**Thesis title:** Serum markers in women with polycystic ovary syndrome: a hypothesis-free multi-omics approach


## Abstract

Polycystic ovary syndrome (PCOS) is one of the most common metabolic and endocrinological disorders in women. PCOS is the major reason behind anovulation related infertility and is clinically characterized by menstrual disorder, chronic amenorrhea, hormonal imbalance and polycystic ovaries. Several phenotypes of the disease exist along withmultiple diagnostic criteria thus making diagnosis of PCOS challenging. Presently, there is no definite molecular marker of the disease and therefore the need for accurate diagnostic marker(s) is well recognized.

The first part of the study involves identification of altered serum proteins in PCOS when compared with controls. Both discovery and validation phase analysis indicated that vitamin D-binding protein, complement component 3, Ig mu chain C region, and Ig gamma-1 chain C region hold promise as effective serum protein markers for diagnosis of the disease.

The second part of the study explores and validates serum metabolic alterations in PCOS patients using proton nuclear magnetic resonance spectroscopy ($^1$H NMR). Significant dysregulation of several amino acids including alanine, leucine, histidine, valine, glutamate, L-glutamine, threonine and proline and energy metabolites viz. lactate, glucose, acetate and 3-hydroxybutyric acid were observed. Amongst these, lactate, threonine, proline, acetate and alanine appear to have highly significant diagnostic potential.

The third part of the study involves liquid chromatography mass spectrometry (LC-MS/MS) based targeted and gas chromatography based untargeted metabolomics approach to identify altered serum metabolic profile in PCOS. A large panel of metabolite markers including riboflavin, sucrose, adenine, N-acetyl glycine, thymine, cystathionine, phosphoric acid, cortisol, and phenylalanine were found to be most significantly altered in these women. These metabolites also hold potential as serum metabolic markers of the disease.

Next, $^1$H NMR was used to discover lipid biomarkers characteristic for PCOS. Altered lipid metabolism majorly characterized by alterations in a number of phospholipid moieties further motivated us to investigate phospholipid specific changes in PCOS using LC-MS/MS. Twelve most significantly altered phospholipid moieties were identified from the four phospholipid classes. Amongst these lipid moieties, PC 38:1, PC 38:2, PC 44:12, SM 42:1, and SM 42:2 were found to have the highest diagnostic capability as markers of PCOS in addition to the lipids identified using NMR.

Interestingly, when all the identified markers were used together, sensitivity and specificity of the panel increased to 84% and 100%, respectively. During significant feature selection, phenylalanine, cystathionine, SM 42:2, SM 42:1, thymine, PC 38:1, PC 44:12, and PC 38:2 were found to be the most promising set of markers for PCOS. It is envisioned that these identified markers can be put to clinical use and is a step towards future development of robust bio-assays for diagnosis of PCOS.



**Keywords:** Polycystic ovary syndrome; biomarker(s); proteomics; metabolomics; lipidomics