Mid-trimester Fetal-placental Velocimetry Response to Nifedipine may Predict Early the Onset of Pre-eclampsia

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Abstract. The effect of nifedipine on fetal-placental blood flow at 22-24 weeks in patients with pregnancy-induced hypertension (PIH) was evaluated. Twenty patients with PIH were submitted to the Doppler evaluation of fetal-placental perfusion at 22-24 weeks. The systo-diastolic (S/D) ratio and the pulsatility index (PI) of uterine, umbilical and middle cerebral arteries and systemic blood pressure were recorded before and 7 days after nifedipine administration (10 mg/ per os 3 times/day until delivery). Statistical analysis was performed with paired and unpaired t-test and the two-tailed Fisher exact test. Nifedipine significantly (p<0.05) decreased the mean systolic pressure in all patients (from 146 to 135 mmHg): 8 patients developed pre-eclampsia (PE) complicated by fetal growth restriction (FGR) (PE group), whilst the remaining were only affected by PIH (PIH group). The gestational age at delivery, neonatal birthweight and 1- and 5-min Apgar scores were significantly (p<0.001) lower in PE than in PIH women. Nifedipine treatment significantly changed the PI and S/D ratio (mean±SEM) of the uterine (PI from 0.66±0.01 to 0.51±0.01; S/D ratio: from 2.00±0.09 to 1.79±0.05) and umbilical (PI: from 1.55±0.04 to 1.40±0.02; S/D ratio: from 2.45±0.09 to 2.31±0.09) arteries and the middle cerebral PI (from 1.45±0.03 to 1.61±0.01) artery only in PIH, but not in PE patients. Fetal-placental blood flow changes after nifedipine may early identify patients at risk of PE.

Predicting pre-eclampsia (PE) remains a difficult task, even though an array of clinical, biochemical and biophysical tests have been developed for this purpose (1). Since none of the current methods combines accuracy, reproducibility and simplicity to become a universal predictive marker of pre-eclampsia, new biochemical and/or biophysical markers and also new algorithms that extract the best information from current screening methods should be developed (1).

PE is a pregnancy-specific syndrome, recognised as a leading cause of maternal and perinatal mortality, and diagnosed by the accompanying increased blood pressure and proteinuria, which affects 3–5% of pregnancies (1-3). Evidence presented over the last decade revealed the pivotal role played by the placenta in the etiopathogenesis of the disease, as PE only occurs in the presence of a placenta and its resolution begins with the removal of the placenta. PE is associated with abnormal placentation, due to altered cytotrophoblast proliferation and invasion of the endometrium and, with the impairment of placental angiogenesis, with the insufficiency and failure of spiral arteries remodeling (1-4). In fact, the vascular endothelium could be an early target for pathophysiological modification in PE (5), and the feature that characterises the pre-eclamptic placenta is its exposure to decreased blood perfusion (6). Doppler velocimetry provides a direct demonstration of this process, because it may detect the persistence of high resistance in utero-placental vessels that are suggestive of a reduced placental and fetal perfusion (7). In addition, the presence of abnormal flow-velocity waveforms, as detected by Doppler ultrasonography, is frequently associated with impaired oxygen and substrate availability to the fetus, fetal growth restriction (FGR) and high maternal and perinatal mortality (7, 8).

It is well known that several placental hormones may have a role in regulating the vascular tone of the pregnant uterus, the placenta and the fetus, acting as vasoconstriction or vasodilatation agents of the utero-placental and fetal circulation (1, 9). In this regard, the influx of extracellular calcium in the vascular smooth muscle fibres of intrauterine tissues is a key factor (10), as vasoconstriction is attenuated by dihydropyridine calcium antagonists which act on the L-type voltage-dependent Ca2+ channels (11). Nifedipine is a dihydropyridine calcium channel blocker, and in vitro
Evidence has shown that it inhibits the Ca\(^{2+}\) influx through potential- and receptor-dependent Ca\(^{2+}\) channels, mediating vasorelaxation (12-16).

On the basis of this evidence, we aimed to evaluate the effect of oral nifedipine therapy on mid-trimester fetal and uteroplacental Doppler velocimetry waveforms in patients with pregnancy-induced hypertension (PIH). We failed to show any effect of nifedipine on blood flow. However, when patients were subdivided according to the outcome of pregnancy, we found that nifedipine did affect utero-placental and fetal perfusion, but only in those women who subsequently did not develop PE with superimposed FGR.

**Materials and Methods**

**Patients.** Twenty (n=20) non-smoking, drug-free volunteers, singleton pregnant women (11 primigravidae, 8 secondigravidae), were studied at 22-24 weeks of gestation. The gestational age was determined on the basis of the last menstrual period and confirmed by ultrasounds. All patients were enrolled at the Department of Gynecology, Obstetrics and Reproductive Medicine of the Second University of Naples, Naples, Italy. Informed consent was obtained from all patients prior to inclusion in the study, for which local Human Investigation Committee approval was obtained. All the patients were affected by PIH (blood pressure ≥140/90 mmHg first diagnosed after 20 weeks of gestation) (17) and exclusion criteria were: fetal bleeding, heart, liver or kidney dysfunctions, chronic hypertension, diabetes or Rh immunization. The maternal age varied from 22 to 31 years (mean±SE: 25.5±1.7), and the weight between 60-83 Kg (mean±SE: 71.5±0.8).

**Protocol.** Each subject received nifedipine, 10 mg per os 3 times a day, until delivery. On the first trial day, the patient rested in hospital for 24 hours. The protocol of investigations included blood pressure measurements and Doppler evaluation before and 7 days after nifedipine administration.

**Doppler study.** Blood flow velocity waveforms were obtained using an EcoDoppler Ansaldo AU-560, with a 3.5 MHz. The systo-diastolic (S/D) ratio and the pulsatility index (PI) were recorded during a period of fetal rest, as previously described (18).

**Middle cerebral artery velocimetric waves:** The cross-section used for the measurement of the biparietal diameter was found and, with an upward movement of the probe, the scissure of Silvius was identified where the artery begins.

**Pregnancy outcome measures.** The outcome measures evaluated in the present study were third-trimester onset PE, defined as 300 mg or more of urinary protein excretion per 24 hours and blood pressure ≥ 140/90 mmHg, first diagnosed after 20 weeks of gestation (18) and fetal growth restriction (FGR), defined as an abdominal circumference more than two standard deviations below the mean for the gestational age and with a birth weight below the tenth percentile for the gestational age (categorized using a validated reference standard (19)). Finally, at delivery, each placenta was placed in ice and taken to the laboratory for histological examination.

**Statistical analysis.** Statistical analysis was performed with paired and unpaired t-test and the two-tailed Fisher exact tests. Statistical significance was set at \(p<0.05\) and data were shown as mean±SEM.

**Results**

**Clinical findings.** Table I represents the clinical characteristics of the patient groups. At the time of Doppler evaluation, before nifedipine treatment, no significant difference was observed both in systolic and diastolic blood pressure between patients who developed PE and those with PIH (Table I). However, 7-day nifedipine treatment significantly \((p<0.05)\) decreased the mean systolic pressure from 146 to 135 mmHg.

Proteinuria was present only in PE patients at the time of hospitalization (Table I). With respect to the pregnancy outcome, the PE group had a significantly \((p<0.001)\) lower gestational age at delivery than the PIH patients. Ten PIH patients delivered by spontaneous vaginal delivery and 2 by caesarean section, due to rupture of membranes with copious loss of amniotic fluid in the absence of labor. On the contrary, only two PE women delivered spontaneously at 36 weeks, the remaining undergoing emergency caesarean section due to acute fetal distress as evaluated by cardiotocography.

The anatomopathological examination of the placentas showed widespread vascular lacunes across the whole surface with many infarctions, widespread calcification and microscopic evidence of necrotic-degenerative transformation of the various cellular components. Numerous clusters of fibrin, resulting from thrombotic phenomena, were also found. These abnormalities were mainly shown by placentas collected from PE rather than PIH patients (Table I).

With respect to the newborns, at birth all the fetuses resulted normal for karyotype and structure. However, those delivered by PE mothers were growth restricted as their fetal weight was significantly \((p<0.0001)\) lower than PIH newborns (Table I), had a greater number of Apgar score less than 7 at the 1st and 5th minute \((p<0.0004\) and \(p<0.0084\), respectively), and a significantly \((p<0.001)\) longer stay in the neonatal intensive care unit (Table I).
<table>
<thead>
<tr>
<th>No.</th>
<th>Weeks at delivery</th>
<th>Proteinuria (g/L)</th>
<th>Placental abnormalities (N)</th>
<th>Birthweight (g)</th>
<th>Birth weight &lt;10th centile</th>
<th>Stay in the NICU (days)</th>
<th>Apgar &lt;7 at 1 min (n)</th>
<th>Apgar &lt;7 at 5 min (n)</th>
<th>Systolic BP (mmHg)*</th>
<th>Diastolic BP (mmHg)*</th>
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<tr>
<td>12</td>
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<td>2</td>
<td>2995±68.2</td>
<td>0</td>
<td>4.8±0.2</td>
<td>0</td>
<td>0</td>
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<td>106.7±1.8</td>
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<td>1.388±0.3</td>
<td>7</td>
<td>2301±38.1</td>
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<td>27.1±1.7</td>
<td>7</td>
<td>5</td>
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<td>103.1±2.1</td>
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*At the time of Doppler evaluation
PIH, pregnancy-induced hypertension; PE, pre-eclampsia.

Doppler findings. Before starting the nifedipine treatment, no significant differences were found in blood flow velocity waveform (PI and S/D ratio, respectively) between the PIH and PE patients (data not shown).

Nifedipine significantly affected both the PI (from 0.66±0.01 to 0.51±0.01, p<0.05) and the S/D ratio (from 2.0±0.09 to 1.79±0.05, p<0.05) of the PIH but not PE patients uterine artery (Table II). Significant changes were also recorded after nifedipine administration in the PI (from 1.55±0.04 to 1.40±0.02, p<0.05) and the S/D ratio (from 2.45±0.09 to 2.31±0.09, p<0.05) of the umbilical artery in PIH but not PE patients (Table II). With respect to middle cerebral artery flow velocimetry waveforms, in PIH patients nifedipine administration significantly changed the PI (from 1.45±0.03 to 1.61±0.01, p<0.05) but not the S/D ratio (3.52±0.16 to 3.31±0.4) (Table II). The changes in this vessel confirms the redistribution of the cardiac load (brain sparing effect) in favor of the vital organs, with a diminution of the blood flow towards those organs which are less essential.

On the contrary, nifedipine treatment did not change either the PI or the S/D ratio values in PE patients.

Discussion

In the present study, we first report that nifedipine modified the blood flow through the uteroplacental, umbilical and fetal vessels in women with PIH, but not in those who later developed PE with superimposed FGR. Other studies provided evidence that nifedipine treatment was not able to modify fetal or uteroplacental velocimetry waveforms in PE (20, 21), but those were conducted in the presence of a gestational disease and not before its development.

The initiating event in PIH appears to be reduced uteroplacental perfusion, as a result of abnormal cytotrophoblast invasion of spiral arterioles, and placental ischemia. The persistency of these events may cause PE and FGR, clinical counterparts of the impaired placenta. In fact, in the present study we reported a higher incidence of placental abnormalities in the PE than in the PIH group. Placental ischemia is thought to lead to widespread activation/dysfunction of the maternal vascular endothelium that results in enhanced formation of endothelin-1 (ET-1) and decreased formation of vasodilators (1, 22).

Nifedipine, a dihydropyridine calcium channel blocker, is an effective antihypertensive drug in pregnancy (23, 24), and in vitro evidence has shown that it mediates vasorelaxation (12-16), decreasing the effect of ET-1 on vasoconstriction of non-pregnant (25) and uteroplacental (26) vessels. With respect to pregnancy, ET-1 is a strongly vasoactive polypeptide involved in the regulation of the uteroplacental blood flow, and the ET-1-induced vasoconstriction is more potent in placental arteries from PE/intra-uterine-growth-restriction patients than in placental arteries from women with normal pregnancies (27). Furthermore, ET-1 placental expression and maternal circulating levels are increased in pre-eclampsia (28), and ET-1 maternal levels are higher in PE than in PIH (29).

In the light of this evidence, we postulate that the effect of nifedipine treatment in changing the uteroplacental and fetal blood flow in PIH patients, but not in those who later developed PE with FGR, depends on the different vasocstriction effect of endogenous ET-1, also underlying the concept that the pathological process responsible for a poor outcome of pregnancy is also responsible for a different etiopathology between PIH and PE/FGR.

Finally, the present study also offers subject for discussion on the strategy of predicting pre-eclampsia beyond simple screening tests (1). In fact, in recent years flow velocity waveform analysis has become an important tool in research and clinical work, to evaluate the prognosis and outcome of delivery (30). There is now compelling evidence that women affected by PIH are at high risk of developing PE and/or FGR (1-4), and that Doppler ultrasound velocimetry of
uteroplacental, umbilical and fetal vessels provides the clinician with important information on the hemodynamics of the respective vascular area (30). In fact, the insufficiency and failure of spiral artery remodeling (1-5) expose the fetoplacental unit to reduced blood perfusion (6, 7), frequently associated with impaired oxygen and substrate availability to the fetus (7, 8). Thus, applied as a screening test, Doppler ultrasound velocimetry has important but limited diagnostic accuracy in predicting pre-eclampsia, with or without superimposed FGR (30, 31). Our data give support to a stepwise strategy for predicting pre-eclampsia, moving from simple screening tests (such as clinical history and blood pressure measurement) to more specific and sophisticated tests that can be applied to selected patients following a sequential algorithm. Confirmation of our findings would shed further light on the prediction strategies of poor outcome in pregnancy.

References


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