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Fractional volume of placental vessels in women with diabetes using a novel stereological 3D power Doppler technique

N.W. Jones [a, b, *], R. Deshpande [a, b], H.A. Mousa [c], P. Mansell [a, d], N. Raine-Fenning [b], G. Bugg [a]

[a] Nottingham University Hospitals NHS Trust, Nottingham, UK
[b] School of Clinical Sciences, University of Nottingham, Nottingham, UK
[c] Department of Fetal Medicine, Leicester Royal Infirmary, UK
[d] School of Biomedical Sciences, University of Nottingham, Nottingham, UK

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ABSTRACT

Objectives: In maternal diabetes the placenta is large with abnormal vascular development and increased villous volume. We used a novel stereological 3D power Doppler ultrasound technique to investigate differences *in-vivo* in the placental fractional volume of power Doppler signal (FrVol-PD) between women with and without diabetes.
Methods: We recruited 17 pregnant women with pre-gestational diabetes and 20 controls, all with anterior placentae. Each subject had ultrasound scans (Voluson 730 Expert) every 4 weeks between 12 and 32 weeks gestation. 3D power Doppler data were acquired and the placenta manually outlined using VOCAL (4D View). Power Doppler signal within the resultant volume was counted in a 3D manner adapting the random but systematic techniques used in stereology.
Results: Subjects were of similar age, BMI and parity. From 16 weeks the mean (SD) placental FrVol-PD was higher in the non-diabetic than in the diabetic group: 16 weeks – 0.125 (0.03) versus 0.108 (0.03), 20 weeks – 0.144 (0.05) versus 0.104 (0.03), 24 weeks – 0.145 (0.05) versus 0.128 (0.03), 28 weeks – 0.159 (0.05) versus 0.133 (0.02) and 32 weeks – 0.154 (0.03) versus 0.123 (0.04). These differences were significant between control and diabetic subjects [$F(1,35) = 4.737$, $p = 0.036$] and across gestation [$F(3,140) = 8.294$, $p < 0.001$].
Conclusion: Using a novel stereological-based ultrasound technique we have demonstrated the reliability of this application in the placenta. This technique shows promise for non-invasive assessment of placental function: further studies are required to identify if structural changes in a diabetic placenta are accompanied by altered function, manifest as reduced perfusion demonstrable in-vivo.

1. Introduction

In women with pre-gestational diabetes the placenta is structurally abnormal. Such placentae are often hypertrophied [1] with possible alteration in capillary volume [2] and branching pattern [3], villous surface area [4] and thickness of the villi [5]. Less is known about placental function in pregnancies complicated by maternal diabetes. Studies have suggested that power Doppler can predict adverse outcomes [6–8], which are more common in pregnancies complicated by maternal diabetes.

3D power Doppler angiography (3D-PD) and specifically the 3D vascular indices generated by the quantification of the Doppler signal within any given volume, have several limitations in the assessment of organ perfusion. The indices are dependent upon machine settings [9]; signal attenuation occurs with increasing depth [10,11] and varies with intervening tissue type [12,13] making comparison between subjects difficult. The technique is also sensitive to motion artefact [14]. Additionally 3D-PD technology provides no information relative to time; flow and perfusion are therefore not measured directly [15]. When the entire placenta has not been sampled and 'sonographic sphere biopsies' are performed questions have been raised about the clinical value of these indices due to large variability and poor reproducibility of the results [16,17].

* Corresponding author. c/o Fetal Medicine Department, Obstetrics and Gynaecology Department, Nottingham University Hospitals NHS Trust, Queen's Medical Centre Campus, Derby Road, Nottingham NG7 2UH, UK. Tel.: +44 1159249924x61924.
E-mail addresses: nia.jones@nottingham.ac.uk, nia.jones@nuh.nhs.uk (N.W. Jones), Peter.mansell@nuh.nhs.uk (P. Mansell), nick.fenning@nottingham.ac.uk (N. Raine-Fenning), george.bugg@nuh.nhs.uk (G. Bugg).

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The Cavalieri principle when applied to stereology is a method that utilises random, systematic sampling to provide unbiased and quantitative data [18] and has been shown to be reliable in both histological [19] and magnetic resonance imaging [20,21] studies. It is based on the assumptions that each area is representative of the whole organ; that, to avoid bias, each area has an equal chance of being sampled and that sampling should be done in a standardised manner [22]. We adapted this measurement technique for the quantification of power Doppler signal in a 3D acquired volume of the placenta.

The aim of this study was to describe a new technique to quantify placental vasculature, test its reliability and then apply it clinically to investigate placental vascularity in women with and without diabetes. Our hypothesis was that the novel measurement technique would be reliable and that it would be able to detect difference between the two groups.

2. Methods

2.1. Study design

This study was conducted at the Queen's Medical Centre campus of the Nottingham University Hospitals NHS Trust. Recruitment occurred at the time of the routine dating scan, facilitated through poster advertisements within the hospital. Approval for the study was given by the Nottingham Research Ethics Committee, in accordance with the principles of Good Clinical Practice and the Department of Health Research Governance Framework for Health and Social Care, 2005.

The experimental design was a prospective, longitudinal observational study with two groups; women with pre-gestational diabetes and a control group of non-diabetic women. Inclusion criterion for the study was a viable singleton pregnancy with an anterior placenta in women aged 18 years or more. Pregnancies affected by structural or chromosomal anomalies were excluded from both groups and women with medical conditions were excluded from the control group. Demographic and outcome data were collected from each subject by direct questioning, reviewing hospital notes and the maternity computerised delivery summaries. Demographic data collected from all subjects included maternal parity, BMI, ethnicity, medical problems and previous obstetric outcomes related to placental dysfunction (growth restriction, pre-eclampsia, placental abruption and stillbirth). Additional information collected from the diabetic subjects included type and duration of diabetes, HbA1c measurements at booking and in the third trimester, diabetic complications of retinopathy, nephropathy, neuropathy or hypertension. Outcome data included gestation at birth, birth weight, mode of delivery, gender of fetus, obstetric outcomes related to placental dysfunction and any pregnancy or labour complication.

2.2. Ultrasound data acquisition

Women were scanned at four weekly intervals at the gestational time points of 12 (range 11–14), 16 (15–18), 20 (19–22), 24 (23–26), 28 (27–30) and 32 (31–34) weeks using a Voluson 730 Expert (GE Medical Systems, Zipf, Austria) with a transabdominal 2–5 MHz 3D convex volume transducer. Women lay in a semi-recumbent position and were asked to remain as still as possible for the examination. A preliminary 2D ultrasound was performed to confirm viability and quantify fetal biometric measures.

The placenta was located and the view optimised by adjustment of scanning depth, focal zone and position of the transducer. The smallest distance between the placenta and probe was sought to minimise the effect of attenuation. The whole placenta was acquired within an acquisition sweep at the earlier gestations but, this was not possible beyond 21 weeks; acquisition of the maximal volume of the placenta therefore became the aim. A default power Doppler probe programme was activated and the power Doppler sector box adjusted to include the maximal placental area. Identical settings were maintained for all subjects as follows: gain 0.0, power 100%, PRF 0.6 kHz, quality 'high', wall motion filter 'low2'., signal rise 0.2, signal persistence 0.6, balance >190. The thermal index for bone (TIB) was 0.3. Acquisition was performed during periods of fetal quiescence and both transducer and maternal movements were minimised. The volume, once captured, was checked for the presence of movement artefact; acquisition was repeated if artefact was evident.

2.3. Ultrasound data analysis: the adapted Cavalieri principle of stereological analysis

For the purpose of this study, we analysed a single volume without visible movement artefact. The observer was blind to the subject's identity and diabetic status. The Virtual Organ Computer-aided AnaLysis tool within the 4D View programme (VOCAL™: GE Medical Systems, Zipf, Austria) was used for the analysis (Fig. 1a) and a novel stereological adapted technique was employed to calculate the fractional volume of power Doppler signal (FrVol-PD) within the placenta.

For accurate recognition of the placenta its contours were first outlined using the VOCAL tool. Using the multiplanar display and the 'colour off' mode, which displays the grey-scale image only and therefore improves visualisation of the utero-placental border [23], the volume was manipulated to visualise the discoid-shaped coronal placental view in the C plane. The placental edge was traced in the longitudinal (A) plane using the manual trace mode as the dataset was rotated through 180° using 15° rotation steps which resulted in 12 separate 2D images being available for measurement. The outlines were adjusted individually in each measurement plane to ensure the most accurate placental contour had been defined.

Analysis of FrVol-PD required orthogonal grids to be placed across the placental images. A computer generated random number sequence was used to determine the site for the first measurement and hence the placement of the grid [18,24]. Subsequent measurement sites were uniformly placed at 2 cm intervals in the longitudinal (A) and transverse (B) planes and at 1 cm intervals in the coronal (C) plane, aided by the use of the measurement mode in 4D View (Fig. 1b). Once the sites for measurement had been selected the reference point in 4D View, which shows the same position in all three image places (longitudinal, transverse, and coronal) was positioned over these marks. The vessel numbers were counted perpendicular to this plane, as follows: horizontal markings in the A plane necessitated vessel counting in the B plane, whilst horizontal markings in the B plane entailed counting in the A plane and vertical markings in the A plane required vessel counting in the C plane. Once the appropriate location for counting was viewed a specially developed acetate counting grid was placed over the computer screen in a random orientation for counting (Fig. 1c).

The next stage was to calculate the fractional volume of power Doppler signal (FrVol-PD) within the traced placenta. The power Doppler signal (PD) was counted if it was located within the traced placental borders and touched the upper, right hand intersection of a cross in the grid (Fig. 2). Visible signal artefact was excluded from counting. The volume of placenta (PI) was calculated by counting the number of crosses inside circles located within the contours of the placenta (Fig. 1c). This number was then multiplied by nine, as one in nine of the crosses were surrounded by circles and Howard et al. [22] report that this method is both reproducible and reduces the time of analysis. For each placenta, analysis was performed on a minimum of ten points using the three different orthogonal planes [22]. The fractional volume of the placenta occupied by PD signal (FrVol-PD) was then calculated according to the following formula:

$$\mathrm{FrVol-PD} = \frac{\sum_{i=1}^{m} P(\mathrm{PD})_i}{\sum_{i=1}^{m} P(\mathrm{PI})_i}$$

i.e. [sum of points counted for power Doppler signal in each plane] ÷ [sum of points counted for placental tissue];where FrVol-PD = fractional volume of power Doppler signal; m = number of planes analysed (aim for ≥10); P = points counted; PD = power Doppler signal; PI = Placenta; i = individual plane.

The vascularisation index (VI) within the traced placental volume, generated by the histogram facility within 4D View was also recorded. This index, which is representative of the percentage of the colour voxels within the defined volume of interest [25], was compared to the stereologically measured FrVol-PD result.

As this is a pilot study, and because there are no comparable data available, the number of subjects recruited was not based upon a power calculation.

2.4. Statistical analysis

Statistical analysis was undertaken using SPSS (Version 15.0, Chicago, IL). The Kolmogorov–Smirnov test was performed to assess the distribution of the data and the results are expressed as mean and standard deviation or median and range accordingly.

Statistical comparison between two groups was performed using the independent samples t test for normally distributed data and the Mann–Whitney test for non-parametric data. Variance components across time were assessed by the repeated measures design. Mauchly's test was used to assess sphericity, which assumes that variance of the differences between data taken from the same participants are equal. The results are presented as an F ratio; the ratio of the variation explained by the model to unexplained factors, with the Huynh–Feldt correction factor applied if sphericity was violated. The Bonferroni correction was applied to address the problem of multiple comparisons.

Comparison of the fractional volume of power Doppler signal was made between control and diabetes groups and results were compared according to pregnancy outcomes related to placental dysfunction (pre-eclampsia and placental abruption). In women with diabetes results were also compared by the duration of diabetes and the presence of maternal vascular complications as evident by the presence or absence of retinopathy, microalbuminuria and hypertension.

Reliability of the technique was performed on a sub-group of subjects. Intra-observer reliability compared the differences in two measurements of the same dataset made by a single observer whilst inter-observer reliability compared the differences in single measurements made by three different observers. Results were expressed as intraclass correlation coefficients (ICC) with absolute agreement together with their 95% confidence intervals [26] [27], and limits of agreement [28].