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Carotid-radial pulse wave velocity as an alternative tool for the evaluation of endothelial function during pregnancy: potential role in identifying hypertensive disorders of pregnancy

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Abstract— **Preeclampsia/eclampsia syndrome, a major cause of maternal mortality and morbidity, has been recognized as a condition with a globally impaired endothelial function (EF). The possibility of identifying early subclinical endothelial damage during pregnancy could be of value in classifying the different hypertensive states of pregnancy, and have a positive impact in the understanding of this syndrome, as well as on the appropriate treatment of these patients. Reactive hyperemia-related changes in carotid-radial pulse wave velocity (PWVcr) were proposed as an alternative tool for the evaluation of EF in patients with cardiovascular risk factors. If impaired EF, which follows hypertensive disorders of pregnancy can be assessed using PWVcr changes remains still unknown. Aims: To assess and compare reactive hyperemia-related changes in PWVcr and FMD in pregnant women (healthy and with hypertensive disorders) and non pregnant women. Methods: Healthy pregnant (HP; *n=13*), preeclamptic (PE; *n=7*), non-proteinuric hypertensive (NPH; *n=6*) and non-pregnant (NP; *n=32*) women were included. Left PWVcr (strain gauge mechano-transducers), left brachial arterial diameter (B-Mode ultrasound) and blood flow velocity (Doppler ultrasound) were measured before (baseline) and after the transient ischemia of the left forearm were determined. Results: One minute after the cuff deflation, PWVcr decreased in HP (6.9 ± 1.5 to 6.0 ± 0.9 m/s, p<0.001) and in NP (8.1 ± 0.9 to 7.4 ± 0.9 m/s; p<0.001). NPH showed a blunted hyperemic PWVcr response (6.6 ± 1.4 to 6.7 ± 1.0 m/s; p=0.91), whereas PE showed a tendency to increase (6.0 ± 0.7 to 6.4 ± 0.8 m/s; p=0.10). Reactive hyperemia PWVcr response (ΔPWVcr in %) differed comparing HP with NPH (-12% vs. +2%; p<0.01) and with PE (-12 vs. +6%; p <0.01), whereas no differences were found between NHP and PE (p=1.00). Conclusion: HP showed an enhanced PWVcr reduction, whereas PE and NPH showed a blunted hyperemic PWVcr response. Carotid-radial PWVcr analysis could have a potential role in the assessment of pregnancy to study EF with a potential clinical application in predicting pregnancy induced hypertension and preeclampsia.**

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I. Introduction

PREECLAMPSIA/eclampsia syndrome is recognized a major cause of maternal mortality and morbidity worldwide [1]. Pathophysiology of preeclmapsia is not complete understood, but is thought to require the achievement of two characteristic stages. The first corresponds to an insufficient placentation which determines a rise in the resistance of the uteroplacental circulation, and the second involves the maternal reaction through the activation of an inappropriate inflammatory response with a globally impaired endothelial function (EF) [2].

The possibility of identifying early subclinical endothelial damage during pregnancy could be of value in recognizing and classifying the different hypertensive states of pregnancy, and have a positive impact in the understanding of this syndrome, as well as on the appropriate and early treatment of these patients.

Celermajer's et al. technique has become the most popular technique to assess EF [3]. It consists in positioning a pneumatic cuff around the upper arm and to determine an arterial occlusion for five minutes (transient ischemia). This maneuver elicits an increment of blood flow in the brachial artery once the cuff is deflated (i.e. reactive hyperemia, RH), which finally stimulates endothelium to release NO resulting in a dilatation of the brachial artery and wall intrinsic alterations [4-6]. Despite of the growing use that has been acquiring FMD, it has not a well defined place in the clinical practice. An explanation that has been given is the great inter-subjects variability reported mostly related to biological, technical and interpretative discrepancies [5], [7].

Recently, RH-related changes in pulse wave velocity (PWV) have been proposed as an alternative tool to evaluate EF [5], [8]. PWV is recognized as the "gold standard" parameter for the evaluation of regional arterial stiffness and has had a wide biomedical application [9]. Previous studies have shown that PWV can be acutely altered by endothelium-related changes in vascular tone and constitutively released NO [10]. It has been reported a reduction in carotid-to-radial PWV (PWV_{cr}) values in response to RH test in healthy young adults and a blunted or low reduction in pathophysiological circumstances, such as hypertension and congestive heart failure [5], However, if impaired EF, which follows hypertensive disorders of pregnancy can be assessed using PWVcr changes, has not been tested already.

In this context the work aim was to assess the EF from pregnant women (normal and with hypertensive states) and non pregnant women by utilization of changes in PWVcr due to RH and to compare it with that collected from FMD analysis.

II. Methods

A. Subjects

The definitions used followed the classification of the gestational hypertensive disorders, as recommended by the report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [11].

Healthy pregnant (HP; n=13), preeclamptic (PE; n=7), non-proteinuric hypertensive (NPH; n=6) and non-pregnant (NP; n=32) women were included in the study. All the preeclamptic women included were *low* in terms of the severity of the syndrome. Following the guidelines for the ultrasonic assessment of endothelial dependent FMD of the brachial artery [12], subjects were asked to abstain from physical activity, tobacco products, and vitamin supplementation for at least 4 hours prior the examination. The study protocol was approved by the ethics committee and all the participants gave written informed consent.

B. Study protocol, laboratory samples and recordings

The first part of the study included anthropometric measurements like height and weight (body mass index was calculated), and a clinic interview for the obtainment of obstetric data. The subjects also underwent routine laboratory assessment to classify the type of hypertensive disorder in pregnancy and to identify the severity of the preeclampsia syndrome, namely: 24-hour proteinuria, uric acid and creatinine.

After that, subjects were instructed to lie in supine position for 15 minutes to establish a hemodynamic steady state in a temperature-controlled (21º-23ºC) room. Heart rate (HR) was continuously determined using the information obtained by the mechano-transducers. Right brachial blood pressure (BP) was measured by using sphygmomanometer every two minutes during the procedure.

To provoke the endothelial stimulus, five minutes of ischemia were induced by occluding the brachial artery. The occlusion was performed inflating a pneumatic cuff placed on the left forearm just below the elbow to at least 50 mm Hg above systolic pressure.

Before (baseline) and after the arterial occlusion and cuff release were carried out, carotid and radial pressure waveforms were simultaneously obtained using strain gauge mechano-transducers (Motorola MPX 2050, Motorola Inc., Corporate 1303 E. Algonquin Road, Schaumburg, Illinois 60196, USA) by placing them on the skin over the carotid and radial arteries (Fig. 1). The signals were recorded and analyzed off-line using software that allows PWVcr calculation, taking into account the given distance (DL) between these arterial sites and the time delay (ΔT) between the carotid and radial waveforms onset. The algorithm used for the detection of the foot waves was explained in previous work [6]. The PWVcr variation coefficient was less than 5%. The study protocol is represented in Figure 2.

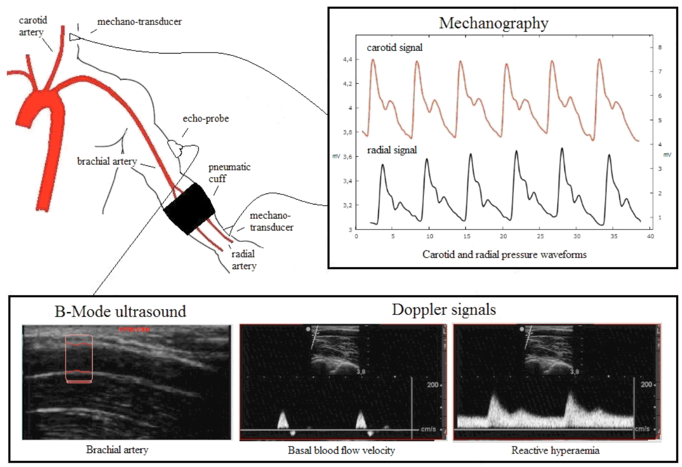

Fig. 1. Schema of the instrumental approach employed to measure the PWVcr (mechano-transducers), brachial artery diameter (B-Mode echography) and blood flow velocity (Doppler signals).

At the same time, left brachial artery was visualized longitudinally above the antecubital crease using high resolution B-Mode ultrasound (SonoSite, MicroMaxx, SonoSite Inc., 21919 30th Drive SE, Bothell, WA 98021, USA; Sampling rate: 12.5 Hz; 5 to 10 MHz probe, Model: L38e). Video-sequences were recorded and analyzed off-line using an automated step- by- step algorithm applied to each digitalized image that allows the brachial diameter obtainment.

In addition, Doppler signals were performed to acquire blood flow velocity in baseline and in specific moments during the reactive hyperaemia period. Doppler signals were used for the characterization of endothelial stimulus. All measurements were done by the same trained operator.

C. Data analysis

As was mentioned, PWVcr was quantified using specific software developed by our group. PWVcr levels corresponding to baseline and to the first 5 minutes after cuff deflation were determined by averaging four beats in each specific time. After that, the percent of PWVcr change (with respect to basal levels) was quantified as follows:

$$\Delta PWVcr[\%] = PWVcr_{after\ cuff\text{-}deflation} - PWVcr_{baseline} / PWVcr_{baseline} \cdot 100$$

(Equation 1)

Demographic and clinical data of the four study groups

Variable	NP *(n=32)*	HP *(n=13)*	PE *(n=7)*	NPH *(n=6)*
Age (years)	27,4 ± 5,8	26,9 ± 8,3	31,4 ± 8,8	30,2 ± 3,9
Gestational age (weeks)	- ± -	34,3 ± 3,7	35,9 ± 2,3	36,3 ± 1,5
Number of gestations (n)	1,1 ± 1,2	3,2 ± 3,9	1,7 ± 1,7	4,5 ± 3,8
FMD (%)	7.1 ± 2.3	9.4 ± 6.4 [a]	4.4 ± 2.6 [a]	5.9 ± 6.0 [a]
Weight (kg)	60,9 ± 9,3	69,0 ± 9,6	97,9 ± 13,8 [a, b]	79,3 ± 10,3 [a, c]
Height (cm)	166,5 ± 6,3	159,8 ± 8,0	159,6 ± 6,7	156,8 ± 5,6
BMI (kg/m^2)	21,9 ± 2,9	27,1 ± 3,9 [a]	38,6 ± 6,6 [a, b]	32,4 ± 5,3 [a]
SBP (mmHg)	125,1 ± 8,4	116,6 ± 13,5	126,6 ± 12,1	129,7 ± 9,6 [b]
DBP (mmHg)	71,6 ± 7,4	67,8 ± 9,8	71,4 ± 7,7	76,7 ± 12,1
Heart rate (bpm)	82,0 ± 8,1	82,5 ± 14,7	89,9 ± 13,0	79,3 ± 8,6
Creatinine (mg/dL)	0,7 ± 0,1	0,6 ± 0,1	0,6 ± 0,1	0,6 ± 0,1
Uric acid (mg/dL)	2,5 ± 1,0	3,2 ± 1,2	5,6 ± 1,0	3,8 ± 0,7
24-hour Proteinuria (g)	0,0 ± 0,0	0,0 ± 0,0	0,5 ± 0,1 [a, b]	0,1 ± 0,1 [a, b, c]

[a, b,] and [c] indicates p< 0.05 cf. NP, HP and PE respectively. All comparisons were determined using ANOVA + Bonferroni test. NP- nonpregnant, HP- healthy pregnant, PE- preeclamptic and NPH- non-proteinuric hypertensive women.

To analyze the endothelial function taking into account the "gold standard" accepted methodology, the FMD was quantified as the percentage change in the arterial diastolic diameter (DD), considering the basal levels and those measured one minute after the cuff deflation:

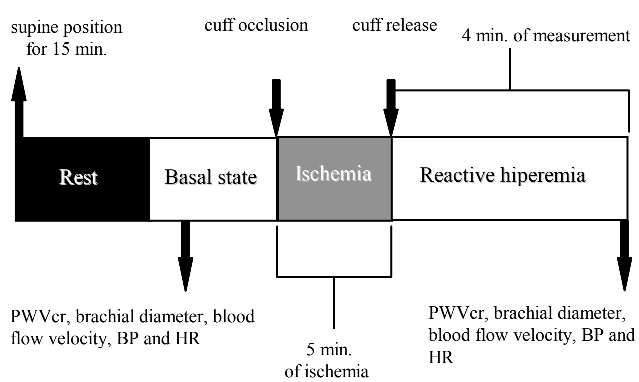

Fig. 2. Representative diagram of the study protocol applied to evaluate changes in arterial biomechanics by means of PWVcr and FMD.

$$FMD[\%] = DD_{after\ cuff\ deflation} - DD_{baseline} / DD_{baseline} \cdot 100$$

(Equation 2)

The mean blood flow velocity (*Vm* [cm/s]) and brachial mean diameter (*Dm*) were used for the shear rate (SR) calculation, an estimate of shear stress without accounting for blood viscosity [7] following the equation:

$$SR = Vm/Dm$$

(Equation 3)

SR was obtained for the characterization of the endothelial stimulus.

D. Statistics

Changes in BP, HR, PWV, arterial diameter and shear rate, were evaluated using ANOVA + Bonferroni test. Differences in the variables (PWV, arterial diameter and shear rate) percentual changes were evaluated using two paired two tailed Student t-test. All data are presented as mean value (MV) ± standard deviation (SD). A $p<0.05$ indicated significant statistical differences.

III. Results

The demographic data, clinical considerations and FMD response are shown in Table 1. Age and gestational age were similar between study groups. Baseline SBP levels were different only in HP and NPH (p=0.036).

Taking into account the RH-test, all groups evoked endothelial stimulus (reactive hyperemia) evaluated by means of shear rate values and the values were similar within the groups (p<0.001 and p=0.78 respectively). No significant changes in heart rate or blood pressure were found before and after the cuff was deflated, ensuring stable hemodynamic conditions (p=0.32). When all the groups are analyzed in terms of FMD response, they showed a dilatation of the brachial artery, however, in HP women the levels were the highest.

Baseline PWVcr was higher in NP than in women with PE or NPH (p=0.017 and p<0.001 respectively), and higher without significance with respect to HP (p=0.06) (Fig. 3).

One minute after the cuff deflation, PWVcr decreased in HP (6.9 ± 1.5 to 6.0 ± 0.9 m/s, p<0.001) and in NP (8.1 ± 0.9 to 7.4 ± 0.9 m/s; p<0.001). However, NPH showed a blunted hyperemic PWVcr response (6.6 ± 1.4 to 6.7 ± 1.0 m/s; p=0.91), whereas PE showed a tendency to increase (6.0

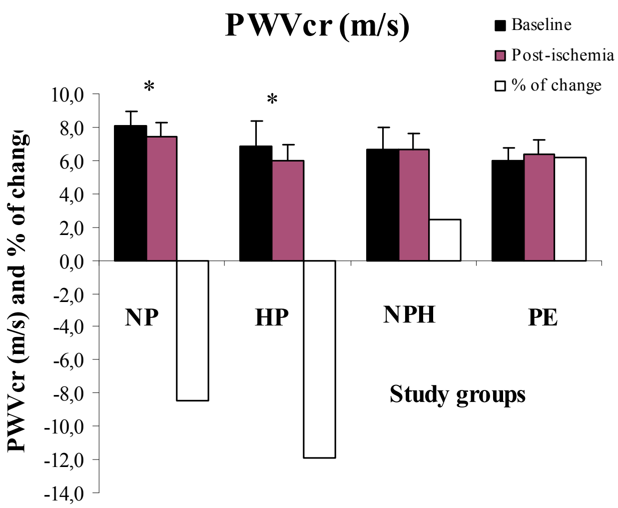

Fig. 3. PWVcr levels and % of change before and after the cuff deflation in the different study groups. * p<0.05 (paired two tailed Student *t*-test) comparing baseline with post-ischemia levels.

± 0.7 to 6.4 ± 0.8 m/s; p=0.10). PWVcr response in % differed comparing HP with NPH (-12% vs. +2%; p<0.01) and with P (-12 vs. +6%; p <0.01). No differences were found between NHP and PE (p=1.00).

IV. DISCUSSION

The exact mechanisms that explain altered hemodynamic responses in hypertensive disorders in pregnancy, particularly preeclampsia, are unclear. However, a global endothelial dysfunction appears to play a key role was mentioned above, and preeclampsia is commonly associated with higher risk of mortality.

In this work, for the first time, the assessment of EF in pregnant women by means of RH-related changes in PWVcr was determined. HP showed the maximal response in terms of PWVcr reduction, which is consistent with an enhanced EF that is observed during normal pregnancy evaluated by other methods [13].

On the other hand, PWVcr changes evidenced in pregnant women with hypertensive disorders not only did not show a reduction behavior, but also a tendency to increase after the cuff deflation. Indeed, as it could be expected, PWVcr changes in PE were higher in PE, probably indicating greater degree of severity in endothelial dysfunction. It is noteworthy, that variables such as baseline levels of PWVcr, blood pressure and gestational age were similar across pregnant women, eliminating potential confounding factors in the analysis.

Therefore, the dissimilar response of PWVcr should represent intrinsic alterations in vascular response associated with pregnancy disorder and not differences in basal conditions.

V. CONCLUSION

Endothelial function can be evaluated by means of PWVcr changes in response to transient forelimb ischemia in pregnant women. Hypertensive pregnant showed a blunted hyperemic PWVcr response compared with healthy pregnant women. This altered behavior may represent an impaired vasodilatory reserve, mainly related with endothelial dysfunction. Carotid-radial PWVcr analysis could have a potential role in the assessment of pregnancy to study EF with a potential clinical application in predicting pregnancy induced hypertension and preeclampsia.

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