Obstet. Gyneo. Dept., Faculty of Medicine, King Abdulaziz University, Jeddah–KSA
*gehan_raouf@hotmail.com

Abstract: Background: Preeclampsia is a heterogeneous condition, potentially involving several separate pathophysiological pathways; currently no clinical screening test is useful for prediction of preeclampsia development. Fourier-transform infrared spectroscopy (FT-IR) holds great promise for clinical chemistry measurements. Methods: FT-IR spectra of serum samples from pregnant women -14 patients and 31 normotensive were obtained. Absorbance ratios, second derivative spectra, ANOVA test and personal correlation statistical analysis were tacking in comparison studies. The parameters studied were proteins and lipids. Results: Different absorbance ratios for specific bands were calculated and plotted versus the patient samples. These ratios yielded statistically significant increase/decrease among the groups under investigation. The results showed that among the normotensive control group three subjects later developed preeclampsia. The results obtained from the IR-measured (amide A/amide B) ratio of serum confirmed, with 92.9 % confidence level, the effectiveness of this technique for the diagnosis of preeclampsia. Normotensive pregnant women who developed preeclampsia were considered as subjects at high risk. Conclusion: This study suggests, for the first time that FT-IR spectroscopy can be successfully used as an accurate and rapid test, for diagnosis and early prediction of preeclampsia, starting from 20 week of gestation.

[Gehan A. Raouf, Abdel-Rahman L. Al-Malki, Nesma Mansouri, Rogaia M. Mahmoudi. T. **Preliminary Study in diagnosis and early prediction of Preeclampsia by Using FTIR Spectroscopy Technique.** Life Science Journal. 2011;8(2):453-464] (ISSN:1097-8135). http://www.lifesciencesite.com.

Key words: Fourier Transform Infrared Spectroscopy (FTIR); Oxidative Stress; Dyslipidemia; Preeclampsia; Serum
Abbreviations: Fourier transform infrared spectroscopy (FTIR);

1. Introduction:

Preeclampsia, which affects 3% to 10% of pregnancies [1], is a pregnancy-specific disorder characterized by hypertension, proteinuria and edema. The efforts to develop screening tests for potential use in clinical practice have yielded disappointing results [2]. Markers were generally chosen on the basis of specific pathophysiological abnormalities that have been reported in association with preeclampsia. Maternal concentrations of these biomarkers have been reported to be either increased or reduced early in gestation before the onset of preeclampsia. Given that preeclampsia is likely to be a heterogeneous condition, potentially involving several separate pathophysiological pathways, it is not surprising that simple clinical indicators are ineffective in identifying women who would benefit from pathway-specific treatment [3]. A variety of substances indicative of endothelial dysfunction are increased in the blood or urine of women with preeclampsia[3,4-5]. Many of these substances are elevated weeks before (as well as during) clinically evident preeclampsia [6,7]. It has been suggested that preeclampsia is a disease of antioxidant inadequacy appearing when the normal antioxidant balance is upset [8].

During the last decade, Fourier transform infrared (FTIR) spectroscopy has proven and accepted to be a powerful tool for the study of biological samples. The primary reason for this is that common biomolecules such as proteins, nucleic acids, and lipids, have characteristic functional groups having unique molecular vibrational modes (vibrational fingerprints) corresponding to specific infrared light frequencies [9,10]. The composition and structure of molecular functional groups can be determined by analyzing the position, width, and intensity of infrared light absorption[12-16].

In this study we have tested FTIR spectroscopy as a potential specific accurate diagnostic tool for identifying normal pregnancy and preeclampsia, The second objective of this study was to define a new biophysical marker that is simple, valid and rapid, with potentially no limitation in clinical practice for early prediction of women – that are at high risk- who might later develop preeclampsia.

2. Materials and Methods

This study was approved by the Bioethical and Research Committee at the Faculty of Medicine, King Abdulaziz University (KAU). An oral voluntary consent was obtained from all the participating subjects.

The main focus of this work was to conduct prospective or cross sectional studies aimed at evaluating the feasibility of using a clinical and biophysical test, performed during pregnancy, before the development of preeclampsia.

Inclusion Criteria:

Cases eligible for inclusion in this study were normotensive pregnant women that have no evidence of proteinuria (control group) and patient group either with mild or sever preeclampsia. Preeclampsia was defined as hypertension (systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥ 90 mmHg after 20 weeks' gestation) and proteinuria (≥ 300mg in a 24 hr urine collection or one dipstick measurement of ≥ 1+) according to the Committee of Terminology of American College of Obstetricians and Gynecologists (ACOG) definition [17]. Severe preeclampsia was diagnosed on the basis of diastolic blood pressure ≥ 110 mmHg or significant proteinuria (dipstick measurement of ≥ 2+) or the presence of severity evidences such as headache, visual disturbances, upper abdominal pain, oliguria, convulsion, elevated serum creatinine, thrombocytopenia, marked liver enzyme elevation, and pulmonary edema.

Exclusion criteria:

Included fetal anomalies, chronic heart disease and inflammatory disorders.

2.1. Selection of women:

- **Age:** The age of 45 women participating in this study was from 16 to 49 years (32.0 ± 7.6 years).
- **Week of gestation:** All the subjects were in second and third trimester. The gestational age was between 20 to 42 weeks**.** Data taken from the medical record of subjects are given in Table 1.
- **Study groups:** 31 normotensive pregnant women were taken as control group, and 14 patients diagnosed with preeclampsia were taken as the patients group. All subjects received obstetrical care at KAU hospital.

2.2. Blood Collection and serum separation

Three ml of non-fasting vinous blood were collected from the subjects and drawn into plain tubes. Blood samples were immediately centrifuged at 4000 rpm for 10 min to separate serum. The serum samples were then removed and stored at – 80^{O} C. All the samples were lyophilized prior to FT-IR measurements.

2.3. Infrared Measurement

The lyopholized samples were dispersed in potassium bromide (KBr) by gently mixing with a pestle in an agate mortar to obtain a homogenous mixture as described by Paul and Robert [14] but with 1% concentration. The mixture was then pressed in a die at 5 metric tons force for 3 s, creating a 1.1 cm diameter transparent disc with imbedded samples. For each sample, the absorbance of three different FT-IR spectra were recorded at room temperature(26^{O} C ± 1^{O} C) in the mid infrared range (4000-400 cm^{-1}) using a Shimadzu FTIR-8400s spectrophotometer with continous nitrogen purge. Those three spectra were then coadded. Typically, 20 scans were singal-averaged for a single spectrum and at spectral resolution of 4 cm^{-1}. To minimize the difficulties arising from unavoidable shifts, baseline correction was applied. Each spectrum was normalized as normalization produces a spectrum in which maximum value of absorbance becomes 2 and minimum value 0. Other normalization methods such as normalization to amide II band had also been tested and gave negligible changes in the results. The parameter studied was proteins and lipids. The absorbance ratios were taken from the raw spectra.

2.4. Statistical analysis

Different absorbance ratios for specific bands were calculated and plotted versus the patient samples. An analysis of variance (ANOVA) and personal correlation was conducted to confirm the results obtained from IR measurements.

3. Results

3.1. IR spectral features and assignments

The infrared spectra were obtained for 45 lyophilized serum samples from pregnant women, including 31 samples from women clinically and laboratory assessed as healthy normotensive and 14 samples from patients already diagnosed as preeclampsia. Each spectrum was normalized and base line corrected. Careful examination of the FTIR spectrum obtained from each sample revealed differences in the intensities of the absorption bands in relation to each other, and band shifts were observed among the groups under investigation. Some spectral features in certain control samples resembled those found in the patient spectra.

Careful inspection of the FTIR spectrum obtained from each sample (Fig 1) revealed that the amide B band centered at 3074 cm^{-1} for control samples (15-32) shifted to 3076 cm^{-1} for control samples (33-45) and to 3080 cm^{-1} in patient group. The symmetric

stretching mode of vibration of the CH_3 band centered at 2871 cm^{-1} for control samples (15-32) shifted to 2873 for control samples (33-45) and to 2869 cm^{-1} in the patient group. Moreover, it is also observed from the figure that the intensity of the weak shoulder at 1740 cm^{-1} for the control samples (15-32) turned to a strong shoulder for the majority of patient and control samples (33-45) spectra (Fig. 1B).

Accordingly, to facilitate data interpretation, the control group was divided into two groups: control-1 (samples from 15-32) and control-2 (samples from 33-45), from subjects whose spectra behaved like patient spectra. According to the medical history of most of the control-2 subjects (Table 1) we may consider this group as subjects at high risk although they are normotensive.

For clarity the sum, of equal numbers, of coadded spectra from control-1 samples, the sum of control-2 spectra and the sum of patient spectra are overlaid and shown in (Fig.1). These spectra were dominated by protein, lipids, phospholipids and carbohydrates bands. The major absorbance bands and their assignments are given in Table 2.

3.2. Ratios measurements

The band maxima of the absorption bands around the frequencies 3301, 3074, 2927, 2873, 1652, 1541, 1398, 1244 cm^{-1} were determined after the entire spectrum for each sample has been normalized and base line corrected for subsequent statistical analysis. The following absorbance ratios (**I**) were calculated and represented graphically.

Amide A/Amide B = $[I_{(3301cm^{-1})}/I_{(3072cm^{-1})}]$, **RI** = $[I_{(1652cm^{-1})}/I_{(2927cm^{-1})}]$, **RII** = $[I_{(1541cm^{-1})}/I_{(2927cm^{-1}}$

The **Amide A/Amide B** ratio from all serum samples is represented graphically in Fig (2). It appears from the Figure that the change in the relative intensity of the amide A and B of NH stretching bands (A3301/A3072 absorbance ratio) has the highest value (<1.6 ±0.12) for patient (except patient number 6) and control-2 groups compared to control-1 group. The arrows in the figure are pointed to those control-2 samples which were clinically considered as normal at the time of sample collection and, after following their files and medical record, at the end of their pregnancy they all developed preeclampsia (samples number 34,35 & 39).

The same results are obtained in case of RI and RII ratios (Fig. not shown). For patient and control-2-group the RI and RII values are always greater than 1.6 and 1.4, respectively, compared to the control-1 value which shows much lower values for these ratios. For phosphate/amide II ratio (Fig. not shown) the values of this ratio are lower than 0.4 for patient and control-2 group relative to the control-1-group.

To determine whether the three groups were statistically different from each other, ANOVA test was performed. In the above mentioned IR results, the hypothesis that one of the two means is bigger than the other is assumed before doing the test. The ANOVA results (Table 2) indicated that for all examined ratios there are highly significant differences between patient and control-1 groups. There is no significant difference between patient and control-2 groups in all examined ratios, except in RI. The variation between control-1 & 2 groups is highly significant in all the above tested ratios.

Also personal correlation (Table 3) and linear regression were done between Amide A/Amide B ratio (the golden standard method to discriminate between patient, normotensive control-1, and normotensive control-2 subjects those at high risk to develop preeclampsia), age, week of gestation, body mass index (BMI) and blood group. The results revealed that there is a strong relation between BMI and the elevated Amide A/Amide B ratio. This is true, as overweight increases the incidence of preeclampsia. Moreover, from linear regression analysis the blood group O^+ seems to play a role and increases the risk of this disease (Fig. 3), although this observation should be studied further more with large number of patients and risk groups.

3.3 Second Derivative Analysis

Since eclampsia/preeclampsia involves the modification of existing serum albumin proteins, as well as lipids profile we analyzed the changes of protein structure between the examined groups in more detail. Fig. (4) shows the mean spectra of control-1, control-2 groups together with the patient group in the regions of the esterified lipid C=O stretching band (1750-1700 cm^{-1}), the amide I (1600-1700 cm^{-1}) and amide II (1500-1600 cm^{-1}) bands. Spectra are shown as second derivative. The second derivative of the original spectra offers a direct way to identify the peak frequencies of characteristic components and thus permits much more detailed qualitative and, eventually, quantitative studies. It is obvious from the figure that there are marked shifts in the position and the intensity of both amide I and amide II bands, which strongly affect the overall serum proteins secondary structure.

These amide I frequencies are compatible with the fact that overall protein structure in the control-1-group consists primarily of β-pleated sheets, α-helix and random coil, as the amide I bands centered at 1690, 1652 and 1641 cm^{-1} respectively, whereas in patient group and control-2-group serum samples have a relatively high proportion of α-helix,

random coil and β-turns, as the increase intensities of bands at 1652, 1641 and 1688 cm^{-1} respectively.
For the esterified lipid C=O band, the second derivative spectrum of control-2 displayed a strong sharp band centered at 1741 cm^{-1} together with a weak shoulder at 1721 cm^{-1}, while the control-1 spectrum exhibited only a strong broader band at 1741 cm^{-1}. The patient spectrum showed a more intense strong sharp band at 1744 cm^{-1}, and a weak band at 1712 cm^{-1}.

3.4 Difference Spectrum

In order to further assess and identify the changes in proteins and lipids in serum samples, typical IR marker bands for proteins and lipids were evaluated by creating a 'difference spectrum' in the range of 1800-1400 cm^{-1} (Fig. 5).

Fig. (5A) shows the difference spectrum between patient and control-1 groups, which showed a significant increase in lipid content, triglycerides, fatty acids and cholesterol in patients group, indicated by the positive bands of ester (1737 cm^{-1}) and the positive bands in region 1400-1480 cm^{-1}.

One can also clearly see that the protein secondary structure of control-1 group has lower percentage of α-helix and β-turn than the patient group indicated by the negative amide I band and shifts.

The difference IR spectrum between control-2 and patient groups is shown in (Fig. 5B). It is obvious from the figure that the protein secondary structure of control-2 group has a higher proportion of α-helix structure than patient group, as indicated by the positive band at 1658cm^{-1}. Meanwhile, the control-2 group has a lower amount of lipid ester C=O, as indicated by the negative band at 1744 cm^{-1}

Table 1: General information for subjects given from medical records in KAU hospital.

Sample No		Gestation Age	Age	Blood Group	BMI	History
Patients	1	42	30	O+	31.3	Mild PET, BP=147/89, protein= 0.12, SVD, ↑ fibrinogen, ↑ ALP, ↑ Neutrophils **medication**=aldomate 500 mg, adulate 20 mg.
	2	40	26	B+	30.0	Mild PET, BP=155/90,SVD, protein= 1.6, ↑ fibrinogen, ↑ ALP, ↑ Neutrophils, history preeclampsia, ↑ BP **medication**= adalate 20 mg.
	3	38	38	A+	35 .0	Mild PET, BP= 139/84, GDM, hypertension, +ve protein urea, CS (trans lie verse baby).
	4	37	22	O+	24.3	Protein=0.162, BP=140/85, ↑ BP, ↑ fibrinogen, ↑ ALP, ↑ Neutrophils, PIH, **medication**=aldomate 250 mg.
	5	37	43	O+	43.2	↑BP, FDM, CS (brain aneurysm), ↑ LDL, ↑ cholesterol, protein= 0.15, ↑ ALP, ↑ Neutrophils, history preeclampsia, **medication**=omeprazole, glucophage 75 mg, omperazol, Insulin.
	6	37	43	O+	35.9	Mild PET, BP= 146/90, SVD, ↑ fibrinogen, ↑ ALP, ↑ LDH, history preeclampsia.
	7	37	26	A+	33.9	Mild PET, protein=0.399, BP= 147/85, protein=trace, ↑ fibrinogen, ↑ ALP, ↑ Neutrophils, history preeclampsia **medication**=omperazol 10 mg, aldomate 250 mg.
	8	36	31	A+	41.2	Mild PET, BP=144/80, 151/76, protein= 0.2, CS, ↑ TSH, ↑ fibrinogen, ↑ LDH, ↑ Eosinophils, history preeclampsia **medication**= thyroxin (hypothyroidism).
	9	35	37	B+	32.9	Mild PET, BP=150/90, protein=0.2, CS, previous PET, ↑ fibrinogen, ↑ ALP, ↑ AST, ↑ Neutrophils, **medication**=adalat 20 mg.
	10	34	27	A+	39.8	previous PET,CS, ↑BP, GDM, **medication**= aldomat 250 mg.
	11	34	24	B+	36.0	BP= 149/90, 168/105, CS, ↑ fibrinogen, ↑ ALP, ↑ LDH, ↑ Neutrophils, **medication**= heparin, aldomt 500 mg.
	12	34	16	O+	23.9	Mild PET, BP= 146/84, protein= +2, SVD, wince, ↑ fibrinogen, ↑ ALP, ↑ LDH, ↑ AST, ↑ APTT, **medications**= adalat, ampecillin1g.
	13	32	26	O+	-	SVD, ↑ Neutrophils, ↑ fibrinogen, **medications** = thyroxin.
	14	31	40	A+	36.0	Mild PET, BP= 148/65, protein= 0.28, ↑ fibrinogen, ↑ ALP, ↑ Neutrophils, **medications**= aldomat 250 mg.
Controls	15	37	37	B+	30.7	BP=115/67, SVD.
	16	37	46	B+	-	BP= 132/76, SVD, ↑ fibrinogen, ↑ ALP **medication**= ampecillin1g.
	17	35	25	O+	-	BP= 104/48, CS.
	18	34	24	B+	-	BP= 95/52.
	19	33	29	A+	-	BP= 132/58.

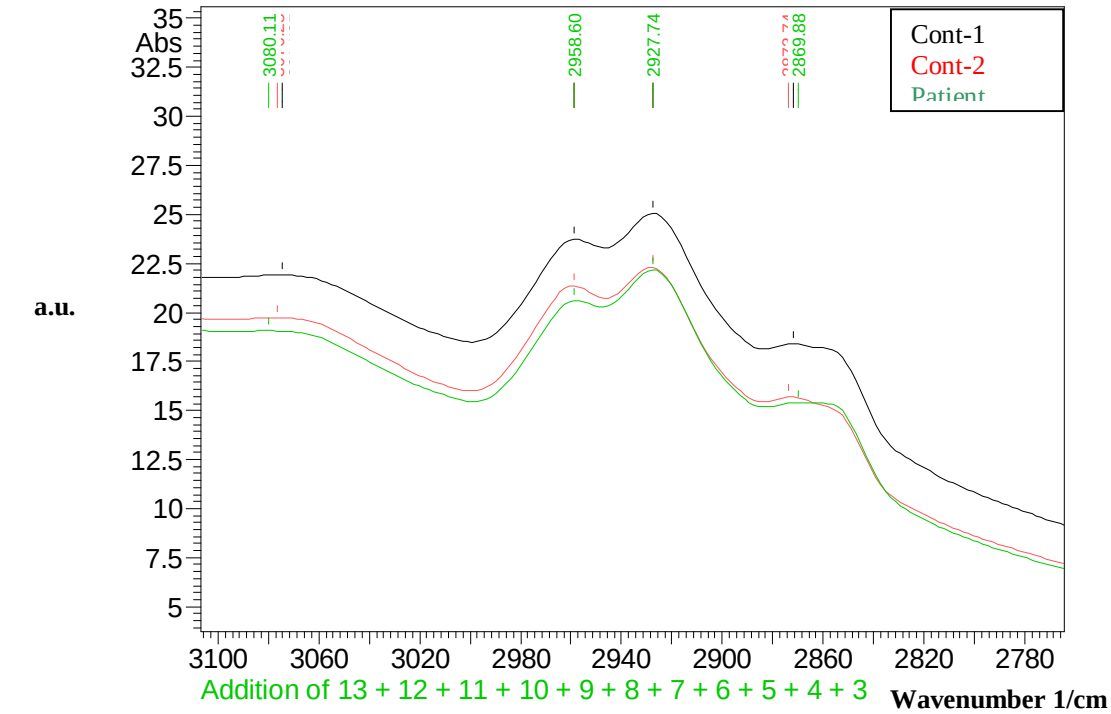
	20	32	38	O+	38.1	BP= 97/63, CS.
	21	32	35	A+	29.8	BP= 117/55.
	22	30	20	A+	28.8	BP= 101/50.
	23	25	28	B+	34.8	BP= 92/56, SVD **medication**= thyroxin 25 mg (Hypothyroidism).
	24	25	36	O^-	47.2	BP= 155/77, BP= 132/85, SVD.
	25	25	35	B+	-	BP= 107/57, CS.
	26	25	27	O+	42.8	BP= 102/53, SVD.
	27	24	27	O+	-	BP= 97/63, CS, ↑ Neutrophils.
	28	22	29	O+	28.2	BP= 117/55, twins, CS.
	29	22	22	A+	-	BP= 101/50.
	30	20	38	O+	-	BP= 92/56, SVD.
	31	20	36	O+	-	BP= 118/59, SVD.
	32	20	28	B+	-	BP= 100/63, CS.
	33	38	43	O+	27.2	BP= 121/76, SVD, anemia during pregnancy, ↑Neutrophils, ↑ALP **medications**= Erythromycin 500 mg.
	34	22	21	O+	16.7	BP= 110/56, SVD. ↑ TSH, ↑glucose, ↑AST, ↑Neutrophils, ↑Basophils.
	35	20	40	A+	33.4	BP= 121/82, CS, ↑Neutrophils,↑Eosinophils, ↑ Fibrinogen, ↑APTT.
	36	20	27	O+	24.7	BP= 90/55, ↑Neutrophils.
	37	35	32	B+	-	BP= 113/60, CS.
	38	35	33	O+	-	BP= 135/87, GDM, SVD, ↑BP, history with disease.
	39	35	38	A+	27.4	BP= 109/59, kidney disease, ↑ total protein, posterior vaginal repair.
	40	33	38	O+	28.0	BP= 122/78, anemia during pregnancy, SVD.
	41	25	28	B+	37.1	BP= 118/74, CS, ↑Eosinophils.
	42	25	42	O+	-	BP= 113/75, SVD, hyperglycemia.
	43	24	49	O+	-	BP= 139/67, diabetes, history preeclampsia, ↑BP, CS, **medications**= aldomat.
	44	24	31	O+	27.3	BP= 113/66, ↑ALP.
	45	21	27	A+	-	BP= 96/63, kidney disease, ↑Neutrophils,↑Eosinophils, Fibrinogen, ↑APTT.

Table 2: The results of ANOVA test and the p-values among control-1, control-2 and patient groups

Multiple Comparisons

LSD

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
RI	Patients	Control 1	.173383*	.044620	.000
		Control 2	-.124244*	.048918	.015
	Control 1	Patients	-.173383*	.044620	.000
		Control 2	-.297627*	.043534	.000
	Control 2	Patients	.124244*	.048918	.015
		Control 1	.297627*	.043534	.000
RII	Patients	Control 1	.112800*	.036778	.004
		Control 2	-.060827	.040321	.139
	Control 1	Patients	-.112800*	.036778	.004
		Control 2	-.173627*	.035883	.000
	Control 2	Patients	.060827	.040321	.139
		Control 1	.173627*	.035883	.000
Amide A / Amide B	Patients	Control 1	.152450*	.028148	.000
		Control 2	-.042808	.030859	.173
	Control 1	Patients	-.152450*	.028148	.000
		Control 2	-.195258*	.027463	.000
	Control 2	Patients	.042808	.030859	.173
		Control 1	.195258*	.027463	.000



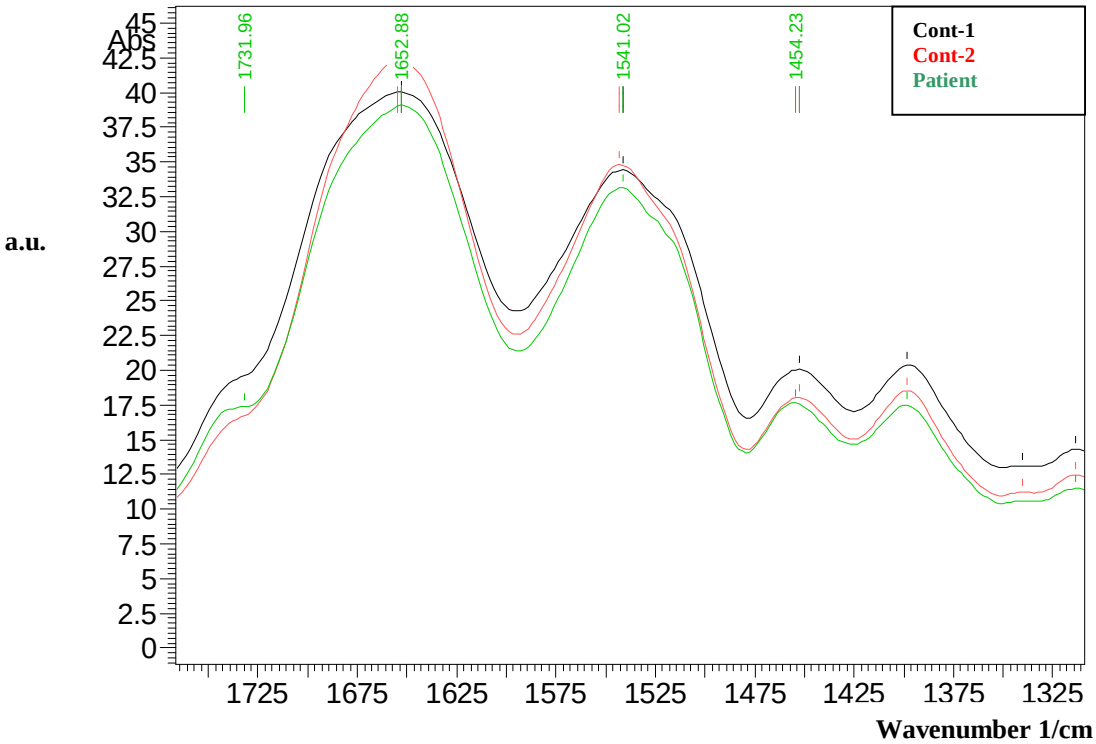


Fig. 1. FTIR spectra represent control-1, control-2 and patient groups in region (3100-2800 cm^{-1}), the arrows pointed to the band shifts in the amide B and CH_3 bands (A). FTIR spectra in region (1700-1300 cm^{-1}), the arrows pointed to the shifts in amide I band together with the variation in the ester band intensity at 1737cm^{-1}(B).

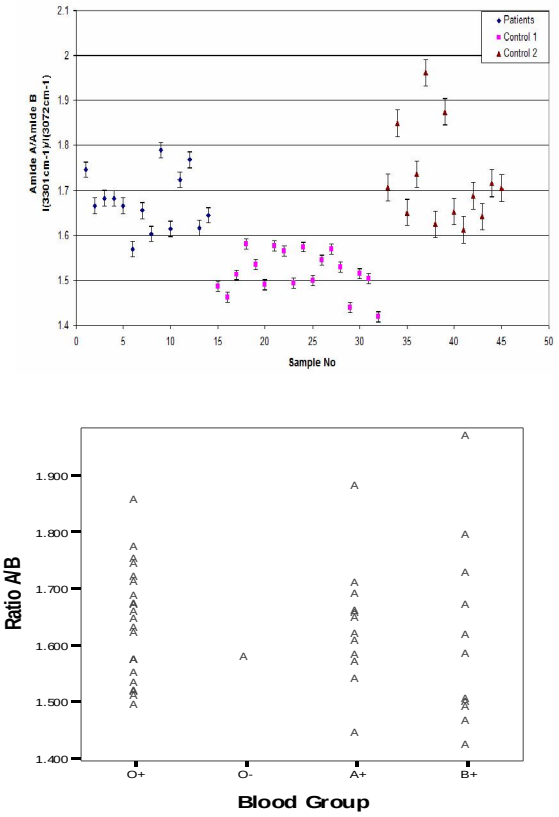


Fig. 3 Linear regression method the figure shows the relation between Amide A/ Amide B ratio and the blood group of all subjects. All ratios over 1.6 are for patient and control-2 groups.

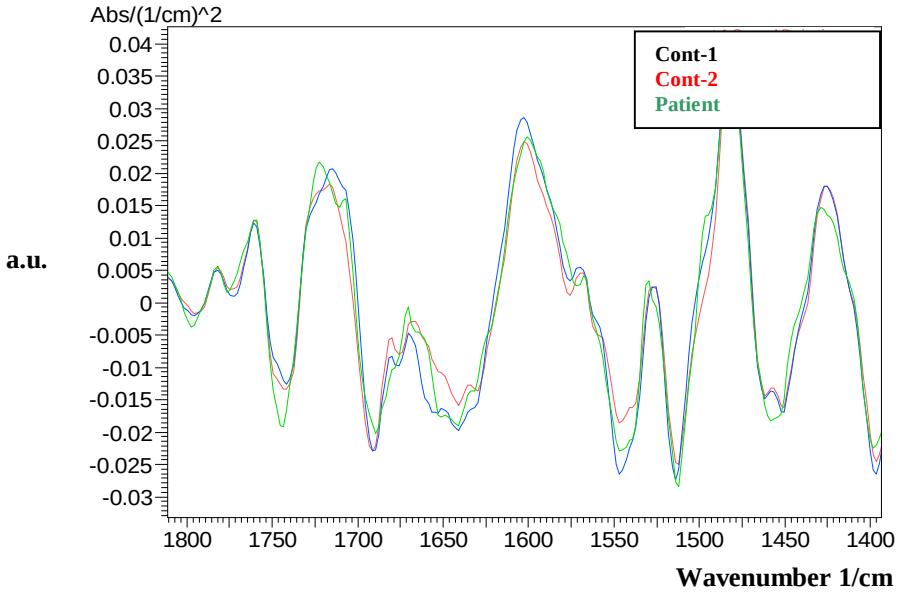


Fig. 4. IR second derivative spectra of serum samples from control-1, control-2 and patient groups in range (1800-1400 cm^{-1}). The arrows pointed to the shift in the band positions and the changes in band intensities for esterified C=O(1750 -1700 cm^{-1}) and amide Ibands(1700-1600 cm^{-1}).

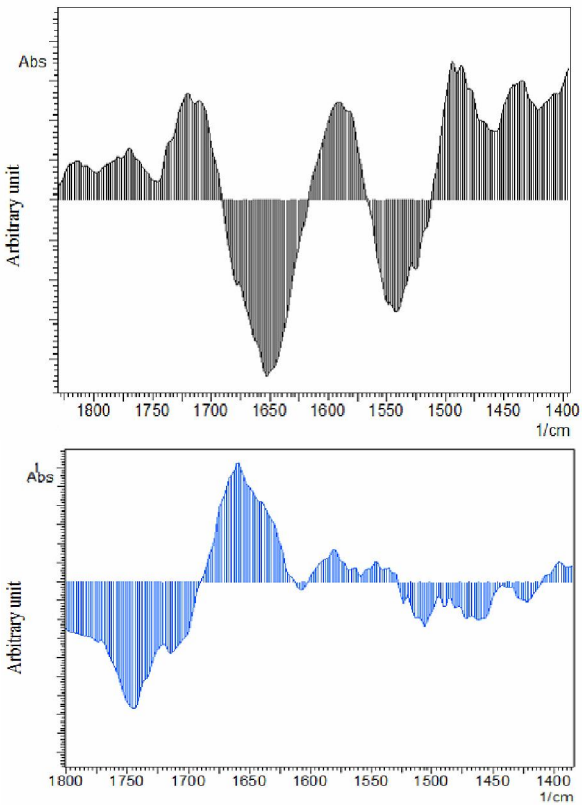


Fig. 5(A) The difference spectrum between patient and control-1 groups showed a significant increase in lipid content, triglycerides, fatty acids, cholesterol and the decreased protein content in patient group than the control-1. (B) The difference IR spectrum between patient and control-2 showed higher proportion of protein α-helix structure than patient group indicated by the positive band at 1658 cm^{-1} and lower content of lipids in control-2 group compared with patient group.

Table 3: Personal correlation between control-1, control-2 and patient samples and the blood group.

Correlations

		Gestation Weeks	Age	Blood Group	Ratio A/B	Weight
Gestation Weeks	Pearson Correlation	1.000	.082	.139	.258	.335*
	Sig. (2-tailed)		.594	.361	.087	.024
	N	45.000	45	45	45	45
Age	Pearson Correlation	.082	1.000	-.078	-.060	.257
	Sig. (2-tailed)	.594		.612	.696	.088
	N	45	45.000	45	45	45
Blood Group	Pearson Correlation	.139	-.078	1.000	-.080	.178
	Sig. (2-tailed)	.361	.612		.599	.242
	N	45	45	45.000	45	45
Ratio A/B	Pearson Correlation	.258	-.060	-.080	1.000	-.074
	Sig. (2-tailed)	.087	.696	.599		.628
	N	45	45	45	45.000	45
Weight	Pearson Correlation	.335*	.257	.178	-.074	1.000
	Sig. (2-tailed)	.024	.088	.242	.628	
	N	45	45	45	45	45.000

*. Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

There is currently no clinically useful screening test to predict the development of preeclampsia[29]. The results obtained from this study will be discussed according to the changes which occurred in the protein secondary structure and the alteration in lipid profiles as a consequence of endothelial dysfunction and oxidative stress which accompany the manifestation of preeclampsia.

A variety of substances indicative of endothelial dysfunction are increased in the blood or urine of women with preeclampsia[4,5]. Many of these substances are elevated weeks before (as well as during) clinically evident preeclampsia [1,6-7].

It has been suggested that preeclampsia is a disease of antioxidant inadequacy appearing when the normal antioxidant balance is upset [8]. Oxidative damage by free radicals or reactive oxygen species (ROS) can result in lipid peroxidation and protein modification [30], causing changes in membrane properties and cell dysfunction [31]. The highly reactive primary products of lipid peroxidation, lipid hydroperoxides, are formed when free radicals attack polyunsaturated fatty acids or cholesterol in membrane and lipoproteins. Lipid hydroperoxides function in normal physiology by regulating enzymes and redox-sensitive genes [32,33]. However, uncontrolled lipid peroxidation, such as that occurring in case of preeclampsia, can result in cellular dysfunction and damage [1, 34,35].
Such damage, known as oxidative stress, is normally prevented by an extensive and multilayered antioxidant system consisting of both low and high molecular weight components [30].

Albumin is the most abundant serum protein. Among the variety of biological mechanisms which have been proposed to explain the beneficial effects of higher albumin concentrations. A direct protective effect of the albumin molecule and the inverse relationship between serum albumin level and mortality risk has been suggested [36-38].

Albumin may represent the major and predominant circulating antioxidant in plasma, which is known to be exposed to continuous oxidative stress [39]. Alterations in the structure of albumin may result in impairments of its biological properties [40]. These modifications could occur in preeclamptic patients and insulin dependent diabetes mellitus patients, which are reasons of the pathological conditions associated with early occurrence of vascular complications, together with functional alterations of albumin.

On the bases of the forgoing consideration, one can explain the significant alteration in the amide I band intensities, together with the observed band shifts in the raw and second derivative infrared spectra of serum samples for control-2 and patient groups compared to the control-1 group. These band shifts are associated with the changes in the conformational structure of proteins Thus, a dramatic change in proteins secondary structure takes place which, in turn, impair the antioxidant effect of serum albumin and blood circulating antioxidant. These changes occurred earlier and were more pronounced in the women who later developed preeclampsia. Our results are also agreed with results obtained by Fraile et al., [41]. They observed that the Amide II/ Amide I ratio is higher for pure albumin as compared with Albumin-lipid systems. Their results revealed that lipids destabilize albumin native structure.

IR spectroscopy support at least modification of secondary structure in albumin upon addition of lipids [41]. The variations in amide A/amide B ratio (the golden standard marker) can be used for diagnostic purposes to differentiate normal pregnancies from preeclamptic patients. For all patients and control-2 this ratio is always higher than 1.6. Moreover, it can be used to predict women who have the potential to develop preeclampsia starting from 20 week of gestation, since the results exhibited a similar behavior between the patient and control-2 group (group at high risk) compared with control-1 group. Meanwhile, the IR second derivative spectra are also capable of differentiating all groups under investigation.

Two out of three samples (samples 34, 35 and 39), who were at 20, 22 weeks of gestation, later developed preeclampsia.

The observed decrease in the intensities of protein bands in our results was also observed by Serge et al., [42] when incubated bovine serum albumin with glucose, revealing structural modifications in the protein. Cohen et al., [43] proved that glycated albumin increases oxidative stress. Our results are consistent with the above mentioned data. Thus, the decreased protein band intensities and the increased glucose band centered at 1033 cm^{-1} were observed for all patient samples. Hence pregnancy is a state of physiological insulin resistance. This state reaches maximal in the third trimester, and is exacerbated in preeclampsia [44,45].

The most outstanding results from this study is that by using Amide A/ Amide B ratio we could early predict preeclampsia, from the thirty one control subjects, in samples number 34, 35 and 39 two of whom were 20, 22 week of gestation. It has been accepted that preeclampsia affects only about 3-10 percent of the all pregnancies. In this study we demonstrated 9.6 % control subjects that had subsequently developed preeclampsia from

normotensive pregnant women. This ratio is more or less comparable with that reported in the literature.

There are scores of reports that lipid peroxidation products are increased in plasma/sera of women with preeclampsia [34, 35]. However, most lipid peroxidation assays have sensitivity and specificity problems [46 ,47]. Morris et al., 1998 [48] found no evidence that circulating lipid peroxidation products (8-iso-PGF2, lipid hydroperoxides, and malondialdehyde) are elevated in preeclampsia once appropriate precautions were taken, including addition of antioxidants, to prevent in vitro oxidation. These findings disagreed with our results, given that even preeclamptic women under medication their IR spectra gave the observed proatherogenic changes in lipid profile (esterified band), significant changes in the amide A/Amide B, RI &RII ratios.

Lipoprotein abnormalities may be involved in the pathogenesis of preeclampsia. Several studies have demonstrated proatherogenic lipid profiles in women months before clinical signs of preeclampsia [49]. Triglyceride levels are elevated, high density lipoprotein (HDL) levels tend to be lower, and small dense low- density lipoprotein(LDL) particles are higher in preeclampsia compared with normal pregnancies [50-53] this shift in LDL particle size to smaller and denser subfractions is thought to be particularly important, as these are highly susceptible to oxidation and may play a critical role in the endothelial dysfunction seen in preeclampsia [3,50]. All of these proatherogenic changes in the lipid profile are also found in cardiovascular disease and diabetic subjects and represent those at high risk for coronary artery disease [54].

The results obtained in this study support the previous work which suggests that there was a positive correlation between preeclampsia and lipid parameters, the significant increased triglycerides and decreased HDL-cholesterol during preeclampsia. provide evidence of abnormal lipid metabolism [55].

The present study has shown that the region of ester C=O stretch (1750-1700cm-1) can be used as a marker for characterizing triglycerides (TG) and cholesterols, which are the main components for VLDL and LDL, respectively. The C=O stretching bands for unsaturated TG and unsaturated Cholesterols exhibit a band at about 1746 and 1738 cm-1, respectively [56].

The peaks at 1745(cholesterol and triglycerides ester C=O), 1710(carbonyl C-O stretch), and 1621cm-1(peptide C=O stretch) positively correlated with LDL oxidation [57].

According to the above mentioned data, the significant shift and increase in the intensity of the band at 1744cm-1 and the appearance of a weak band at 1712cm^{-1} in patient second derivative spectra compared to control-1& 2 can be attributed to the oxidation of LDL during preeclampsia.

5. Conclusion:

FTIR spectroscopy provide a new accurate method in recording changes of serum protein secondary structure and concentration during normal pregnancy and preeclampsia. It also permit the follow up of dyslipidemia that takes place in serum samples starting from 20 week of gestation. The novel golden standard biophysical marker is the amide A/ amide B ratio obtained from the original spectrum and it can give us a screening test in diagnosis and prediction of women who are at great risk to develop preeclampsia. The results obtained from the IR measured ratio Amide A/Amide B of serum revealed, with 92.9 % confidence limits, the possibility of accurate diagnosis of preeclampsia, as well as 100% differentiation between the control-1 group and the risk (control-2) group. These results are to be evaluated taking into consideration the accuracy level of the highest useful method currently used for prediction and diagnosis of preeclampsia (Doppler ultrasonography), which ranges from 20-60 %, with positive predictive value of 6-40%.

Acknowledgement

This research work was supported by King Abdulaziz City for Science and Technology grant number M.S.11-23.

Corresponding author

Gehan A. Raouf

Medical Biophysics Lab., King Fahd Medical Research Centre; Biochemistry Dep., Faculty of Science, King Abdulaziz University, 21551 Jeddah – KSA B.O.Box:42805

gehan_raouf@hotmail.com

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