A longitudinal study of resting peripheral blood flow in normal pregnancy and pregnancies complicated by chronic hypertension and pre-eclampsia

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Abstract

Objectives: To investigate the hypothesis that reduced resting tissue blood flow precedes the clinical onset of pre-eclampsia in women at risk of the disease. Methods: We used venous occlusion plethysmography to compare resting calf muscle blood flow in 18 normal pregnant controls, 18 pregnant women with chronic hypertension, and 23 pregnant women at increased risk of developing pre-eclampsia. Calf blood flow was measured at 16, 20, 24, 28, 32 and 36 weeks of gestation. Results: Blood flow increased with gestation in normal pregnancy (P = 0.004) and chronic hypertension (P = 0.006), but not in the ‘at risk’ women who did not develop pre-eclampsia (P = 0.36). In contrast, blood flow decreased significantly in eight out of the 23 women ‘at risk’, who developed pre-eclampsia (P < 0.0001, ANOVA). The decrease in flow preceded the clinical diagnosis of the pre-eclampsia by several weeks. Moreover, a significant inverse correlation was observed between resting blood flow and plasma uric acid concentrations (r = −0.86, P = 0.03) in the women that developed pre-eclampsia. Conclusions: We have shown that reduced resting blood flow precedes the clinical onset of pre-eclampsia independently of hypertension per se. These findings support the notion that impaired tissue blood flow may be involved at an early stage in the pathophysiology of the disease. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Blood flow; Endothelial function; Hypertension

1. Introduction

Pre-eclampsia is a leading cause of maternal and perinatal mortality and morbidity worldwide [1]. Although the primary cause remains unknown, there is considerable evidence that abnormal placentation, resulting in placental ischaemia, releases an unknown factor(s), which in turn causes the generalised vascular endothelial cell dysfunction associated with the disease [2–4]. It has been suggested that the predominance of endothelial-dependent vasoconstriction over vasodilatation results in the cardiovascular maladaptations seen in pre-eclampsia [5,6]. Endothelial-dependent vasodilatation is impaired in pre-eclampsia [7,8] and the clinical presentation of the disease is certainly suggestive of impaired tissue perfusion.

Whilst intuitively one might expect tissue blood flow to be reduced in pre-eclampsia, there is little published evidence to support this hypothesis. We have previously reported that calf blood flow in women with pre-eclampsia is significantly reduced during the third trimester [9]. Calf blood flow was studied because it relates predominantly to skeletal muscle, which lacks arterio-venous shunts. The blood flowing through the calf therefore has to traverse the microvascular bed and is more likely to reflect nutritive blood flow than that, for example, in the skin. In this study we have measured calf blood flow longitudinally to...
investigate the hypothesis that impaired tissue perfusion in pre-eclampsia precedes the clinical onset of the disease.

2. Methods

2.1. Subjects

Resting lower limb muscle blood flow was measured in the calves of 18 nulliparous normal pregnant controls, 18 women with chronic hypertension, and 23 women pregnant for the second time, and who had pre-eclampsia in their first pregnancy (high risk group). Measurements were made at 16, 20, 24, 28, 32, and 36 weeks’ gestation. All the women were recruited from the antenatal clinic at the Chelsea and Westminster Hospital, London. They were non-smokers and were chosen to be similar in age and booking body mass index. Patients with a history of peripheral vascular disease, peripheral neuropathy or any medical disorders other than chronic hypertension were excluded. Chronic hypertension was defined as pre-existing hypertension diagnosed by clinical history prior to pregnancy, high blood pressure at the booking clinic, and persistent hypertension after the pregnancy was over. All the chronic hypertensives had essential hypertension without evidence of end-organ damage. Creatinine clearance was measured in all the hypertensives on entry into the study, to evaluate renal function. Cases with renal impairment were excluded. Pre-eclampsia was diagnosed when patients developed both hypertension and proteinuria, and confirmed retrospectively when both hypertension and proteinuria reversed after the pregnancy. Proteinuria was defined as more than 500 mg per litre in a 24-h urine specimen. Hypertension was defined as an absolute blood pressure greater than 140 mmHg systolic or 90 mmHg diastolic taken twice, at least 6 h apart. The first and fifth Korotkoff sounds were used to determine the systolic and diastolic component, respectively [10].

Preliminary studies had shown that the mean resting blood flow in the third trimester of normal pregnancy was 3.91 ml/min per 100 ml, whereas the mean value in a group of pre-eclamptic patients, at the same stage of pregnancy, was 1.96 ml/min per 100 ml. The differences between these values were highly significant \((P<0.001)\) [9]. Power calculations, based on these data, showed that a sample size of six in each group should be sufficient to achieve statistical significance with \(\alpha\) of 0.05 and \(\beta\) of 0.02. In order to obtain six women with pre-eclampsia, we recruited 23 ‘high risk’ women. The Local Ethics Committee approved the study, and informed consent was obtained from each patient at the time of recruitment.

2.2. Blood flow measurement

The strain gauge plethysmography technique is widely used for the non-invasive assessment of limb blood flow. It has been used to measure limb blood flow in normal non-pregnant women [11] and patients with essential hypertension [12], and has been established as a useful technique for use during pregnancy [13]. Whilst blood flow is often measured in the forearm, we used the calf for the following reasons. Firstly, the calf is less liable to involuntary movement artefacts. Secondly, in severe cases of pre-eclampsia the arms may be required for intravenous therapy and therefore not be readily available for the investigation. Finally, a large amount of control data has been gathered from the calf using the plethysmographic technique [11]. Moreover, previous studies, in which simultaneous assessments from both forearm and calf were made, showed that the values of measured parameters obtained from these tissues were similar [14]. Subjects rested for at least 15 min before the study and leg oedema assessed by applying a firm pre-tibial pressure for 5 s for evidence of pitting. Observations were made in the left lateral position to prevent aorto-caval compression and with the right mid-calf at the level of the heart. Room temperature was kept within the range of 23–24°C. Arterial blood pressure was measured non-invasively in the calf and the ipsi-lateral arm, using a Dinamap Vital Sign Monitor (Type 1800, Critikon, Tampa, FL, USA). The averaged values of systolic and diastolic blood pressures were calculated from triplicate measurements at each site.

Blood flow was estimated using the Filtrass strain gauge plethysmograph (Filtrass, DOMED, Munich, Germany) [15]. This system is based on the standard strain gauge plethysmography described by Gamble et al. [14]. The plethysmograph is mercury free with an integrated automatic calibration device, which allows a touch free calibration, thus reducing artefacts due to investigator manipulation. The sensor is calibrated automatically in triplicate, by a computer driven programme at the start of each study. The relative merits of the Filtrass over the standard strain gauge in terms of the quality of calibration and reproducibility have recently been validated [15]. The Filtrass program is computer-assisted and allows the selection of pre-recorded protocols for measuring blood flow and other microvascular parameters such as filtration capacity in the calf or forearm. Briefly, the congestion pressure cuff, which is attached to a compressor pump, built into the apparatus, was placed around the right thigh and enclosed in a rigid corset, to reduce filling volume and thus filling time. Changes in calf circumference in response to a rapid increase in cuff pressure were measured using a passive inductive transducer with an accuracy of \(\pm5\) µm. The files were recorded and saved for subsequent ‘off-line’ analysis. In order to measure blood flow \((Q_a)\), the venous congestion pressure was raised rapidly to 40 mmHg and held for 20 s. This procedure was repeated three times with the congestion pressure being returned to zero for 5 min between each pressure challenge. Since the 40-mmHg cuff pressure step occludes venous return from the limb but not arterial inflow, the initial swelling rate reflects arterial
Inflow. The value of Qa was estimated by measuring the first 3 s of each volume response curve. Venous blood samples were obtained after each study to estimate uric plasma acid concentrations. The obstetric records of the pregnant groups were reviewed after delivery to confirm reversal of hypertension and proteinuria and also to record the birth weights of the infants adjusted for gestational age at delivery. Resting blood flow was expressed as ml/min per 100 ml of tissue [11].

2.3. Statistical analysis

All the data were normally distributed and are presented as means±S.D. Analysis of serial measurements of blood flow was by summary measures. A linear regression was fitted for each subject’s data over time. The slopes of the lines, which represent the rates of change in blood flow per week, were taken as summary measures for the subjects in each group [16]. The summary measures of the groups were compared for statistical significance using analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. The relationship between blood flow and the other parameters was assessed by Pearson correlation coefficient. Statistical significance was assumed when P was less than 0.05. The Statistical Package for Social Sciences (SPSS) (version 8) was used for all analyses.

3. Results

Table 1 shows the clinical and demographic characteristics of all the groups. The chronic hypertension group had higher booking blood pressures compared to the other two groups, the differences being highly significant (P=0.001 and 0.0001, for systolic and diastolic pressures, respectively). Of the 18 members of the hypertension group, four withdrew from the study because of job relocation. However, there was no evidence of differences in the demographic or clinical data between these women and the rest of this group. Of the remaining 14 chronic hypertensives, four required drug treatment (alpha-methyldopa) for control of blood pressure after 36 weeks of gestation. Out of the 23 women with a previous history of pre-eclampsia, eight had recurrence of the disease. These women are referred to as the pre-eclampsia group. The mean gestational age at onset of the disease was 34.1±1.8 weeks. Babies born to the pre-eclamptic women were smaller when compared to those in the other groups (P=0.03) (Table 1).

Resting calf blood flow was similar in all groups at 16 weeks of gestation (P=0.791, ANOVA). However, flow increased with gestation between 16 and 36 weeks in the normal pregnancy group, rising from 2.5±1.6 to 4.8±1.3 ml/min per 100 ml tissue (units=QaU; mean±S.D.; the increase was highly significant (P=0.004). In the group with chronic hypertension (none of whom developed superimposed pre-eclampsia), blood flow increased from 2.5±1.4 to 4.4±0.8 QaU between weeks 16 and 36, respectively. This increase was statistically significant (P=0.006). In women with previous pre-eclampsia but without recurrence, the increase in flow between weeks 16 (2.5±0.9 QaU) and 36 (3.2±1.1 QaU) was not significant (P=0.36). A subgroup analysis of the chronic hypertension group showed no significant difference in blood flow between the untreated and treated (alpha-methyldopa) groups at 36 weeks (4.4±0.8 vs. 4.3±1.1, respectively; P=0.9).

In contrast to the normal pregnancy, chronic hypertensive and ‘at risk’ (non pre-eclamptic) groups, the women who subsequently developed pre-eclampsia, showed a

<table>
<thead>
<tr>
<th></th>
<th>Normal pregnancy (n=18)</th>
<th>Chronic hypertension (n=18)</th>
<th>‘At risk’ (n=23)</th>
<th>P-value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normotensive</td>
<td>Pre-eclampsia</td>
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<td></td>
<td></td>
<td></td>
<td>(n=15)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Age</td>
<td>30.1±1.1</td>
<td>34.0±1.17</td>
<td>31.2±0.98</td>
<td>30.3±0.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5±0.52</td>
<td>23.9±0.8</td>
<td>25.6±1.4</td>
<td>25.8±0.95</td>
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<tr>
<td>Parity</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Calf circumference (mm)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>16 Weeks</td>
<td>358.8±13.6</td>
<td>359.8±12.2</td>
<td>360.0±14.1</td>
<td>361.8±13.4</td>
</tr>
<tr>
<td>36 Weeks</td>
<td>360.5±13.7</td>
<td>362.1±12.5</td>
<td>362.8±14.2</td>
<td>363.0±13.6</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>106.2±10</td>
<td>126±16.6</td>
<td>113.9±8.6</td>
<td>121.5±6.0</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>63.2±5.6</td>
<td>83.8±14</td>
<td>68.5±5.2</td>
<td>72.8±4.3</td>
</tr>
<tr>
<td>Infant birth weight</td>
<td>3.36±0.7</td>
<td>3.39±0.5</td>
<td>3.55±0.4</td>
<td>2.69±0.8</td>
</tr>
</tbody>
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* Data are presented as mean±S.D. BMI, body mass index; N/A, not applicable.
* P-values less than 0.05 were considered significant (ANOVA) with Bonferroni correction for multiple comparisons.
progressive decline in the resting calf blood flow. The value fell from $3.0 \pm 1.8 \text{ QaU}$ at 16 weeks to $1.4 \pm 0.6 \text{ QaU}$ at 36 weeks’ gestation ($P<0.00001$, ANOVA) (Fig. 1). That the reduction in blood flow was progressive is shown by the significant differences between 16–20 weeks’ gestation ($P=0.02$) and 28–32 weeks’ gestations in these patients ($P=0.04$). The reductions in blood flow were similar among all the women within the pre-eclampsia subgroup (Fig. 2). The similarity in the pattern of reduction in blood flow in the pre-eclampsia subgroup is illustrated in Fig. 2. No correlation was found between resting blood flow and maternal age, booking BMI and blood pressure in any of the study groups. We found a significant inverse correlation between limb blood flow and plasma uric acid levels in the pre-eclampsia sub-group ($r=-0.86$, $P=0.03$). However, by contrast, the relationship between blood flow and plasma uric acid levels in the normal pregnancy, chronic hypertension and non-recurrent pre-eclampsia groups was positive ($r=0.83$, 0.96 and 0.89, respectively; $P=0.02$).

4. Discussion

In this study we have compared changes in the values of resting calf blood flow obtained during normal pregnancy with those obtained in patients with chronic hypertension and pre-eclampsia, at the same gestational stage. Whereas blood flow increased with gestation in normal pregnancy and chronic hypertension without superimposed pre-eclampsia, flow decreased progressively in the ‘high risk’ pregnancies complicated by pre-eclampsia. There was no significant change in blood flow in the women with a previous history of pre-eclampsia but without the disease. The reduction in flow in the pre-eclamptic patients preceded the onset of the clinical manifestation by an average of 14 weeks.

Although the clinical presentation of pre-eclampsia is suggestive of generalised impairment of tissue perfusion, this is the first report to confirm that reduction in tissue perfusion precedes the multisystem clinical manifestations of the disease. The data are also consistent with increasing recognition that end-organ failure or dysfunction is preceded by gradual deterioration of nutritive blood flow. However, reduced tissue perfusion preceding organ dysfunction has been reported in other multisystem disorders such as diabetes mellitus [17] and septic shock [18,19]. Although we have not provided biochemical evidence that the decreased blood flow observed in the pre-eclampsia group was detrimental to tissue metabolism, there is evidence that anaerobic metabolism, as determined by calculated base deficit, is increased in pre-eclampsia...
Essential hypertension is associated with endothelial dysfunction and impaired resting forearm blood flow [21] and damage to the microvascular bed of vital organs such as the retina and the kidneys in long-standing disease [22,23]. In the present study, there was no significant difference between the peripheral tissue blood flow measured, at the same gestational stage, in normal pregnancies and in those of chronic hypertensives without superimposed pre-eclampsia. In this respect, our data is at variance with previous reports that organ perfusion is reduced in chronic hypertension either with or without superimposed pre-eclampsia [23]. However, in our study, the chronic hypertension group had only moderate disease, and no underlying end-organ dysfunction. It is therefore possible that the severity of disease in our chronic hypertension group was not great enough to interfere with the normal adaptive increase in the tissue perfusion during pregnancy. This is supported by evidence that maternal and perinatal outcome, in chronic hypertension, is influenced by the disease severity [24,25], moreover this is particularly true in the presence of organ damage [26,27]. In the present study it was difficult to determine the effects of medical treatment on resting blood flow in the subgroup of chronic hypertensives that developed severe hypertension after 36 weeks, because no data were available after starting treatment in this subgroup. However, a subgroup analysis of these women showed no significant difference between the treated and untreated subgroups.

These data confirm those from an earlier study in which we showed that resting calf blood flow was reduced during the third trimester in pre-eclampsia, when compared either to normal pregnancy or non-pregnant controls [9]. The highly significant inverse correlation between blood flow and uric acid suggests that changes in blood flow may suggest a relationship between blood flow and disease severity although the clinical utility of uric acid levels in pre-eclampsia is not universally accepted [28]. We believe that the reduction in resting blood flow is an index of the pathophysiological change(s) associated with pre-eclampsia, per se, rather than the accompanying hypertension. This follows from the observation that resting calf blood flow increased normally in chronic hypertension without superimposed pre-eclampsia. Since all subjects adopted the same posture for these studies, it is unlikely that the reduction in blood flow in pre-eclampsia was due to aorto-caval compression. Moreover, we believe it is unlikely that the reduction could have resulted from measurement error due to interstitial leg oedema since none of the groups showed either difference in the level of pitting oedema or significant changes in calf circumference during the course of the study. It is also unlikely that the reduction in blood flow in the pre-eclampsia group, who were multiparous,
could be explained by vascular remodelling attributable to their previous pregnancies. This is because a reduction in blood flow was not observed in the subgroup of the ‘at risk’ women, who did not develop the disease.

Martin et al. [29], using a plethysmographic technique, observed that blood flow was higher in the forearm than the calves in a heterogeneous group of hypertensive pregnant women but not in normal pregnant controls. However, when they looked at calf blood flow alone, there were no differences between non-pregnant and pregnant controls and hypertensive women. Ginsburg and Duncan [30] also failed to show any difference in forearm blood flow in pregnancies complicated by pre-eclampsia. These may be explained by the selection criteria governing the populations of women studied by the different groups of workers.

It is possible that the reduction in tissue blood flow observed in the pre-eclampsia group resulted from the generalised endothelial dysfunction of the disease. This is because endothelial-dependent vasodilatation is known to be impaired in pre-eclampsia [7,8]. Furthermore, retrograde endothelial cell transmission of information between post-capillary venules and pre-capillary arterioles is thought to play a key role in detecting altered tissue nutritional status by modulating pre-capillary arteriolar tone [11,31,32]. Since the mechanism is endothelial conduction pathway dependent, any dysfunction, giving rise to a down-regulation of endothelial-dependent mechanisms, may result in the failure of arteriolar relaxation in response to increased tissue demand. Although our studies did not set out to explain the possible mechanisms involved in retrograde endothelial cell signal transmission, possible candidates could include nitric oxide (NO) [33], prostacyclin/thromboxane pathway [34], and endothelin [35]. However, in a more recent study, Anumba et al. [36] have shown that L-arginine-NO pathway is unlikely to be responsible for the impaired vascular reactivity in pre-eclampsia.

In spite of the significant reduction in peripheral blood flow that we have observed, we accept that this study has a number of potential limitations. We measured blood flow in the calf, which is not representative of all muscle groups; skeletal muscle blood flow has been shown to vary with muscle fibre composition [37]. Although the calf circumferences of the three groups were similar, differences in adipose tissue composition could, by adding a further cause of heterogeneity, influence the applicability of our findings [38]. Furthermore, although calf muscle blood flow was presumed to represent nutritive blood flow, we did not exclude contributions from non-nutritive flow through the arterio-venous shunts of the feet by using an ankle occlusion cuff [39]. This procedure, besides giving additional discomfort to the patients, might also have had long-term haemodynamic consequences and interfered with other aspects of the protocol. Whilst skin provides an accessible and convenient tissue for investigating microvascular haemodynamics, its usefulness is limited by the fact that it subserves both nutritional and thermoregulatory functions. Moreover, the commonest method used for its assessment, laser Doppler fluximetry, only measures red cell flux and not total volume flow. Since the major tissue component of the calf is muscle, which has a high muscle to skin ratio and lacks arterio-venous (A-V) anastomotic channels, the measured flow will relate more to the support of local tissue metabolism than the control of body temperature. In addition, most of the arterial flow will traverse microvascular beds. It is for these reasons that we believe that we are measuring nutritive blood flow.

Normal tissue function depends on adequate perfusion for nutrient exchange. The gestation-related increase in tissue blood flow, observed in the normal pregnant group in this study, is consistent with reports from other workers [40]. The increase may represent an adaptive response to meet the increased metabolic requirements of pregnancy.

In summary, we have shown that whereas calf resting microvascular blood flow increases during normal pregnancy and chronic hypertension without superimposed pre-eclampsia, a gradual decline occurs in pregnancies complicated by pre-eclampsia. Moreover, the decrease in blood flow precedes the onset of disease, as diagnosed using conventional clinical criteria. We suggest that resting calf blood flow could be used for the early detection of this condition.

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References
