RAMAN SPECTRAL PREGNANCY FINGERPRINTS IN MATERNAL SERUM

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INTRODUCTION

Raman Spectroscopy (RS) is a non-destructive method of analysis, which is based on the inelastic light scattering of monochromatic light. The pattern of light scattering provides a structural fingerprint of the sample based on the vibrational response to the incoming light which is affected by components in the sample. Modern instrumentation with improved filters, computer algorithms and increased sensitivity allowed for important advancements in the clinical application of RS. Mira M-1 (Metrohm, CA, USA) is a hand-held and high-performance Raman spectrometer which uses Orbital Raster Scan (ORS) latest technology. We recently reported our attempts to identify fingerprints of placental hypoxia in fetal perfusates, obtained in *ex vivo* model. The aim of the present study was to apply this methodology in population of pregnant patients.

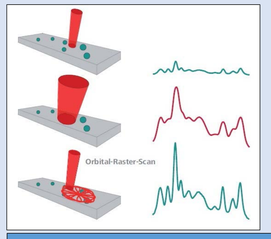

Figure 1: Orbital Raster Scan (ORS) technology is used by Mira M-1, Raman Spectrometer (Metrohm, CA, USA).

MATERIALS AND METHODS

The whole study was approved by TTUHSC as per IRB. Maternal blood samples were collected from 15 patients in the first and third trimester. The blood samples were centrifuged at 3500 rpm for 15 min. The serum was aliquoted and stored at -80^0 C. Raman spectrometer was used to analyze serum samples of first and third trimester. Raman spectra were collected using Mira Cal software (Metrohm, CA, USA). The data was quantified by Principal Component Analysis (PCA) and other statistical analysis in *RStudio* programming language. Feature vectors were collected at each time point from Raman spectra data, obtained from serum samples of all patients. Spectral characteristics of Raman spectra were analyzed using KnowItAll® spectroscopy software and databases (Biorad, PA, USA).

Sample's ID	Gravity	Parity	Fetal weight (gm)	Fetal gender	Maternal BMI (kg/cm^2)	Pre-Eclampsia (Y or N)	Mother's Drug Use (Y or N)
BM03	1	0	3090	F	22.9	N	N
BM04	1	0	3680	M	41.5	N	N
BM06	1	0	2900	M	33.7	N	N
BM09	4	0	4930	M	43.6	Y	N
BM10	4	3	3220	F	NR	N	N
BM11	3	2	3700	F	31.6	N	N
BM19	1	0	3430	F	41.2	N	N
BM21	2	1	2650	F	20.6	N	N
BM23	1	0	3180	M	38.6	N	N
BM24	2	1	3510	F	41.3	N	N
BM27	6	4	3370	M	32.6	N	N
BM31	7	5	4100	F	35.3	N	N
BM34	1	0	3790	F	23.1	N	N
BM36	4	2	2860	M	38	N	N
BM61	5	4	3460	F	21.1	N	N

Table 1: Samples IDs and patients' characteristics.

RESULTS AND DISCUSSION

The data, obtained with Raman spectrometer could be used as point-of care test in prenatal diagnosis. Since changes, observed during gestation are associated with placental maturation, the RS analyses might represent a useful tool for rapid analyses of placental fingerprints in maternal circulation. The quest to identify detectable serum biomarkers of abnormal pregnancy or placental function has been an ongoing focus in an attempt to improve maternal and fetal care. The first trimester of pregnancy is associated with the absence of the contact between maternal circulation and intervillous space of the placenta. The early onset of the blood flow in the intervillous space - prior to weeks 8 and 9 post conception due to defective trophoblastic invasion and plugging of the maternal spiral arterioles is associated with abnormal pregnancy development. This early onset of placental dysfunction is associated with oxidative stress of developing placenta and detectible markers of such stress in maternal circulation. Maternal serum markers in early pregnancy have been extensively studied in association with the pregnancy complications. The maternal serum soluble vascular endothelial growth factor (VEGF) receptor 1 (sFlt-1), soluble Endoglin and placental growth factor (PlGF) at 6-10 weeks of gestation were lower in the women with early pregnancy loss, compared to healthy pregnancy. In general, placental biomarkers herald the development of placental dysfunction and are present well before subsequent pregnancy complications become clinically apparent.

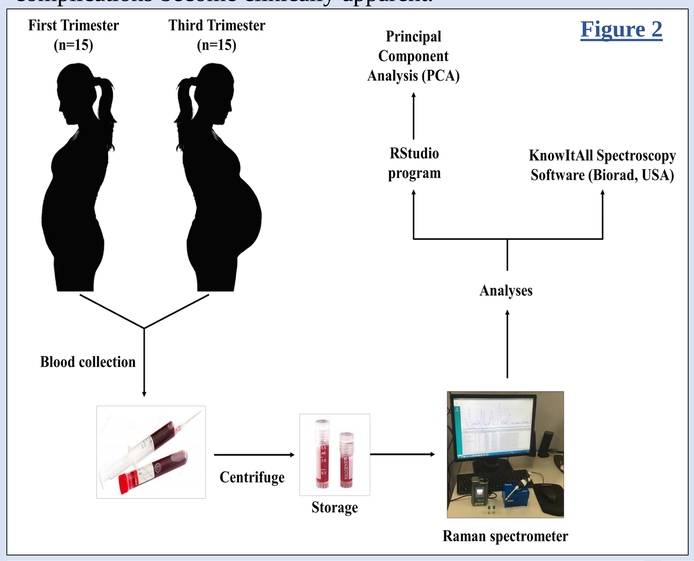

Figure 2

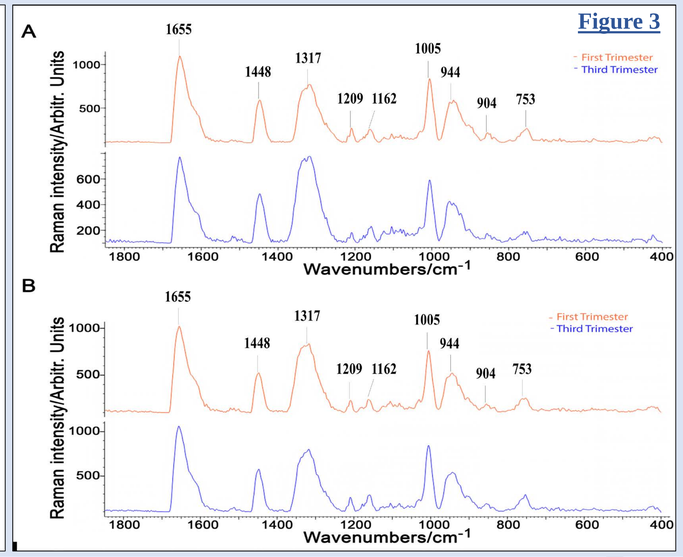

Figure 3

Range	Classification	Bond	Mode	Group
1680-1650	R-O-N=O : Nitrates	N=O	Stretching	1
1665-1630	CH=CH cis : Alkenes	C=C	Stretching	1,2,3,4
1660-1640	R2C=CH2 : Alkenes	C=C	Stretching	1,2,3,4
1660-1615	R-O-NO2 : Nitrates	NO2	Antisymmetric stretching	1,2,3,4
1490-1400	R-CO-NH-C : Amides	CNH	Combination	1,2,3,4
1360-1300	R-NH-CO-NH2 : Ureas	N-C-N	Antisymmetric stretching	3
1350-1310	R-CO-NH-C : Amides	CNH	Stretching	1,2,3

Table 2. Characteristics of the serum Raman spectra, obtained from first trimester and third trimester of pregnant patients (**Group 1:** Non separatable first trimester; **Group 2:** Non separatable third trimester; **Group 3:** separatable first trimester and **Group 4:** separatable third trimester).

Figure 2: Experimental design of serum Raman spectra, collected from first trimester and third trimester of pregnant patients.

Figure 3: Analysis of average serum Raman spectra from first trimester and third trimester of two different groups: **(A)** Separatable and **(B)** Non separatable.

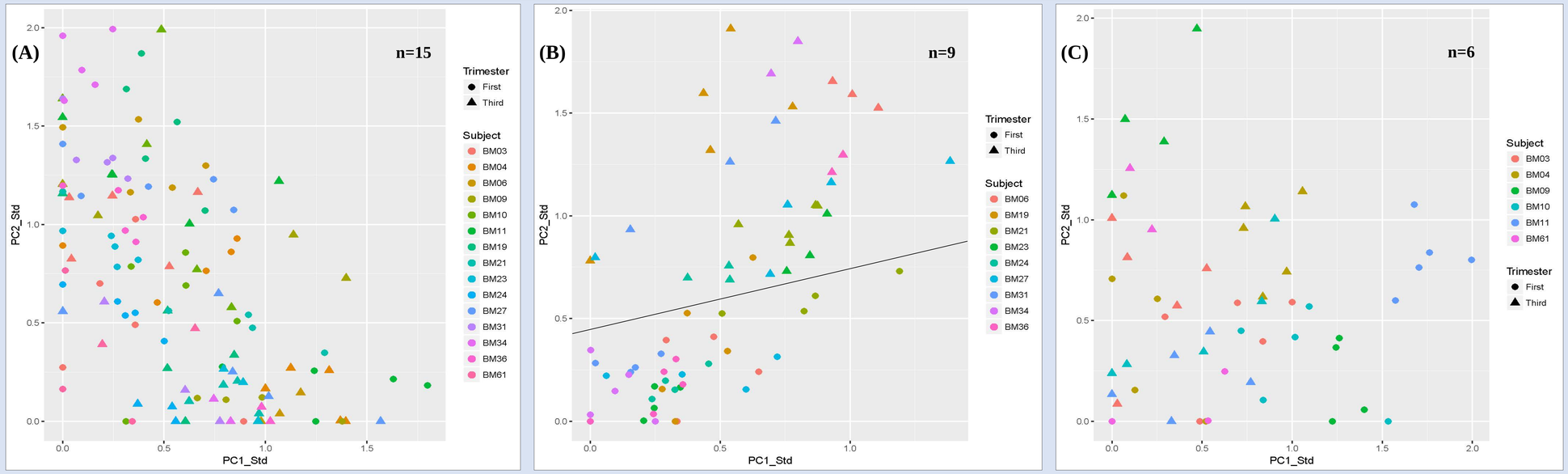

Figure 4: (A) PCA on Raman spectra, obtained from serum samples of all 15 patients in first trimester and third trimester of pregnancy. **(B)** PCA on Raman spectra, obtained from serum samples of specific 9 patients out of 15 patients in first trimester and third trimester of pregnancy (Separatable group). **(C)** PCA on Raman spectra, obtained from serum samples of remaining 6 patients in first trimester and third trimester of pregnancy (Non separatable group). [PC1 and PC2: Principal Components 1 and 2; BM: Serum samples of patients].

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