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Circulating soluble fms-like tyrosine kinase-1 is placentally derived in normal pregnancy: First *in vivo* evidence

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ABSTRACT

Circulating sFlt-1 increases significantly in pregnancy compared to the non-pregnant state and even more so in the event of preeclampsia. We set out to determine if circulating sFlt-1 is placentally derived in normal pregnancy. Paired uterine and peripheral vein samples were collected at time of caesarean section. A follow up peripheral sample was collected in the postpartum period. There was a significant sFlt-1 gradient between uterine and peripheral veins. sFlt-1 levels dropped significantly when the placenta has been removed, i.e. postnatally. This is in keeping with the placenta being the main site of sFlt-1 production in normal pregnancies.

1. Introduction

Soluble fms-like tyrosine kinase-1 (sFlt-1) is an anti-angiogenic factor that increases significantly in the maternal circulation during normal pregnancies and even more so in preeclampsia (PE) [1]. Its physiologic role in normal pregnancies is not known. However, when present in extremely high levels, it causes widespread endothelial dysfunction leading to the development of PE and it is now used as a circulating biomarker for this disease. The study of placental and sFlt-1 biology, is fundamental for the understanding of preeclampsia. Numerous studies have shown *in vitro* sFlt-1 expression and secretion in normal and preeclampsia placentas (higher in PE placentas) [2]. Other sources of sFlt-1 have also been proposed such as smooth muscle cells, endothelial cells and PBMCs [3,4]. Placental production has been assessed *in vivo* by comparing the uterine and peripheral sFlt-1 levels. These studies showed a gradient of sFlt-1 in preeclampsia patients (higher levels in uterine vein than periphery) but not in normal pregnancy [5–7]. This suggested that the placenta is an important source of sFlt-1 in preeclampsia but not in normal pregnancies. This was proposed to argue against placental production in the absence of PE. These studies did not a) adjust for placental location, b) interrogate postpartum levels or c) use automated assays. We set out to determine whether maternal sFlt-1 is placentally derived in normal pregnancy by accounting for these factors.

2. Methods

Ultrasound to assess placental location was performed prior to elective caesarean section in women with normal pregnancy course. We aimed to find patients with extreme lateral placentas where we could sample both uterine veins, and all patients who consented for the study had their placenta location recorded. Serum samples from the antecubital (peripheral) and uterine vein (UV) were collected during caesarean section prior to delivery of the fetus. Accessing bilateral samples was technically more difficult but was performed in a small number of patients with extreme lateral placentas (and normal pregnancies) who consented to the procedure, in whom both uterine veins were surgically and safely accessible (n = 3). In the other cases, the obstetrician performing the caesarean section collected samples either from the left or the right uterine vein at their discretion. Samples were classified as contralateral or ipsilateral depending on the relative placental location to the UV. Centrally located placentas were excluded as well as cases where there was any uterine activity (i.e. contractions). Post-natal serum samples were collected at day 1–2 from the peripheral vein. The study was conducted at the John Radcliffe Hospital, Oxford, United Kingdom and all patients gave written informed consent (Central

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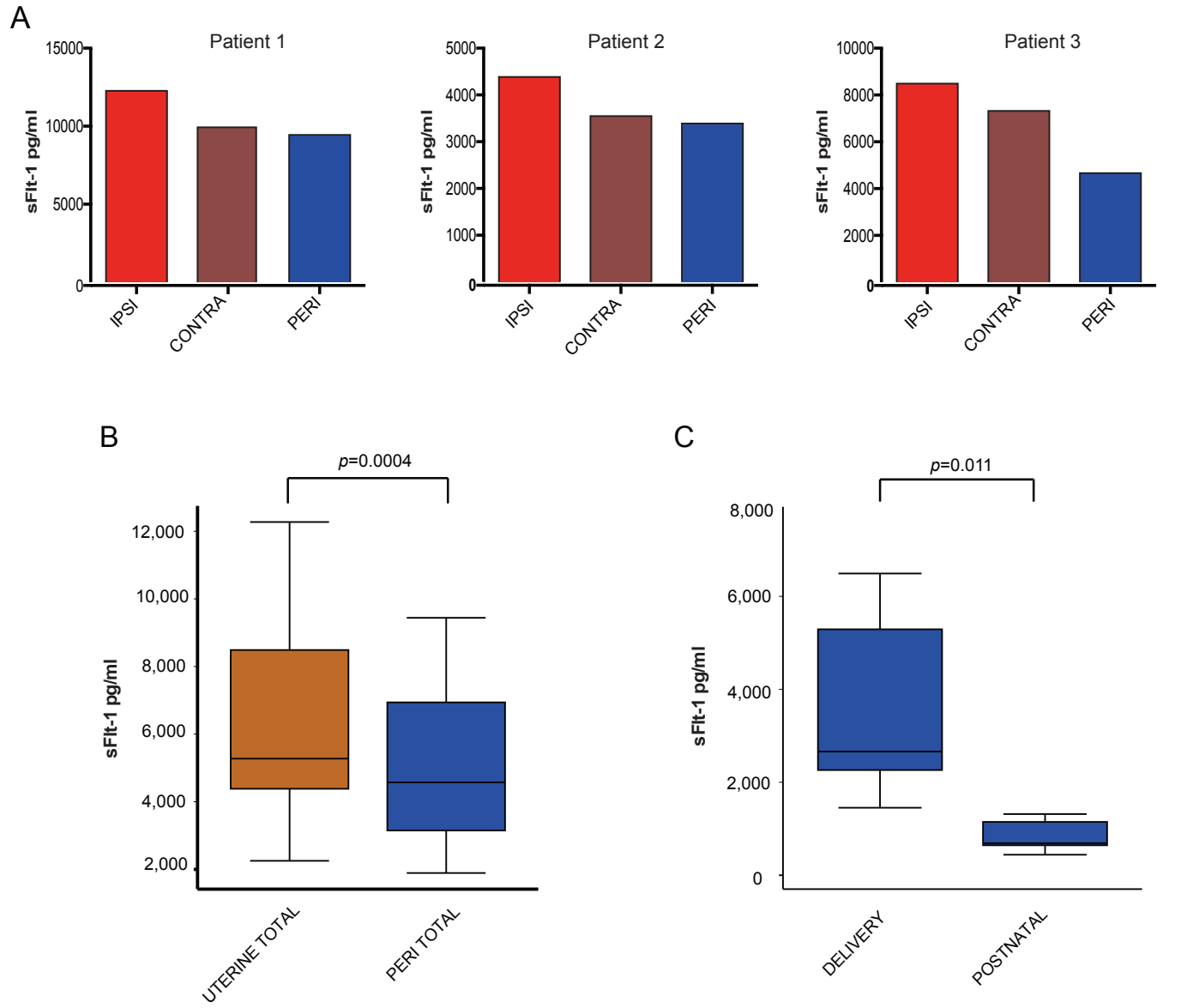

Fig. 1. sFlt-1 levels in uterine and peripheral veins. (A) Shows three patients who had simultaneous bilateral uterine vein samples and a peripheral (PERI) vein sample taken at caesarean section. The placenta was located by ultrasound prior to the procedure. The uterine vein sample taken from the ipsilateral side as the placenta was identified as IPSI and the contralateral side to the placenta was identified as CONTRA. (B) Shows sFlt-1 (pg/ml) measured in paired uterine vein and peripheral vein (PERI) (n = 17 paired samples). (C) Shows sFlt-1 (pg/ml) taken from the peripheral vein just prior to delivery (DELIVERY) and day 1–2 post-natally (POSTNATAL), n = 8 paired samples. Significance was determined to be $p < 0.05$.

Oxfordshire Research Ethics Committee C). sFlt-1 was measured on a Roche e411 analyzer (Roche Diagnostics Limited, Burgess Hill, UK). Inter-assay percentage coefficient of variation was 5.1% at 102 pg/mL and 2.8% at 1043 pg/mL.

3. Results

Seventeen (uterine and peripheral) paired samples were collected in total. Median gestational age was 39.4 (IQR 39.1-40) weeks. We first analysed bilateral samples from 3 patients with extreme lateral placentas. In these patients, sFlt-1 levels from ipsilateral samples were consistently higher than from contralateral ones (Fig. 1A). In order to further validate the hypothesis that placentally derived factors show a concentration difference between the ipsilateral and contralateral side, we measured hCG in these 3 samples. hCG was consistently higher in the ipsilateral samples than in the contralateral ones (20,327 vs 19,668 UI/L; 14,374 vs 13,309 UI/L and 21,329 vs 20,339 UI/L respectively). We then proceeded to analyse the entire study group. Overall median UV sFlt-1 was 5276 pg/ml (IQR 4384–8488) and overall median peripheral sFlt-1 was 4573 pg/ml (IQR 3150–6940); (n = 17 paired samples, paired *Wilcoxon* test $p = 0.0004$) (Fig. 1B).

The sFlt-1 level from ipsilateral UVs was 1.30 (1.15–1.6) times higher than their peripheral counterparts whilst from contralateral UVs it was 1.12 (1.04–1.34) times higher. The median difference on sFlt-1 level between UVs and periphery was 1580 pg/ml (703–2833) on ipsilateral (n = 11) samples and 429 pg/ml (157–2413) on contralateral ones (n = 6). Postnatal samples (Fig. 1C) showed an 80% drop of sFlt-1 postpartum [(median sFlt-1: 688.5 pg/ml (IQR 644.8–1147.5); n = 8; paired *Wilcoxon* test $p = 0.011$).

4. Discussion

We first demonstrated that, in cases of extreme lateral placentas, samples acquired from the uterine vein nearest to the placenta

(ipsilateral) have a higher concentration than the contralateral vessel. The likely placental origin was further confirmed by the concomitant measurement of beta-HCG. These samples also showed that there is an overall sFlt-1 gradient between the uterine vein and the periphery. Accordingly, there was a significant difference in the sFlt-1 concentration between the uterine and peripheral veins in the entire population (n = 17 paired samples). Importantly, after the placenta is removed (i.e. postnatal period), sFlt-1 levels drop significantly. Taken together, this data is in keeping with the placenta being the main site of sFlt-1 production in normal pregnancy. Peripheral sources may also contribute, although their contribution is estimated to be minimal as there is no difference between sFlt-1 in the radial artery vs radial vein [6]. Previous studies have not shown a gradient in uterine vs. peripheral samples. We believe the discrepancy is due to methodology. We accounted for placental location and used an assay with a low CV. These studies are also scarce and relatively small sized (mostly due to difficulty in obtaining these samples) and larger studies would be ideal. To our knowledge, this is the first study to show, *in vivo*, that the placenta is a significant source of circulating maternal sFlt-1. This data will allow the generation of mathematical models to better understand sFlt-1 biology (fundamental for understanding preeclampsia), namely the contribution of placental and other potential sources, distribution and rate of sFlt-1 clearance in maternal circulation.

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6. Declarations of interest

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.03.013.

References

[1] R.J. Levine, S.E. Maynard, C. Qian, K.H. Lim, L.J. England, K.F. Yu, E.F. Schisterman, R. Thadhani, B.P. Sachs, F.H. Epstein, B.M. Sibai, V.P. Sukhatme, S.A. Karumanchi, Circulating angiogenic factors and the risk of preeclampsia, N. Engl. J. Med. 350 (7) (2004) 672–683.

[2] S.E. Maynard, J.Y. Min, J. Merchan, K.H. Lim, J. Li, S. Mondal, T.A. Libermann, J.P. Morgan, F.W. Sellke, I.E. Stillman, F.H. Epstein, V.P. Sukhatme, S.A. Karumanchi, Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia, J. Clin. Invest. 111 (5) (2003) 649–658.

[3] A. Rajakumar, H.M. Michael, P.A. Rajakumar, E. Shibata, C.A. Hubel, S.A. Karumanchi, R. Thadhani, M. Wolf, G. Harger, N. Markovic, Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt-1 (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women, Placenta 26 (7) (2005) 563–573.

[4] S. Sela, A. Itin, S. Natanson-Yaron, C. Greenfield, D. Goldman-Wohl, S. Yagel, E. Keshet, A novel human-specific soluble vascular endothelial growth factor receptor 1: cell-type-specific splicing and implications to vascular endothelial growth factor homeostasis and preeclampsia, Circ. Res. 102 (12) (2008) 1566–1574.

[5] E. Bujold, R. Romero, T. Chaiworapongsa, Y.M. Kim, G.J. Kim, M.R. Kim, J. Espinoza, L.F. Goncalves, S. Edwin, M. Mazor, Evidence supporting that the excess of the sVEGFR-1 concentration in maternal plasma in preeclampsia has a uterine origin, J. Maternal-fetal Neonatal Med. 18 (1) (2005) 9–16.

[6] A.M. Holme, M.C. Roland, T. Henriksen, T.M. Michelsen, In vivo uteroplacental release of placental growth factor and soluble Fms-like tyrosine kinase-1 in normal and preeclamptic pregnancies, Am. J. Obstet. Gynecol. 215 (6) (2016) 782 e1–782 e9.

[7] M.C. Paasche Roland, B. Lorentzen, K. Godang, T. Henriksen, Uteroplacental arteriovenous difference in soluble VEGFR-1 (sFlt-1), but not in soluble endoglin concentrations in preeclampsia, Placenta 33 (3) (2012) 224–226.