Clinical science

49 Maternal haemodynamics in normal pregnancies: New insight on the influence of maternal characteristics

Hemodynamics
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Background: There is growing interest in non-invasive haemodynamic assessment for both clinical and research purposes. In order to allow for application, comparison and interpretation of haemodynamic parameters, there is a need to construct device-specific reference ranges. Furthermore, there is a paucity of data demonstrating the influence of maternal characteristics on central haemodynamic parameters. The purpose of this study was to assess stroke volume (SV), cardiac output (CO) and systemic vascular resistance (SVR) in a low-risk obstetric population, construct gestational-age (GA) specific reference ranges and delineate the effect of maternal characteristics.

Methods: This was a prospective cohort study of 824 patients with a GA ranging from 5 to 42 weeks. The inclusion criteria were women with a viable, singleton pregnancy, aged 16 and above with an uncomplicated pregnancy. Exclusion criteria included any medical disorder or pregnancy complication. The non-invasive device employed in this study was USCOM-1A®. All measurements were performed under standardised conditions. USCOM-1A® employs continuous wave Doppler, with a non-imaging probe in the suprasternal notch to obtain velocity time integrals of transaortic blood flow at the left ventricular outflow tract. For each haemodynamic variable, a normal distribution with mean conditional on GA was considered first. Once the distribution of the data had been determined with respect to GA, maternal characteristics were added to the model to test whether they provided a significant improvement in prediction of the mean/median value. Improvements in model fit were evaluated using the generalised likelihood ratio test, with statistical significance at $p < 0.05$.

Results: Maternal age had an effect on CO ($p < 0.001$). The estimated median CO was constant above the age of 32 years, but was around 0.5 L/min higher for women aged 25 or younger. Maternal weight ($p < 0.001$), height ($p < 0.001$) and their interaction ($p = 0.002$) also affected CO. In women with a height less than 1.60 m, there was no association between median CO and weight. In those with a height exceeding 1.60 m, an increase in weight was associated with an increase in CO. SV was primarily associated with height ($p < 0.001$), although some positive association with weight ($p < 0.001$) can also be observed within the normal BMI range. Greater height ($p < 0.001$) was associated with lower median values of SVR with an estimated difference of around 120 dynes·s·cm^5 between 1.60 m and 1.80 m. Advancing maternal age was associated with higher median SVR with an estimated difference of around 50 dynes·s·cm^5 between 25 and 35 years. Smokers had a lower SVR of73.5 dynes·s·cm^5 (95% CI; 8.6–138.4 dynes·s·cm^5).

Conclusion: We provide USCOM-1A® specific reference ranges for SV, CO and SVR in uncomplicated pregnancies. This will enable clinical application and comparison in pathological conditions. Maternal haemodynamics are significantly influenced by maternal age, height, weight and smoking status.

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Basic science

50 Erythropoietin production adequacy and its placental expression in preeclampsia

Placenta and decidua
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Introduction: The erythropoietin (EPO) participation in preeclampsia (PE) may be associated with its angiogenesis-stimulating and antiapoptotic effects in hypoxia conditions, including placental genesis.

Objectives: To study serum EPO-level and its placental expression level in preeclampsia.

Methods: In case-control study there were 11 women without preeclampsia (the 1st group), 14 – with moderate PE (the 2nd group) and 14 – with severe PE (the 3rd group). EPO-level of mother's blood serum was determined by means of solid-phase chemiluminescent enzyme immunoassay (sandwich-type) with coefficient estimation of EPO-production adequacy (CAepo – relation of observed serum EPO logarithm to tentative EPO logarithm, in normal limits 0.8–1.2). Immune histochemical test was used to estimate EPO-expression in symplastotrophoblast, capillary endothelium, placenta villi stroma macrophages (monoclonal antibodies to Epo (N-19): sc-1310-R, Santa Cruz Biotechnology, Inc., California, USA). Quantitative investigation was performed in 10 randomly selected fields of vision by magnification 400. We used statistical software programs: SPSS 12.1, Statistica for Windows 6.0, STADIA 6.3 prof. (Mann–Whitney test, Pearson's chi-squared test, nonparametric correlation analysis with calculation of Spearman's correlation coefficient).

Results: Blood serum EPO-level in moderate PE (59.25 ± 13.54 mIU/ml; 95% CI 30.34–88.11) appeared to be a bit higher and in severe PE (39.88 ± 10.60 mIU/ml; 95% CI 18.78–64.97) – lower that in the 1st group (43.02 ± 8.24 mIU/ml, 95% CI 25.21–60.83). The cases of EPO inadequate production ($p = 0.005$) were registered only in PE, the detection rate was the highest in severe PE (50.0% of cases). CAepo in severe PE (0.87 ± 0.04; 0.75–0.98) was less (p1-3 = 0.021) compared with the 1st group (1.03 ± 0.03; 95% CI 0.95–1.11). EPO-expression in placenta tissues during the pregnancy not complicated by PE was oftener determined in syncytiotrophoblast (19.73 ± 0.54; 95% CI 18.52–20.93) in comparison with vascular endothelium (9.46 ± 0.60; 95% CI 8.13–10.78; $p = 0.003$) and villi stroma macrophages (12.27 ± 0.51; 95% CI 11.15–13.40; $p = 0.003$). In moderate PE erythropoietin amount in indicated placental structures (26.20 ± 0.46, 95% CI 25.21–27.19; 18.87 ± 0.50, 95% CI 17.80–19.93; 19.87 ± 0.48, 95% CI 18.84–20.89 respectively) was notably higher that analogic indices in the 1st group (p1-2 < 0.001). And EPO-expression was oftener determined in villi syncytium ($p = 0.001$). EPO-representation in placenta of women with severe PE in symplast (33.06 ± 0.76; 95% CI 31.45–34.67), in vascular endothelium (26.00 ± 0.40; 95% CI 25.15–26.85) and in villi stroma macrophages (27.29 ± 0.52; 95% CI 26.19–28.40) was higher (p1-3 < 0.001; p2-3 < 0.001) compared with the 1st and 2nd groups. EPO-expression in syncytium of the 3rd group exceeded the same one ($p < 0.001$) in other placenta structures. We have detected positive correlation relationships of EPO-expression indices in symplastotrophoblast, capillary endothelium, stroma macrophages with case frequency of inadequate EPO-production ($r = -0.377$, $p = 0.014$; $r = -0.429$, $p = 0.005$; $r = 0.444$; $p = 0.003$ respectively) and negative – with CAepo ($r = -0.377$, $p = 0.014$; $r = -0.429$, $p = 0.005$; $r = -0.398$; $p = 0.009$ respectively).

Conclusions: Considerable increase of placenta EPO-expression was registered in association with its inadequate production in PE, especially in severe PE. We suppose that the decreasing of mother's serum EPO-level is probably connected with its renal and perhaps placental production disturbance.

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Basic science

51 Acetyl Salicylic Acid (aspirin) reversed TNF-α inhibition of trophoblast–endothelial interaction

Endothelial dysfunction, anti-angiogenic factors

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Introduction: Early administration of low dose Acetyl Salicylic Acid (aspirin, ASA) has demonstrated a reduction in preterm preeclampsia in some clinical trials. However, whether the mechanism of this preventive strategy of aspirin is via placental function and the pathways involved have not been investigated.

Objectives: The aim of this study is to investigate the effect of ASA on trophoblast integration and related cytokine or angiogenic/invasive pathways in an in vitro model of preeclampsia.

Methods: Red fluorescent-labeled human uterine myometrial microvascular endothelial cells (UtMVECs) were seeded on Matrigel. The endothelial cellular networks formed within 4 h. Green fluorescent-labeled trophoblastic HTR-8/SVneo cells were then co-cultured with the endothelial networks in the presence/absence of TNF-α (0.5 ng/ml) and/or ASA (0.1 mM) for 24 h. Red and green fluorescent images were captured. The effects of TNF-α and ASA on HTR-8/SVneo cells integration into endothelial cellular networks was quantified by Image Analysis software (Image J). The conditioned media were collected to measure IL-6, the free VEGF and PLGF by ELISA. Enzyme immunoassays (EIA) were performed to measure PGF1α, the stable metabolite of vasodilator PGI2. Cells were also retrieved from Matrigel to extract mRNA. The mRNA expression of cytokine angiogenic factors (VEGF, and PlGF), invasion markers (MMP-2 and PAI-1), endothelial cell activation markers (E-selectin), eNOS and cyclooxygenase (COX)-2 were examined by quantitative PCR.

Results: ASA reversed the inhibitory effect of TNF-α on trophoblast cells integration into endothelial cellular networks. In the conditioned medium, TNF-α increased PGF1α production (128%, $p < 0.05$), aspirin alone decreased PGF1α production (19%, $p < 0.01$) and reversed the TNF-α effect on PGF1α production (19%, $p < 0.01$). TNF-α decreased PlGF and increased VEGF and IL-6 production. TNF-α also inhibited the mRNA expression of eNOS, MMP-2 and stimulated COX2, PAI-1 and E-selectin, however, ASA did not reverse TNF-α effects on these molecules.

Conclusion: The results showed that ASA may have a beneficial effect on trophoblast cells integration into endothelial cellular networks via inhibiting the metabolism of vasodilator PGI2, not the known factors of cytokine, angiogenic, invasion markers and endothelial activation markers.

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Basic science

52 Altered ACVR2A expression modifies the response of vascular endothelial cells to normotensive and preeclamptic activin a concentrations

Endothelial dysfunction, anti-angiogenic factors

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Introduction: Circulating activin A concentrations are significantly higher in pre-eclampsia (PE) compared with those in a normotensive pregnancy. We and others have previously identified ACVR2A, which codes for the activin A binding receptor, as a maternal PE susceptibility gene with reduced expression in PE. The PE-associated rs1424954 genetic polymorphism also results in decreased receptor expression.

Objective: Given that PE is characterised by systemic vascular endothelial dysfunction, we used an in vitro cell-line model to investigate how reduced ACVR2A expression modifies the response of maternal vascular endothelial cells to normotensive and PE activin A concentrations.

Methods: The SGHEC-7 cell line was used as a model of vascular endothelial cells. ACVR2A expression was silenced using siRNA transfection and confirmed with real-time PCR and immunoblotting. A non-targeting siRNA was used as a negative control (NC). Following a 72 h transfection, SGHEC-7s were treated for 24 h with activin A concentrations representative of normotensive (0, 10 ng/ml) and PE (50 ng/ml) pregnancies. SGHEC-7 proliferation was determined using the electrical impedance-based xCELLigence system, while permeability was measured with a Millipore fluorescence-based assay. Student's t test and one-way ANOVA with Bonferroni's post-hoc test where appropriate were used for statistical evaluations. Each experiment was performed at least $n = 3$ times. A p-value of < 0.05 was considered statistically significant.

Results: ACVR2A siRNA markedly reduced ACVR2A mRNA and protein levels by 78% ($p < 0.05$) and 54% ($p < 0.01$) respectively. Activin A at 10 ng/ml significantly increased proliferation of both NC and ACVR2A siRNA transfected cells by at least 43% ($p < 0.01$) above that of 0 ng/ml. These increases were inhibited by at least 23% ($p < 0.01$) by the PE 50 ng/ml concentration. Nevertheless, at all tested concentrations of activin A, proliferation of ACVR2A siRNA transfected cells was significantly reduced by at least 32% ($p < 0.01$) compared with that of NC cells. The PE concentration of 50 ng/ml also significantly increased permeability of NC cells by 39% ($p < 0.01$) compared with that of the 0 ng/ml baseline. In contrast, permeability of ACVR2A siRNA transfected cells was significantly increased by 36% ($p < 0.01$) even at 0 ng/ml, with no further increase observed at 50 ng/ml, when compared with that of NC cells.

Conclusions: This study demonstrates that decreased expression of the ACVR2A receptor, in addition to activin A, can also result in dysfunctional vascular endothelial proliferation and permeability. Therefore, the altered expression of the ACVR2A receptor, resulting from a genetic predisposition, may modify the threshold required for activin A to impact vascular endothelial function, thereby exacerbating systemic vascular endothelial dysfunction in PE.

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