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A diagnostic panel of urinary biomarkers for pre-eclampsia in low and high-risk women

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Introduction

- Pre-eclampsia (PE) is a pregnancy-specific disease affecting 2-7% of women and remains a leading cause of maternal and prenatal morbidity and mortality in the western world
- Early detection/diagnosis of PE allows appropriate monitoring and targeting of therapeutic strategies
- Proteomics is a rapidly advancing technique, which gives functional insight into gene expression in living organisms
- Urine is an ideal medium for study, as it is readily available, easily obtained, less complex than other bodily fluids and potentially a rich source of biomarkers
- Analysis of urine has previously proven informative in the diagnosis of other diseases such as acute kidney injury, renal transplant rejection, tumours of the kidney and renal tract and assessment of cardiovascular risk[1]
- Proteomics could prove to be a powerful resource in identifying potential biomarkers for prediction of PE

Aims

1) To identify a distinctive PE urinary proteome profile
2) To use selective reaction monitoring (SRM) to quantify selected differential protein expression for potential biomarker discovery

Methods

- Urine (1.8 ml) was collected following written informed consent from 12 clinically diagnosed PE women and 12 gestation-matched control women using ISSHP guidelines
- Protein recovery was optimised using acetone precipitation and immunodepletion techniques
- Qualitative profiles were carried out using SDS-PAGE on urine samples from each group
- Gel regions from each group were also profiled using LC/MS/MS
- ScaffoldQ+ and Mascot v2.2 were used to identify and quantify relative protein levels
- Subsequent selection of candidate proteins were then subjected to SRM quantification in a diagnostically challenging cohort (Figure 1)

Acknowledgements

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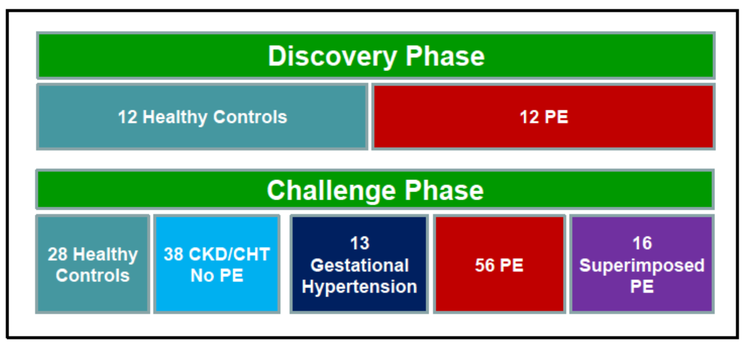

Figure 1: Study Design. CKD: Chronic Kidney Disease; CHT: Chronic Hypertension

Results

Qualitative assessment in 'Discovery Phase'

- Sufficient protein concentrations were obtained after depletion from the volumes used
- ScaffoldQ+ confirmed 82 proteins with high confidence (minimum 2 peptides at 95% probability) and 195 proteins at minimal stringency (1 or more peptides at >0% probability) that could be manually validated
- Seven proteins were detected uniquely in PE subjects and one present only in control samples

Quantitative assessment in 'Challenge Phase'

- 25 peptides were quantified by SRM
- 10 were differentially expressed between 'Cases' and 'Controls'
- Following step-wise regression, 2 peptides remained independently differentially expressed. ROC are shown in Figure 2

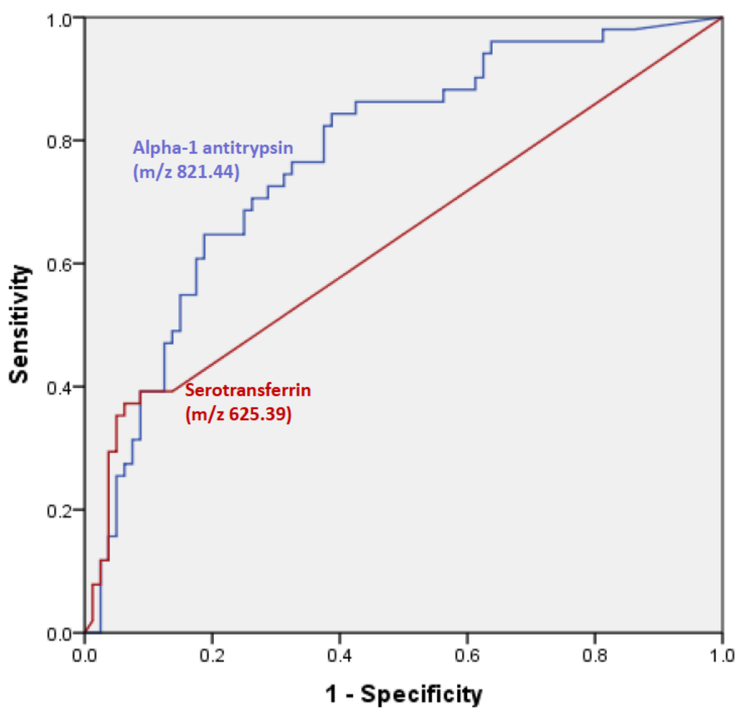

Figure 2 – ROCs of independent differentially expressed markers in challenge phase between cases (54 women) and controls (83 women)

Alpha-1 antitrypsin (m/z 821.44) ROC Area 0.77 (SE 0.34)
Serotransferrin (m/z 625.39) ROC Area 0.64 (SE 0.04)
Combined ROC Area 0.78 (SE 0.04)

Subgroup analysis in 'Challenge Phase'

i) Low risk women

- 13 peptides were differentially expressed between 'PE' and 'Healthy controls/Gestational Hypertension'
- Three peptides with the highest AUC ROC are shown in Figure 3

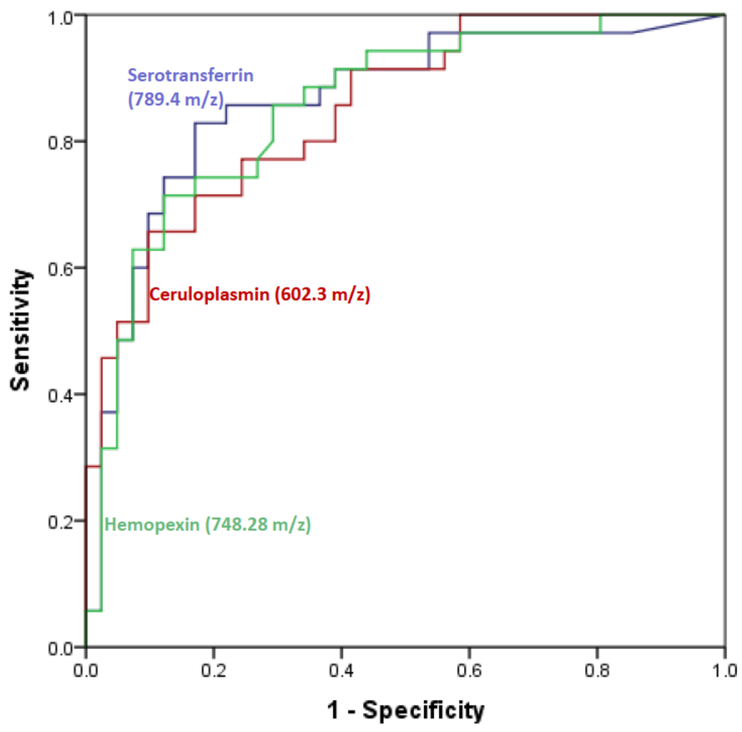

Figure 3 – ROCs of differentially expressed markers in challenge phase between low risk PE (35 women) and healthy controls (28 women)/gestational hypertension (13 women)

Ceruloplasmin 602.3 m/z: ROC Area 0.83 (SE 0.43)
Hemopexin 748.28 m/z: ROC Area 0.85 (SE 0.44)
Serotransferrin 789.4 m/z: ROC Area 0.86 (SE 0.44)

Subgroup analysis in 'Challenge Phase'

ii) High risk women

- Serotransferrin (625.39m/z) was differentially expressed between high risk controls (38 women) and women with superimposed PE (16 women) but AUC ROC was 0.75 (SE 0.82)

Conclusions

- We have successfully optimised a workflow for urine with varying protein concentrations
- Proteins with differential levels between groups have been identified and targeted in subsequent SRM
- A urinary proteomic signature can be identified in time-of-disease PE samples
- Validation of this proteomic profile and identification of a similar mid-trimester signature for prediction of PE will facilitate stratification of care and surveillance

References

[1]Bramham *et al.*, 2009 QJM;102(8):523-38

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