NOVEL APPLICATION OF HANDHELD RAMAN SPECTROSCOPY (RS) DEVICE AS POINT OF CARE DIAGNOSTIC TOOL IN PERINATAL MEDICINE

Suheung Lee[1], Gandhi K[1], Maher J[1], Ventolini G[1], and Schlabritz-Loutsevitch N[1]

[1]Department of Obstetrics and Gynecology, Texas Tech University Health Sciences Center at the Permian Basin, Odessa, TX, USA. Email: Natalia.schlabritz-lutsevich@ttuhsc.edu

INTRODUCTION

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[1]. Timely point-of care diagnosis is particularly relevant in light of increased maternal morbidity and mortality in the United States[2]. If we are to reverse these trends we must do a better job of identifying sick mothers before they are critically ill.

Current medical communities have an urgent need to develop rapid point-of-care techniques that effectively provide diagnostic information in a short period of time and allow instant analyses and distribution of data among providers. However, the most commonly used diagnostic modalities in clinical settings either lack easy accessibility, or take considerable time to provide results. There has been a recent report regarding an application of Mira M-1 (Metrohm, CA, USA), a hand-held Raman spectrometer, for rapid diagnosis of placental hypoxia[3]. The report combined Principal component analyses with the specific Raman Spectral software in order to dissect the patterns, associated with the pregnancy progression[3].

Raman spectroscopy (RS) is the methodology which allows for an investigation of physiology at cellular and tissue levels using photon scattering[3]. It is a non-destructive and non-invasive method. Each peak in a Raman spectrum is associated with a unique part of the molecule and can be used for identification and confirmation. Orbital Raster Scanning (ORS) is a novel Raman spectroscopy sampling technique that helps to drive more accurate measurements and comprehensive analysis[3]. ORS increases the sensitivity of Raman measurements up to 10 fold by rastering a tightly focused laser beam over a larger area[3]. (*raster: A scanning pattern of parallel lines that form the display of an image projected on a cathode-ray tube of a television set or display screen[4]) RS could be used as the specific metabolic fingerprint and therefore represents a unique diagnostic tool (Figure 1, Monograph, Introduction of Raman spectroscopy, Metrohm, USA).

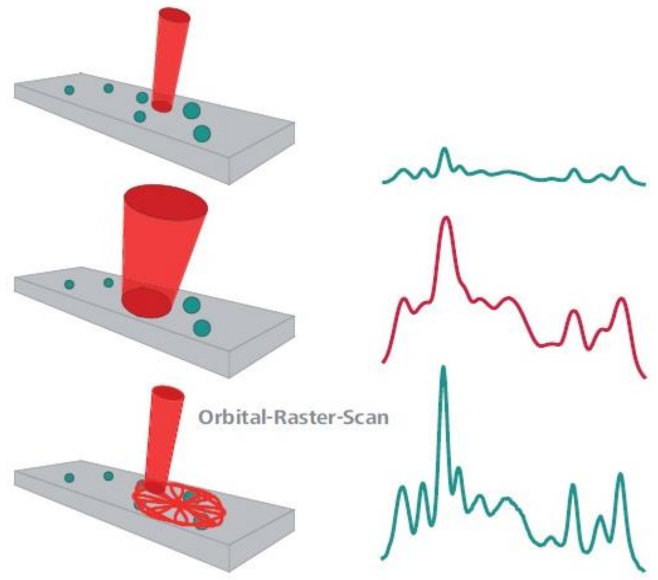

Figure 1: Dispersive spectrometers use tightly-focused beam (top), resulting in a high spectral resolution, but components in heterogeneous samples can be missed completely. Simple broadening of the beam would result in a loss of special resolution (center). The ORS technique (bottom) scans a larger sample area and maintains high spectral resolution that is required for analyte identification. [Monograph, Introduction of Raman spectroscopy, Metrohm[4]]

OBJECTIVES

In this study we aimed to identify the Raman Spectral pattern of normal pregnancy, as well as the one of pregnancy from obese women during 1st and 2nd trimester, using Mira M-1 device.

MATERIALS AND METHODS

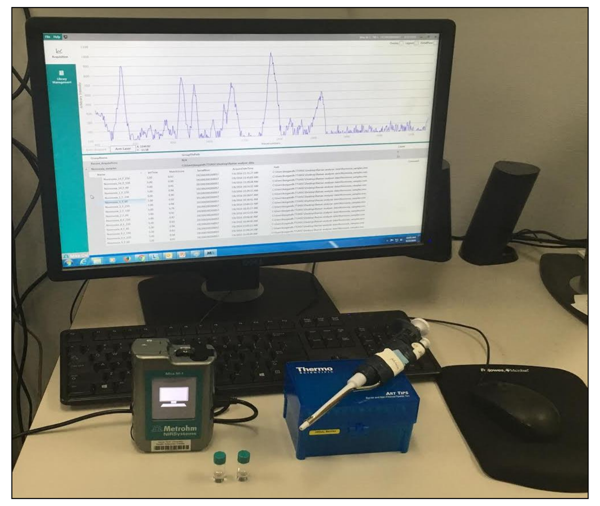

Figure 2a: Experimental setup for Raman spectroscopy instrument (Mira M-1, Metrohm, USA).

- Obese (n=7, BMI > 30) and non-obese pregnant women (n= 8 with BMI < 30) were enrolled into this study in the first and second trimester of pregnancy (Institutional Protocol # L17-136). Raman spectrum (RS) was obtained, and Raman Spectroscopy analysis of serums of Obese vs. Non-obese Women in the 1st and 2nd trimester of pregnancy was performed.
- Each serum sample was aliquoted in cryogenic vials, stored at -80°C until it was used. The samples were thawed on ice before analyses. RS of each aliquot was obtained with Mira M-1, and the spectra were analyzed with MiraCal software (Metrohm, USA).

Figure 2b: A schematic diagram that displays the overall method of RS using Mira M-1 device and RS analysis with MiraCal software (Metrohm, USA)

RESULTS

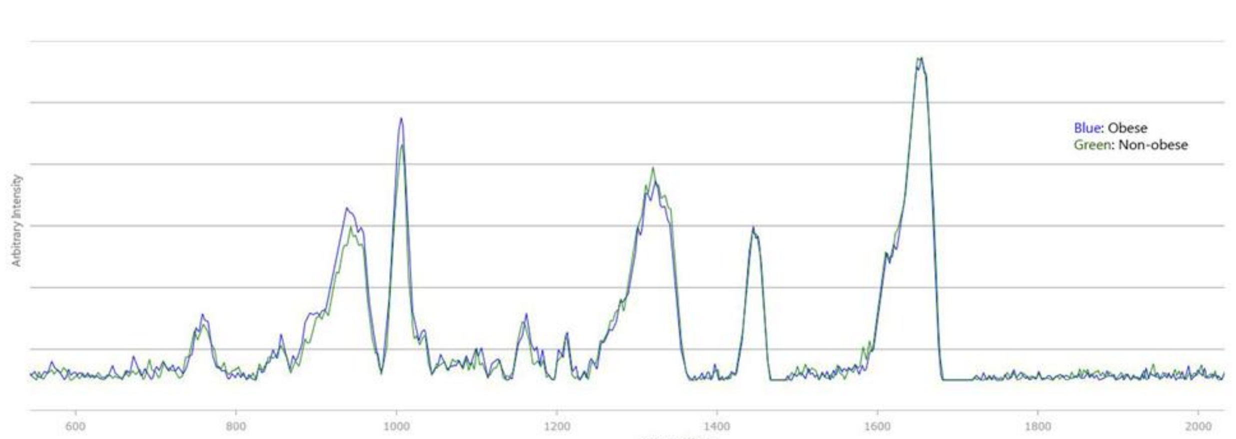

Figure 3: Raman Spectroscopy Analysis of Serums of Obese vs. Non-obese Women in the 1st trimester of Pregnancy

Raman shift (cm^{-1})	Range (cm^{-1})	Classification	Bond	Mode
765.26	790-650	CH=CH cis: Alkenes	C-H	Out-of-plan deformation
	770-620	R-CO-NH-C: Amides	N-H	deformation
	850-750	R-O-N=O: Nitrites	N-O	stretching
890.68	895-885	$R_2C=CH_2$: Alkenes	CH_2	Out-of-plane deformation
	960-875	COOH: Carboxylic acids	OH	Out-of-plane deformation
937.98, 955.59	960-875	COOH: Carboxylic acids	OH	Out-of-plane deformation
1005.1	1060-900	$C-CH_3$: Alkane	CH	Rocking vibration
	1010-940	$CH=CH_2$: Alkenes	CH	Out-of-plane vibration
	1020-905	C-C≡CH: Alkynes	CH	Stretching
1162.42	1190-1140	$R_2N-CO-NH_2$: Urea	N-C-N	Symmetric stretching
1299.94	1320-1290	$R_2C=CH_2$: Alkenes	CH	Deformation
	1310-1250	R-CO-NH-C: Amides	CNH	Stretching
1324.15	1350-1310	R-CO-NH-C: Amides	CNH	Stretching – open cis
	1360-1300	$R_2N-CO-NR_2$: Urea	NCN	Asymmetric stretching
1655.3	1670-1645	HO-C=C-CH: Aldehydes	C=O	Stretching
	1660-1640	$R_2C=CH_2$: Alkenes	C=C	Stretching
	1680-1640	$R-CO-N-R_2$: Amides	C=O	Stretching

Table 1a: Raman Spectra of Serums from Obese Women in the 1st trimester of Pregnancy

RESULTS

Raman shift (cm^{-1})	Range (cm^{-1})	Classification	Bond	Mode
1007.3	1060-900	$C-CH_3$: Alkane	CH	Rocking vibration
	1010-940	$CH=CH_2$: Alkenes	CH	Out-of-plane vibration
	1020-905	C-C≡CH: Alkynes	CH	Stretching
1158.02	1190-1140	$R_2N-CO-NH_2$: Urea	N-C-N	Symmetric stretching
1319.75	1350-1310	R-CO-NH-C: Amides	CNH	
	1360-1300	$R_2N-CO-NR_2$: Urea	NCN	Asymmetric stretching
1648.7	1670-1645	HO-C=C-CH: Aldehydes	C=O	Stretching
	1660-1640	$R_2C=CH_2$: Alkenes	C=C	Stretching
	1680-1640	$R-CO-N-R_2$: Amides	C=O	Stretching

Table 1b: Raman Spectra of Serums from Non-obese Women in the 1st trimester of Pregnancy

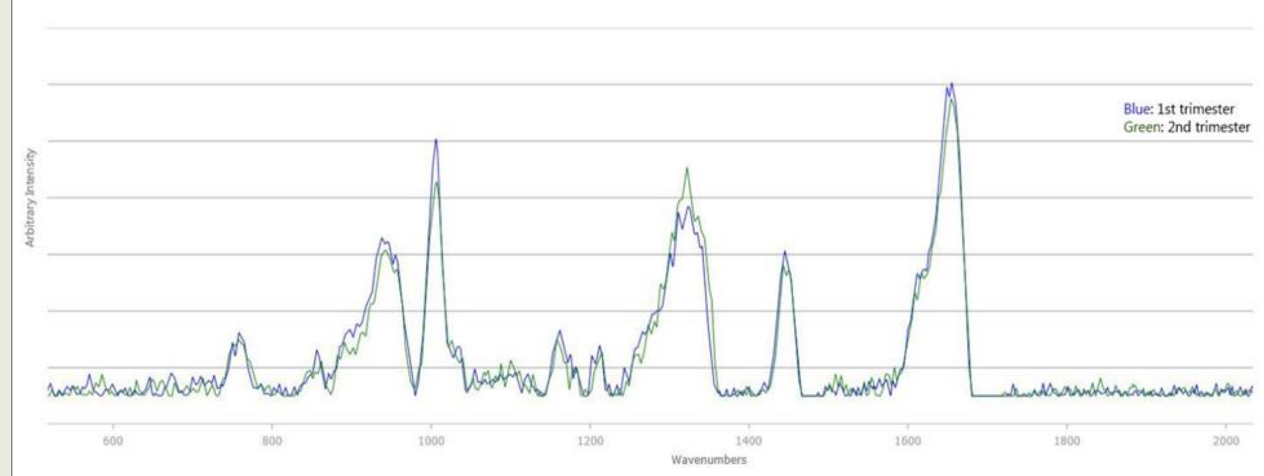

Figure 4: Raman Spectroscopy Analysis of Serums from 1st and 2nd trimester of Obese pregnant women

Raman shift (cm^{-1})	Range (cm^{-1})	Classification	Bond	Mode
935.78	960-875	COOH: Carboxylic acids	OH	Out-of-plane deformation
1006.2	1060-900	$C-CH_3$: Alkane	CH	Rocking vibration
	1010-940	$CH=CH_2$: Alkenes	CH	Out-of-plane vibration
	1020-905	C-C≡CH: Alkynes	CH	Stretching
1162.42	1190-1140	$R_2N-CO-NH_2$: Urea	N-C-N	Symmetric stretching
1200.93	1255-1200	CCCC: Alkanes	C-C	Rocking vibration
	1225-1200	$CH_2=CH-O$: Vinyl Ethers	C-O-C	Asymmetric
	1295-1145	$R-NH_2$: Amines	N-H	Rocking vibration
	1225-1075	Phenyl-C=O: Aryl ketones	Phenyl-C	Stretching
1324.15	1350-1310	R-CO-NH-C: Amides	CNH	Stretching – open cis
	1360-1300	$R_2N-CO-NR_2$: Urea	NCN	Asymmetric stretching
1445.17	1450-1325	HO-C=C-CH: Aldehydes	CH	Rocking
	1490-1400	R-CO-NH-C: Amides	CNH	Combination; stretching bending cis
1649.8, 1655.3	1670-1645	HO-C=C-CH: Aldehydes	C=O	Stretching
	1660-1640	$R_2C=CH_2$: Alkenes	C=C	Stretching
	1680-1640	$R-CO-N-R_2$: Amides	C=O	Stretching

Table 2a: Raman Spectra of Serums from 1st trimester pregnant women in the Obese group

Raman shift (cm^{-1})	Range (cm^{-1})	Classification	Bond	Mode
942.39	960-875	COOH: Carboxylic acids	OH	Out-of-plane deformation
1007.3	1060-900	$C-CH_3$: Alkane	CH	Rocking vibration
	1010-940	$CH=CH_2$: Alkenes	CH	Out-of-plane vibration
	1020-905	C-C≡CH: Alkynes	CH	Stretching
1156.92	1190-1140	$R_2N-CO-NH_2$: Urea	N-C-N	Symmetric stretching
1321.95	1350-1310	R-CO-NH-C: Amides	CNH	Stretching – open cis
	1360-1300	$R_2N-CO-NR_2$: Urea	NCN	Antisymmetric stretching
1442.96	1450-1325	HO-C=C-CH: Aldehydes	CH	Rocking
	1490-1400	R-CO-NH-C: Amides	CNH	Combination; stretching bending cis
1654.2	1670-1645	HO-C=C-CH: Aldehydes	C=O	Stretching
	1660-1640	$R_2C=CH_2$: Alkenes	C=C	Stretching
	1680-1640	$R-CO-N-R_2$: Amides	C=O	Stretching

Table 2b: Raman Spectra of Serums from 2nd trimester pregnant women in the Obese group

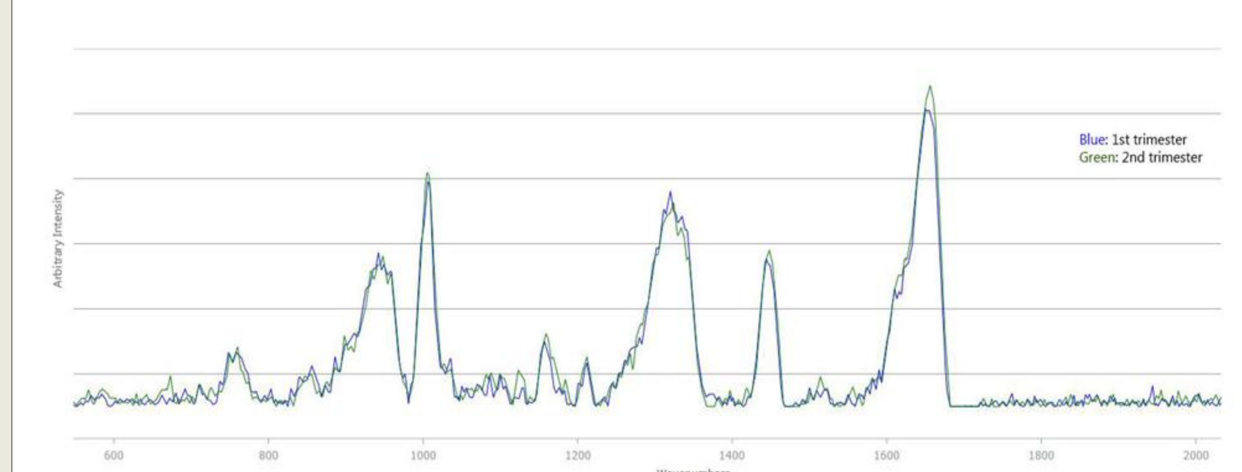

Figure 5: Raman Spectroscopy Analysis of Serums from 1st and 2nd trimester of Non-obese pregnant women

Raman shift (cm^{-1})	Range (cm^{-1})	Classification	Bond	Mode
1319.75	1350-1310	R-CO-NH-C: Amides	CNH	Stretching – open cis
	1360-1300	$R_2N-CO-NR_2$: Urea	NCN	Antisymmetric stretching

Table 3a: Raman Spectra of Serums from 1st trimester pregnant women in the Non-obese group

Raman shift (cm^{-1})	Range (cm^{-1})	Classification	Bond	Mode
1324.15	1350-1310	R-CO-NH-C: Amides	CNH	Stretching – open cis
	1360-1300	$R_2N-CO-NR_2$: Urea	NCN	Antisymmetric stretching

Table 3b: Raman Spectra of Serums from 2nd trimester pregnant women in the Non-obese group

RESULTS

- Raman spectra of obese and non-obese pregnant women in the 1st trimester of pregnancy were also compared. There were subtle but notable differences in Raman spectra between the two groups, indicating changes of chemical compositions in serum. From the obese group, we detected 8 Raman peak shifts that were not present in the non-obese group (Table 1a). The non-obese group also had 4 Raman peaks unique to its own group (Table 1b).
- We found differences between the 1st and 2nd trimester of pregnancy in the obese women, whereas the non-obese women did not display differences during these pregnancy windows (Tables 2 and 3).

CONCLUSIONS

This study demonstrates feasibility of utilization of handheld RS device to detect differences in RS during pregnancy. We identified Raman peaks that were unique to each group, indicating the possible impact of obesity that generates chemical and molecular compositions in the blood that are different from the ones in the non-obese condition. This finding implies that RS can be used for early detection of pathologic changes due to obesity during pregnancy. Medical professionals can initiate active management for obese pregnant mothers to prevent detrimental effects from obesity, earlier than the time that current established protocols currently indicate.

We also detected differences in RS between the 1st and 2nd trimester groups in the obese pregnant women. This finding implies the progressive nature of physiologic impacts from obesity; we can argue that obesity causes chemical and molecular changes in the blood throughout the pregnancy. It is notable, that this pattern was not found between the 1st and 2nd trimester group of the non-obese pregnant women. The absence of changes in RS among non-obese pregnant women during the 1st and 2nd trimester of pregnancy further emphasizes that obesity causes changes in chemical and molecular patterns.

REFERENCES

1. Cuffe, J. S. M., Holland, O., *et al.* 2017
2. Arabin, B. & Baschat, A. 2017
3. Schlabritz-Loutsevitch, N., Gandhi, K. *et al.* 2017
4. https://www.news-medical.net/whitepaper/20151214

ACKNOWLEDGEMENTS

The authors are thankful to Clinical Research Institute for the help with this study.