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Short communication

Analysis of syncytial nuclear aggregates in preeclampsia shows increased sectioning artefacts and decreased inter-villous bridges compared to healthy placentas

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ABSTRACT

Syncytial nuclear aggregates (SNAs) are increased in pregnancy complications and include 'true' syncytial knots and inter-villous bridges. Apparent nuclear overlay caused by sectioning artefacts are frequently counted from single sections. Haematoxylin and eosin stained serial sections were assessed for frequency of SNA subtypes in placentas from normal, preeclamptic and fetal growth restricted (FGR) pregnancies. There were more sectioning artefacts and syncytial knots and fewer bridges in samples from pre-eclampsia compared to controls. There were no significant differences between FGR and control samples. This suggests the villous tree in preeclampsia has less inherent structural support and trophoblast cell dynamics are different.

1. Introduction

In the human placenta, nuclei within the syncytiotrophoblast layer can form clusters, termed syncytial nuclear aggregates (SNAs) [1]. SNAs are reported to be increased in number and develop earlier in pregnancy complications, including pre-eclampsia (PE) and fetal growth restriction (FGR), as compared to normal pregnancies [2–4]. While FGR and PE can co-exist FGR often occurs without PE, particularly at term and these diseases are recognised as having separate placental phenotypes [5]. Although referred to by other papers as "syncytial knots" [6,7], SNAs encompass syncytial knots which attach to one villus and bridges which attach to two villi [8]. Some apparent SNAs are actually sectioning artefacts revealed through serial sections to be branching points or kinks in the villus [9]. This observation may be important as branching patterns of placental villi are altered in pregnancy pathologies [10]. We aimed to examine SNA subtypes in PE and FGR and compare them to healthy pregnancies to provide more detail on SNAs found in pregnancy pathologies and to cast light on their origin.

2. Methods

Placental tissue was collected with informed consent (08/H1010/55 North West Research Ethics Committee) control samples were collected from uncomplicated pregnancies (≥37 weeks gestation, maternal BMI ≤29.9, between the 10th–95th individualised birthweight centile and no maternal or fetal morbidities during pregnancy [1]). PE was defined as two blood pressure readings of 140/90 mmHg 4 h apart and ≥300 mg of protein in a 24-h urine sample. FGR was defined as an individualised birthweight ratio (IBR) below the 5th centile and had no hypertension, proteinuria or underlying maternal disease.

Villous tissue was dissected out from central, mid and edge regions to sample the whole of the placenta in control and complicated pregnancies. Tissue was fixed in 4% neutral buffered formalin and wax embedded. Ten serial 5 μm sections were made from each block, placed on 3-aminopropyltriethoxysilane coated slides and stained with haematoxylin and eosin. Three areas were imaged per dissected region using microscopy and traced through the 10 serial sections; a total of 9 stacks were collected per placenta. These stacks were examined using the Basic Aid Evaluating Serial Sections v1.0 (BAESS) [11] software program to label the apparent SNAs according to the subcategories of knot, bridge and sectioning artefact as previously described [1]. Data were analysed using Mann–Whitney *U* test between disease and control, *P* value returns of ≤0.05 were regarded as statistically significant.

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Table 1
Demographic data for participants whose placentas were used in this study. Symbols (* = $P \leq 0.05$) compared to control, (** = $P \leq 0.01$) compared to control.

Demographic characteristic	Median (range)/number (%)		
	Normal	FGR	Preeclampsia
Maternal age (years)	35 (26–38)	33 (20–37)	27 (19–36)
Gravidity	3 (1–4)	2 (1–5)	1 (1–3)
Parity	1.5 (0–3)	1 (0–2)	0 (0–1)
BMI (kg/m^2)	22.6 (18–28.8)	24.2 (20.7–29.2)	32.4 (18.2–46)
Ethnicity			
Black African or Caribbean	0 (0)	2 (28.6)	1 (14.3)
Caucasian	6 (100)	4 (57.1)	4 (57.1)
Pakistani	0 (0)	1 (14.3)	1 (14.3)
Other	0 (0)	0 (0)	1 (14.3)
Systolic blood pressure (mmHG)	117 (110–123)	120 (110–140)	159 (154–170)
Diastolic blood pressure (mmHG)	72 (63–81)	72 (65–90)	104 (98–110)
Protein (creatine ratio mg/mmol)	None detected	None detected	214 (52–333)
Cigarette smoking	0 (0)	0 (0)	0 (0)
Gestation at delivery (weeks)	40.925 (39.14–41.71)	39 (36.57–41.71)	39 (27.7–42.42)
Mode of delivery			
Vaginal delivery	3 (50)	4 (57.1)	4 (57.1)
Caesarean section	3 (50)	3 (42.9)	3 (42.9)
Birthweight (g)	3650 (3180–4040)	2350 (2120–3060)**	2800 (880–4340)
Individualised birthweight centile	53 (13.8–70)	1 (0–4)**	25 (0–98)
Fetal gender			
Female	3 (50)	5 (71.4)	3 (42.9)
Male	3 (50)	2 (28.6)	4 (57.1)
Placental weight (g)	470.0 (426–669)	357.0 (224–562)*	486.0 (289–728)

3. Results and discussion

Demographic data of participants are shown (Table 1). Examples of a syncytial knot and bridge identified in serial sections are shown (Fig. 1A). Sectioning artefacts (Fig. 1B) and syncytial knots (Fig. 1C) were increased in preeclamptic compared to control placentas ($P = 0.0082$ and $P = 0.035$ respectively). Inter-villous bridges were decreased in preeclamptic placentas compared to controls ($P = 0.0082$) (Fig. 1D). Examples of single sections of normal, PE and FGR villous placenta are shown (Fig. 1E–G).

Participants in each group were comparable except for differences related to the definition of the disorders such as significantly smaller birthweights and IBRs (both $P \leq 0.01$) from FGR pregnancies [12] and increased blood pressure and proteinuria in PE. SNA number is known to increase as gestation progresses, so having comparable gestational lengths means that significant changes caused by time are less likely in this study [13]. There was a trend towards increased BMI in women with PE ($P = 0.0513$), which may reflect the increased risk of PE with elevation of BMI [14,15] which can affect cell turnover but does not affect SNA number [16,17]. This observation gives weight to theories of exaggerated ageing of the trophoblast in PE [18,19].

A greater incidence of syncytial knots in PE compared to normal placenta may reflect an increased rate of addition of new nuclei to the syncytium resulting in a greater incidence of knots. Jones and Fox thought it possible that knots combined to from syncytial bridges [8], if true a dysregulation of this process in preeclampsia could lead to increased syncytial knot formation but decreased syncytial bridge formation.

SNAs revealed to be sectioning artefacts were increased in tissue from pregnancies with PE relative to controls, and those counted as bridges were decreased. This agrees with previous evidence that the branching structure of the placenta is altered in PE [20,21]. A decrease in inter-villous bridges has not been observed before and has implications for placental stability. Bridges have been hypothesised to stabilise the placenta against structural damage caused by blood flow through the inter-villous space [8]. We speculate that a reduction in inter-villous bridges could leave the placenta vulnerable to the physical effects of high maternal blood pressure, or conversely, high inter-villous blood pressure could inhibit bridge formation or destroy bridges. Furthermore, reinforcement of placental structure by bridges might contribute to equalising the exposure to perfusing maternal blood between adjacent villi.

There were significantly more syncytial knots in PE than control tissue but this was not true for FGR samples (Fig. 1C). FGR is associated with reduced rates of cytotrophoblast proliferation [21], whereas, increased cytotrophoblast fusion may occur in PE [22,23] to repair syncytial damage. Data regarding SNAs and syncytial knots in FGR are conflicting; Tomas et al. [24] found no changes in SNA number (but an increase in SNA size) in FGR compared to normal placentas. Other groups have reported more SNAs in FGR [2,3]. This could be due to the wide variety of causes of FGR which may affect placental structure in different ways. Conditions likely to cause FGR without changing cell turnover include inflammation and chromosomal abnormality [25,26]. There are also different clinical manifestations of FGR, with and without abnormal flow in the umbilical artery, which have not been taken into account in this study. Further study is needed to understand the relationship between SNAs and different causes of FGR.

Overall, this study suggests a different cell turnover process in PE placentas that alters the incidence and type of SNAs. Given that SNAs in single sections are increased under conditions of oxidative stress [27], a better understanding of the formation of different types of SNA could improve our understanding of disease mechanisms in a highly variable, clinically important disease.