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Pilot study of comparative placental morphometry in pre-eclamptic and normotensive pregnancies suggests possible maladaptations of the fetal component of the placenta

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ABSTRACT

Objective: Adequate maternal, intervillous and fetal blood flow are all necessary for fetal well-being. Compromise to any part of this exchange would be detrimental to pregnancy outcome. Pre-eclampsia is associated with reduced maternal spiral artery flow, resulting in reduced placental perfusion. This in turn creates an ischaemic environment, which may predispose to morphological changes in placental villi. This pilot study sought to assess whether there were morphological alterations in the fetal component of the placenta which could be detrimental to exchange and therefore pregnancy outcome.

Study design: This study utilized morphometric image analysis to examine some features of the fetal component of the placenta in normotensive (NT) and pre-eclamptic (PE) groups. The features examined included: density of placental villi (expressed as percentage of field area occupied by placental tissue); stem vessel carrying capacity (expressed as percentage of stem villus area occupied by vessel lumina); the thickness of the stem arterial walls relative to artery size (expressed as percentage of artery area occupied by arterial wall) and the extent of fibrosis associated with villi (expressed as percentage of field area occupied by fibrosis).

Results: There were significant differences between NT and PE placentae in density of placental villus arrangement NT: 51.89 ± 6.19, PE: 64.78 ± 6.93 ($P < 0.001$); carrying capacity of stem villi NT: 17.20 ± 11.78, PE: 8.67 ± 8.51 ($P < 0.001$); relative thickness of stem villi arterial walls NT: 74.08 ± 12.92, PE: 86.85 ± 10.55 ($P < 0.001$); and extent of fibrosis NT: 0.727 ± 0.310, PE: 1.582 ± 0.707 ($P < 0.001$).

Conclusion: These significant differences between normotensive and pre-eclamptic placentae suggest possible fetal maladaptations in response to the intervillous ischaemia, compounding the existing maternal compromise to materno-fetal exchange. Further investigations would, however, be necessary in order to make more conclusive deductions.

1. Introduction

Pre-eclampsia is a condition unique to pregnancy, characterised by abrupt onset of hypertension and proteinuria. It is associated with an increase in maternal morbidity and mortality, and with a fivefold increase in perinatal mortality [1–3]. Pre-eclampsia occurs in about 2–8% of pregnancies in developed countries, but this figure can be threefold higher in developing countries. Worldwide, pre-eclampsia and eclampsia account for over 50,000 deaths annually [3]. In South Africa, hypertensive disorders of pregnancy (HDP) account for 19% of all maternal deaths, and of these, 83% are due to pre-eclampsia and eclampsia [2]. Resolution of the disorder occurs with delivery of the placenta, hence much research has been focused on the placental role in this syndrome.

As far back as 1939, researchers had associated the development of pre-eclampsia with reduced placental perfusion [4]. During early placental development, extravillous trophoblast invasion into the placental bed is inadequate, resulting in failure of the physiological conversion of the spiral arteries that is characteristic of normal pregnancy [5–13]. This in turn leads to decreased perfusion of the intervillous space. In this ischaemic environment one would expect structural compensations of the villi such as increased surface area or decreased diffusion distance. Alternatively, there could be increased damage such as fibrotic deposition or necrotic areas.

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Studies on the comparative morphology of pre-eclamptic versus normotensive placentae have demonstrated interesting and sometimes conflicting results. Villous surface area has been shown to be altered disproportionately to volume in pregnancies associated with certain hypoxic conditions, including pre-eclampsia [14]. Intrauterine growth restriction (IUGR) has been associated with changes in placental morphology such as decreased surface areas, impoverished growth of peripheral villi and decreased fetal angiogenesis. This was not the case for pre-eclampsia alone, however [15–18]. Significant hyper-ramification of the capillary loops in terminal villi of pre-eclamptic placentae has been observed, together with irregular vessel profiles and narrow vessel lumina [19,20]. The prevalence of inflammation, infarction, ischaemia, haemorrhage, and syncytial knots was found to be increased in both pre-eclampsia and IUGR [21], and it has long been established that the placentae from pre-eclamptic pregnancies display increased syncytial knotting [22,23].

Clarity in the relationship between placental function and morphology on the one hand, and pre-eclampsia on the other hand is still lacking. Kingdom and Kaufmann suggested that changes in placental morphology will depend on intraplacental oxygen status, which in turn depends on both maternal supply and fetal extraction [24]. Pre-eclampsia is generally recognized as being characterized by reduced maternal flow with *uteroplacental* hypoxia (inadequate oxygenated blood entering the intervillous space). This, however, is only one aspect of the equation. If the villous elements of the placenta do not transport available oxygen to the fetus efficiently, a situation of *post placental* hypoxia could arise, in which a compromised villous flow compounds the reduced maternal flow. Added to this, the efficiency of exchange could also be affected by any impedance to intervillous flow (such as could result from an increase in density of villi/reduction of intervillous space/fibrosis of villi).

Utilizing morphometric image analysis, this study compared some villous parameters which could affect the haemodynamics of materno-fetal exchange. This was with a view to investigating possible morphological changes in the villi occurring in response to, but also compounding, the effects of the placental ischaemia associated with pre-eclampsia.

2. Materials and methods

2.1. Patient selection and sample collection

The study was conducted in Durban, South Africa, as an offshoot of a larger pre-eclamptic study. Following institutional ethical approval and informed consent, thirty normotensive (BP $\leq$ 130/80 mm Hg) and 30 pre-eclamptic (SBP $\geq$ 140 mm Hg, DBP $\geq$ 90 mm Hg, proteinuria) African patients were selected by a research midwife. Patients between the ages of 18 and 40 years and of parity 0–5 were included in the study. Exclusion criteria included chronic hypertension, pre-existing seizure disorder, eclampsia, pre-gestational diabetes, placental abruption, gestational diabetes, thyroid disease, asthma, chronic renal disease, intrauterine death, cardiac disease and infection with HIV. Demographic, clinical and neonatal data were collated.

2.2. Sample preparation and morphometry

Placental samples were immediately fixed in buffered formaldehyde and processed using a Sakura VIP 500 Automated Tissue Processor. A single 3–5 μm section from each sample was prepared for general morphological examination using a Mayers Haematoxylin and Eosin stain [25]. Numerous photomicrographs were taken at different levels of magnification, and utilized for the morphometry using a Nikon Eclipse E600 microscope interfaced with the Software Imaging Systems (Germany) image analysis package. A single observer did all the measurements in order to minimize inter-observer bias.The following morphology parameters were assessed:

(i) *Density of placental villus arrangement*: Using the 4× objective, the four quadrants of each slide were photographed. Colour thresholds, saturation and intensity were individually set for each image, binary images were created (Fig. 1A and B), and phase analysis was performed to determine the area of the field that was occupied by placental villi. This was then expressed as a percentage of the total field area. The average of the measurements for the four quadrants was determined for each slide.

(ii) *Percentage of stem villus area occupied by stem vessel lumina*: Utilising the features of stem villi outlined by Benirschke and Kaufmann [26], two stem villi were selected in each section on the basis of clarity of morphology for measuring purposes. Stem villi at different levels of branching (and therefore different calibres) were included. For each stem villus, the sum of all the vessel luminal areas was expressed as a percentage of total villus area.

(iii) *Relative thickness of stem arterial walls*: Utilising the micrographs from (ii) above, the main arterial vessel was selected in each. The area of the artery was established using the outer adventitial boundary. The lumen area was subtracted from this in order to ascertain the area occupied by arterial wall. This was expressed as a percentage of total vessel area in order to compensate for differing calibres of stem villi.

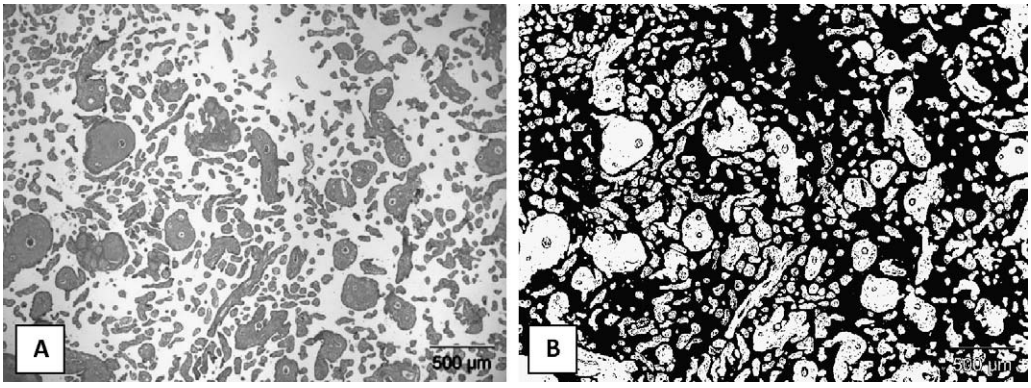

Fig. 1. (A) Light micrograph of H and E stained placental tissue. (B) Binary image of micrograph (A). The combined area occupied by white in micrograph (B) was expressed as a percentage of the total field area.