Is endothelial function impaired among women with placenta-mediated fetal growth restriction? Evidence from a prospective cohort study using peripheral artery tonometry

Michal Kirshenbaum[1], Lior Topaz[1], Micha Baum[1], Shali Mazaki-Tovi[2], and Yoav Yinon[3]

[1]Sheba Medical Center
[2]Sheba Medical Center, Tel-Hashomer, Israel 52621; Sackler School of Medicine, Tel Aviv University, Tel Aviv Israel 69978
[3]Chaim Sheba Medical Center

August 28, 2020

Abstract

Objective- To assess maternal endothelial function in singleton pregnancies complicated by intrauterine growth restriction (IUGR) due to placental dysfunction. Design- Prospective cohort study. Setting- 37 pregnant women who underwent endothelial function assessment using EndoPATTM device. Population or Sample- Study population included two groups: 1. Pregnancies with estimated fetal weight below 10th percentile and abnormal umbilical artery flow (n=15); 2. Pregnancies with normal fetal growth without placental complications matched by gestational age (n=22). Exclusion criteria included diseases with potential vascular dysfunction or smoking. Methods- EndoPAT device evaluates changes in peripheral vascular flow and tone in reaction to temporal ischemia. Normal post-ischemic endothelial reaction is an increase in vascular flow. A ratio of the readings before and after ischemia is used to calculate the score for endothelial function, called reactive hyperemic index (RHI). Low RHI value indicates endothelial dysfunction. Main outcome measures- RHI values. Results- Mean gestational age at endoPAT examination was comparable between the IUGR and control group (32.5 ± 2.2 vs, 31.6 ± 3.2, respectively; p=0.21). Mean RHI was significantly lower in the IUGR group compared to the control group (1.32 ± 0.16 vs 1.51 ± 0.31, p= 0.02, respectively). As expected, mean gestational age at delivery and neonatal birth weight were lower in the IUGR group compared to the control group (35.4 ± 2.3 vs 37.3 ± 2.6 p=0.04; 1640 ± 414 grams vs 2785 ± 587 grams, p<0.001 respectively). Conclusions- Pregnant women with isolated IUGR due to placental dysfunction had impaired endothelial function.

Tweetable abstract:

Pregnant women with isolated IUGR due to placental dysfunction were found to have impaired systemic endothelial function.

Funding statement:

Introduction:

Normal placental angiogenesis is a crucial process that establishes feto-maternal circulation. Inadequate placental angiogenesis, defined as defective remodeling of the uteroplacental arteries, can lead to placental complications such as early pregnancy loss, preeclampsia and intra-uterine growth restriction (IUGR) [1].

The physiological vascular changes observed in pregnancy, including an increase in cardiac output and reduction in peripheral resistance and blood pressure, are largely governed by alteration in endothelial function

mediated via nitric oxide and vascular endothelial growth factor [2,3]. Moreover, normal pregnancy is associated with a reduction in central blood pressure, arterial stiffness and wave reflection [4]. There is a large body of evidence indicating that endothelial dysfunction is involved in the pathophysiology of preeclampsia. Studies, which have investigated endothelial function by measuring flow mediated dilatation (FMD), demonstrated decreased vasodilatation response in preeclamptic women compared to normal pregnant women [56]. Moreover, augmentation index, a measure of systemic arterial stiffness, is elevated in pregnancies complicated with hypertensive disease and particularly preeclampsia [78]. In addition, maternal vascular function might help in defining a subset of patients who will develop severe preeclampsia characteristics [9]. These findings supports the perception that vascular dysfunction is the underlying pathophysiology of preeclampsia. In line with this hypothesis, several studies have shown that endothelial dysfunction is more common in women with a history of preeclampsia many years after the affected pregnancy, compared to women with previous normal pregnancy. These findings might explain their increased risk of future cardiovascular disease [10–12]. Similar to preeclampsia, isolated intra-uterine growth restriction (IUGR) is also associated with future maternal increased risk of ischemic heart disease[13]. Moreover, endothelial function, measured by FMD, was significantly reduced in women with previous isolated placenta-mediated IUGR 6-24 months after delivery [14]. Nevertheless, maternal endothelial function has never been evaluated during pregnancies complicated by isolated placenta-mediated IUGR.

There are several noninvasive methods for endothelial function assessment, all of which are based on the change in diameter and flow in arteries in response to post ischemic reactive hyperemia[15]. A widely used method for assessing endothelial function is flow mediated dilation (FMD). Nonetheless, the clinical application of FMD is limited due to its dependence on the operator's skills , its technical difficulties and problems with calibration between laboratories, which may preclude its use in widespread clinical practice [16]. Therefore, new noninvasive techniques have been developed in-order to make endothelial function assessment more accessible in general medical practice. Reactive hyperemia-peripheral artery tonometry (RH-PAT, EndoPAT^{TM}-2000, Itamar Ltd., Caesarea, Israel) has been recently developed as a simple and potentially more reproducible method. Previous studies validated the use of EndoPAT^{TM} and found a reliable correlation with conventional FMD [1718].

Therefore, our aim was to compare endothelial function of healthy women during pregnancy complicated with IUGR to those with normal pregnancies using the EndoPAT^{TM} method.

Methods:

Subjects-

This was a prospective observational cohort study, in which women who attended the MFM clinics at a single tertiary center, were enrolled during an 18 months period. The study group included women with pregnancies complicated by isolated placenta-mediated IUGR. IUGR was defined as estimated fetal weight below the 10thpercentile using population-based growth curves for singletons accompanied by an abnormal umbilical artery Doppler flow, defined as pulsatility index above the 95th percentile or absence/ reverse of end-diastolic velocity [19]. The control group included women with uncomplicated pregnancies with appropriate for gestational age (AGA) fetus matched to the study group by gestational age at testing. Exclusion criteria for both groups included hypertensive diseases of pregnancy, chronic hypertension, renal disease, diabetes mellitus, smoking, body mass index >30 kg/m^2 , aspirin treatment and betamethasone administration within a week prior to the EndoPAT test. Patients with fetal anomalies or genetic abnormalities were also excluded. The abnormal umbilical artery Doppler flow and lack of fetal anomalies and genetic abnormalities indicated that placental insufficiency was most likely the underlying cause of IUGR.

The study was approved by the institutional review board of Sheba medical center. All women gave informed written consent before entering the study.

Endothelial function assessment-

Maternal endothelial function was assessed using the Endo-PAT^{TM} 2000 device. Unlike previously used

techniques of duplex ultra-sonography to assess flow-mediated vasodilation, the Endo-PAT[TM] is totally non-operator-dependent method, which assesses digital flow mediated dilatation during reactive hyperemia, as a measure of endothelial function. The device records endothelium-mediated changes in the digital pulse waveform known as the PAT (Peripheral Arterial Tone) signal, measured with a pair of novel modified plethysmographic probes, situated on the index finger of each hand. Endothelium-mediated changes in the PAT signal are elicited by creating a downstream hyperemic response. Hyperemia is induced by occluding blood flow through the brachial artery for 5 minutes using an inflatable cuff on one hand. The response to reactive hyperemia is calculated automatically by the system. Reactive Hyperemia Index (RHI) is the post to pre occlusion PAT signal ratio in the occluded side. These values are normalized to measurements from the contra-lateral arm, which serves as control for non-endothelial dependent systemic effects. Higher RHI values represent adequate endothelial cell hyperemic response and well endothelial function. Based on previous validation studies, RHI >1.67 was considered as normal [1820].

All examinations were performed in the same temperature controlled room, during the morning, after a night fasting. Figure 1 shows examples of normal and abnormal EndoPAT tests.

Statistical analysis-

On the basis of previous validation studies, which defined normal endothelial function as RHI >1.67 and revealed a variance of ~ 15% in RHI measurement, 11 subjects in each group were required to detect a difference between groups of at least 15% (corresponding to absolute difference in RHI of 0.25 units) with 80% power and 5% type 1 error [21,22]. Normally distributed data were described as mean ± standard deviation (SD), and non-normally distributed data were described as median and intra-quartile range (IQR). Univariate analyses were performed by t-test or Mann-Whitney test as appropriate for continuous variables and by Chi-square or Fisher's exact test for categorical variables. Significant difference was defined as p<0.05. All analyses were conducted using SPSS 24 (SPSS Inc., Chicago, IL).

Results:

A total of 37 patients were enrolled and included in the study: 15 patients with IUGR consisted the study group and 22 patients with AGA fetus consisted the control group. Maternal characteristics and obstetric outcomes of the study groups are resented in table 1. Maternal age, parity and BMI were comparable between the groups (table 1). As expected, patients in the IUGR group were delivered earlier compared to the controls (36.7 vs 37.7 weeks, p=0.04) and the mean neonatal birth weight was significantly lower among the IUGR group compared with the normal controls (1634 ± 428 grams vs 2785 ± 587 grams respectively, p<0.01).

The results of endothelial function assessment are described in table 2. The median gestational age at EndoPAT test was 32 weeks in both groups (table 2). Systolic and diastolic blood pressure did not differ between the two groups at the time of endothelial function assessment (113 vs 110 mmHg, p= 0.08; 74 vs 67 mmHg, p=0.06, respectively). The median RHI value was significantly lower in the IUGR group compared with the normal control group (1.31 vs 1.47 respectively, p=0.04). All women in the IUGR group had an abnormal RHI value (<1.67), compared with 68% of the control group (p=0.056).

Disscusion:

In the current study, we found that RHI values of women with pregnancies complicated with placenta-mediated IUGR were significantly lower compared with RHI values of pregnant women with normal intra-uterine growth. The lower RHI values indicate systemic vascular dysregulation and endothelial dysfunction.

Of note, although the median RHI value was significantly lower among women in the IUGR group, the median RHI value in women with normal fetal growth was also below the level defined as normal in the general population. This finding is in correlation with previous studies that assessed endothelial function in different stages during normal pregnancy, and found that from the third trimester onward, there is a gradually deterioration in endothelial function [2324]. Therefore, it is possible that there is a physiological decrease in endothelial function during normal pregnancies. This finding may account for the lack of significant difference

3

in the rate of endothelial dysfunction between the two groups, according to the definition used in the non-pregnant population. Of note is the fact that despite the physiologic endothelial dysfunction demonstrated in the control group, the absolute lower RHI levels among women with IUGR pregnancies indicates that endothelial function is impaired in women with normotensive IUGR.

It is well established that women with preeclampsia exhibit endothelial dysfunction both during pregnancy and years after delivery[11,25,26]. However, the data regarding IUGR pregnancies without hypertension or preeclampsia are limited. Endothelial cell activation markers were reported to be higher only in preeclamptic but not in IUGR pregnancies, supporting the hypothesis that endothelial dysfunction in IUGR pregnancies is confined to the uteroplacental circulation and is not systemic as in preeclampsia [27]. On the other hand, Yinon et al. have showed that patients with previous early onset placenta-mediated IUGR without preeclampsia had endothelial dysfunction years after delivery similar to patients with previous early-onset preeclampsia. Moreover, patients with previous IUGR had the most severe degree of endothelial dysfunction even in comparison to patients with previous preeclampsia [14]. These findings correlate with epidemiological data indicating increased risk for cardiovascular disease in women who had pregnancies complicated by IUGR[13]. The findings of our study support the aforementioned studies and show that endothelial function in women with pregnancies complicated by IUGR is already impaired during pregnancy. It is well known that multiple key angiogenic factors play a crucial role in placental angiogenesis, amongst them are sFLT-1, sEng, VEGF and PlGF, which coordinate to generate the placental villous tree, and abnormal expression of these angiogenic factors will result in endothelial dysfunction [28][29]. However, it is still unknown whether maternal exposure to these angiogenic factors released from the ischemic placenta during pregnancy result in endothelial damage and future maternal vascular disease or whether endothelial dysfunction is a preexisting condition leading to both placental dysfunction in pregnancy resulting in preeclampsia and IUGR and to increased risk of future cardiovascular disease later in life. We believe that our data support the later theory in which maternal vascular dysfunction is a predisposing factor leading to both abnormal placental development and future cardiovascular disease. However, in order to prove this hypothesis, it would be preferable to assess endothelial function prior to conception.

Our finding of impaired endothelial function in women with pregnancies complicated with IUGR may explain the increased risk of these women for future cardiovascular disease. Therefore, intervention such as lifestyle modification or prophylactic treatment may prove beneficial in reducing long- term morbidity and mortality among these women.

The strength of this study lies in its prospective nature and the well characterization of the study groups including patients with isolated IUGR without hypertensive diseases. Only cases of IUGR that were accompanied with abnormal umbilical artery Doppler flow were included, thus ensuring that all cases of IUGR were due to placental insufficiency. Moreover, to best of our knowledge, this is the first study investigating endothelial function in IUGR pregnancies using the EndoPAT^{TM} method. The main limitation of this study is the small number of patients studied, which may limit our ability to detect differences between the groups. However, despite the small sample size, significant difference in RHI was found between the two groups.

Conclusion:

Our study found that endothelial function among women with pregnancies complicated with IUGR due to placental insufficiency was significantly impaired compared to women with normal pregnancies. More studies are needed in order to assess the effectiveness of endothelial function test using EndoPAT device early in gestation to predict IUGR and the implication of endothelial dysfunction during pregnancies complicated with IUGR on these women's future health.

Disclosure of interests: The authors have no financial, personal, political, intellectual or religious interests to declare.

Contribution to authorship:

Michal Kirshenbaum- Conceived and planned the experiment, performed the endothelial function assessment

4

using the EndoPAT device and wrote the manuscript.

Lior Topaz- Performed the endothelial function assessment using the EndoPAT device.

Micha Baum- Helped in planning the experiment and recruiting the patients.

Shali Mazaki-Tovi- Helped in planning the experiment, recruiting the patients and interpreting the final results.

Yoav Yinon- Conceived and planned the experiment, contributed to the interpretation of the results and consulted in writing the manuscript.

References:

1. Bardin N, Murthi P, Alfaidy N. Normal and Pathological Placental Angiogenesis. *Biomed Res Int* . 2015;354-359

2. Savvidou MD, Donald a E, Nicolaides KH. Assessment of endothelial function in normal twin pregnancy. *Ultrasound Obstet Gynecol* . 2001;17(3):220-223.

3. Khalil A, Jauniaux E, Cooper D, Harrington K. Pulse wave analysis in normal pregnancy: A prospective longitudinal study. *PLoS One* . 2009;4(7):e6134

4. Macedo ML, Luminoso D, Savvidou MD, McEniery CM, Nicolaides KH. Maternal wave reflections and arterial stiffness in normal pregnancy as assessed by applanation tonometry. *Hypertension* . 2008;51(4):1047-1051.

5. Yoshida a, Nakao S, Kobayashi M, Kobayashi H. Flow-mediated vasodilation and plasma fibronectin levels in preeclampsia.*Hypertension* . 2000;36(3):400-404.

6. Germain AM, Romanik MC, Guerra I, et al. Endothelial dysfunction: A link among preeclampsia, recurrent pregnancy loss, and future cardiovascular events? *Hypertension* . 2007;49:90-95.

7. Spasojevic M, Smith SA, Morris JM, Gallery EDM. Peripheral arterial pulse wave analysis in women with pre-eclampsia and gestational hypertension. *BJOG An Int J Obstet Gynaecol* . 2005;112(11):1475-8.

8. Rönnback M, Lampinen K, Groop PH, Kaaja R. Pulse wave reflection in currently and previously pree-clamptic women. *Hypertens Pregnancy* . 2005;24(2):171-80.

9. Di Pasquo E, Ghi T, Dall'Asta A, et al. Maternal cardiac parameters can help in differentiating the clinical profile of preeclampsia and in predicting progression from mild to severe forms. *Am J Obstet Gynecol* . 2019;(221(6)):633e1:633e9.

10. Lippincott MF, Carlow A, Desai A, et al. Relation of Endothelial Function to Cardiovascular Risk in Women With Sedentary Occupations and Without Known Cardiovascular Disease. *Am J Cardiol* . 2008;102(3):348-352.

11. Chambers JC, Fusi L, Malik IS, Haskard DO, Swiet M, Kooner JS. Association of maternal endothelial dysfunction with preeclampsia.*JAMA* . 2001;285(12):1607-1612.

12. Evans CS, Gooch L, Flotta D, et al. Cardiovascular system during the postpartum state in women with a history of preeclampsia.*Hypertension* . 2011;58(1):57-62.

13. Smith GCS, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129 290 births. *Lancet* . 2001;357(9273):2002-2006.

14. Yinon Y, Kingdom JCP, Odutayo A, et al. Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: Insights into future vascular risk. *Circulation* . 2010;122:1846-1853.

15. Vogel RA. Measurement of endothelial function by brachial artery flow-mediated vasodilation. *Am J Cardiol* . 2001;88(2):31-34.

16. Alley H, Owens CD, Gasper WJ, Grenon SM. Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery in Clinical Research. *J Vis Exp* . 2014;(92).

17. Rubinshtein R, Kuvin JT, Soffler M, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. *Eur Heart J* . 2010;31(9):1142-8.

18. Kuvin JT, Patel AR, Sliney KA, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude.*Am Heart J* . 2003;146(1):168-74

19. Gordijn SJ, Beune IM, Thilaganathan B, et al. Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound Obstet Gynecol* . 2016; 48(3):333-9.

20. Bruno RM, Gori T, Ghiadoni L. Endothelial function testing and cardiovascular disease: Focus on peripheral arterial tonometry.*Vasc Health Risk Manag* . 2014; 26;10:577-84

21. Brant LCC, Barreto SM, Passos VMA, Ribeiro ALP. Reproducibility of peripheral arterial tonometry for the assessment of endothelial function in adults. *J Hypertens* . 2013; 31(10):1984-90.

22. Liu J, Wang J, Jin Y, Roethig HJ, Unverdorben M. Variability of peripheral arterial tonometry in the measurement of endothelial function in healthy men. *Clin Cardiol* . 2009;32(12):700-4.

23. Fujita K, Tatsumi K, Kondoh E, et al. Endothelial function progressively deteriorates during normal pregnancy. *Hypertens Pregnancy* . 2013;32(2):129-138.

24. Savvidou MD, Kametas NA, Donald AE, Nicolaides KH. Non-invasive assessment of endothelial function in normal pregnancy. *Ultrasound Obstet Gynecol* . 2000;15(6):502-7

25. Orabona R, Sciatti E, Vizzardi E, et al. Maternal endothelial function and vascular stiffness after HELLP syndrome: a case–control study. *Ultrasound Obstet Gynecol* . 2017;50(5):596-602.

26. Agatisa PK, Ness RB, Roberts JM, Costantino JP, Kuller LH, McLaughlin MK. Impairment of endothelial function in women with a history of preeclampsia: An indicator of cardiovascular risk. *Am J Physiol - Hear Circ Physiol* . 2004; 286(4):1989-93.

27. Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Divergent metabolic and vascular phenotypes in pre-eclampsia and intrauterine growth restriction: Relevance of adiposity. *J Hypertens* . 2004;(22):2177-2183.

28. Levine RJ, Maynard SE, Qian C, et al. Circulating Angiogenic Factors and the Risk of Preeclampsia. *N Engl J Med* . 2004; (22):2177-2183.

29. Lam C, Lim KH, Karumanchi SA. Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. *Hypertension* . 2005;46(5):1077-85

Table 1. Maternal characteristics and obstetric outcomes

	IUGR	Control	P
Maternal age, mean SD	30.8	31.6	0.3
G median (IQR)	2 (1,3)	2 (1,3)	0.4
BMI	26.2 ± 5.3	26.8 ± 4.2	0.35
Gestational age at delivery, median (IQR)	36.7 (35.6, 37.2)	37.7 (35.3, 39.3)	0.04

	IUGR	Control	P
Neonatal weight, mean SD	1634 ± 428	2785 ± 587	<0.001

BMI- body mass index

Table 2. Endothelial function assessment

	IUGR	Control	P
Gestational age at EndoPAT, median (IQR)	*32.(31,33)*	*32 (31,33)*	*0.18*
Systolic BP prior to EndoPAT, median (IQR)	*113 (110,118)*	*110 (100,114)*	*0.08*
Diastolic BP prior to EndoPAT, median (IQR)	*74 (67, 78)*	*67 (60, 76)*	*0.07*
RHI, median (IQR)	*1.31 (1.215,1.345)*	*1.47 (1.29,1.57)*	*0.02*
RHI < 1.67 n (%)	*15 (100)*	*15 (68)*	*0.05*

BP- blood pressure

Hosted file

`Tables and figures BJOG.docx` available at https://authorea.com/users/354535/articles/478073-is-endothelial-function-impaired-among-women-with-placenta-mediated-fetal-growth-restriction-evidence-from-a-prospective-cohort-study-using-peripheral-artery-tonometry