State the objective of the project

Preeclampsia (PE) is a pregnancy-related disease, affecting 3–7% of all pregnancies. PE is a major cause of maternal and perinatal mortality and morbidity worldwide. However, most women still might deliver a healthy baby if PE would be detected early and treated with regular prenatal care. Regardless of unknown origin, it is extremely important to search for a novel sensitive method of quantitative analysis of PE biomarkers in urea of pregnant women in its early stage. PE is related to loss of podocytes (glomerular visceral epithelial cells, maintaining glomerular filtration barrier) and consequently kidney injury. Urinary podocytes have been proposed as a source of a new marker for PE. A sensitive detection of podocytes in urine (podocyturia) might be a useful method for both diagnostics of PE and prediction of the severity of the disease.

The main goal of this project is to adopt and optimize the chemical derivatization method, developed by us previously, for ultra-sensitive identification and quantitative analysis of urinary PE biomarkers (mainly podocin) by LC-ESI-MS/MS. We also plan to apply stable isotope labeled ionization enhancers for a comprehensive shotgun proteomics analysis of urine proteins for early and late PE and to check applicability of the developed ionization enhancers in early prediction of PE. This interdisciplinary project will be performed by collaborating scientists from Research and Development Centre of Regional Specialist Hospital in Wroclaw and Faculty of Chemistry, University of Wroclaw.

Methodology

The proposed project involves the collection of urine samples from patients with diagnosed PE and investigations of possible preeclampsia biomarkers by derivatization of tryptic digests obtained from urine sediment. We will apply derivatization by ionization enhancers developed by us previously. Then the derivatized peptides will be analyzed by tandem mass spectrometry methods. The identified biomarker fragments will be resynthesized and derivatized with the ionization enhancers and their isotopologues to obtain internal standards. To improve the specificity and precision of the proposed strategy we plan to use multiple fragments of podocin as well as other detected proteins for the quantitative analysis. Additionally we will also apply the proposed derivatization method for sensitive shotgun proteomics analysis of urine proteins at different stages of PE. The tryptic peptides isolated from urine samples collected at different stages of PE will be derivatized using ionization tags labeled with different combinations of stable isotopes, e.g. urine peptides for early, medium, late and very late stage of PE will be derivatized by 2,4,6-triphenylpyryllium, 2,4,6-triphenylpyryllium-d_5, 2,4,6-triphenylpyryllium-d_{10}, and 2,4,6-triphenylpyryllium-d_{15} groups, respectively. The derivatized samples will be combined and the changes in relative concentrations of podocin and possible other proteins will be quantified by LC-ESI-MS/MS.

Expected impact of the research project.

It may be expected that the results obtained during this project may improve the actual state of knowledge of preeclampsia biomarkers, allowing the development of new method of diagnosis based on chemical derivatization and mass spectrometry. We believe that application of the proposed method will improve a detection limit of the biomarker up to 100 times, similarly to results obtained by us previously on model peptides and protein hydrolysates. This may revolutionize the diagnosis of early stage of PE, because the proposed approach may overcome the problems related to insufficient amount of peptide biomarker in biological samples. We hope that the shotgun proteomics analysis of urine proteins collected at different stages of PE should be useful in discovery of new biomarkers of PE, contributing to the improvement of prediction and diagnosis of the disease and allowing early therapeutic intervention.