PROPOSED TITLE

Identification of non-invasive signatures for differential diagnosis of biopsy-proven diabetic nephropathy and non-diabetic kidney disease by metabolomics and peptidomics approach

INVESTIGATORS

Principal investigator

Dr.Sujoy Ghosh,
Associate Professor,
Dept.of Endocrinology and Metabolism,
4th Floor, Ronald Ross Building,
IPGMER & SSKM Hospital, Kolkata-20.
Phone : 9674625823
E-mail: drsujoyghosh2000@gmail.com

Co-principal investigator

Dr. Soumen Kanti Manna
Associate Professor
Biophysics & Structural Genomics Division
Saha Institute of Nuclear Physics, Kolkata, India
Phone: +91 33 23374632 (Ext 4626)
Email: soumenmanna@gmail.com

Project assistant

Madhurima Basu
PhD student
Dept.of Endocrinology and Metabolism,
4th Floor, Ronald Ross Building,
IPGMER & SSKM Hospital, Kolkata-20.
Phone no:9051948176
Email:mbasu08@yahoo.com

Introduction

Chronic Kidney disease (CKD) in diabetes is one of the most prevalent microvascular complications (1) which lead to end-stage renal disease (ESRD) worldwide (2). Among all cases of kidney failure, about 44% is associated to diabetes. Diabetic patients with kidney disease may suffer not only from kidney failure but also cardiovascular disease associated morbidity and mortality and high treatment cost (3). Kidney disease in diabetes is the most common complication among the both type of diabetes, which usually manifest after 10-15years after diagnosis to Type-1 diabetes or Type-2 diabetes (4).

Differential diagnosis of diabetic vis-a-vis non-diabetic kidney disease based on clinical manifestation and biochemistry is often challenging. This is believed to lead to underrepresentation of the non-diabetic kidney disease in epidemiological studies. More importantly, misclassification might lead to deleterious consequences for the patient as the management regimes of these two diseases are different altogether. Since histopathological investigation, the gold standard for diagnosis, is invasive with high risk and the facility for biopsy is not available commonly, identification of a non-invasive or, at least, minimally-invasive signature may significantly improve therapeutic outcome. It may also yield tools for disease surveillance and therapeutic monitoring.

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Review of existing literature

Metabolomics

Previous studies have revealed changes in metabolic signature associated with kidney damage (reviewed in 1, 2). Most of these studies dealt with acute kidney injury (3, 4), chronic kidney disease (5, 6) or renal carcinoma (7, 8). Based on serum and urinary metabolomic analysis, results of earlier studies (1, 9) also suggested dysregulation of a number of metabolic pathways including glycolysis, TCA cycle, purine metabolism, lipid metabolism and amino acid metabolism to be associated with diabetic kidney disease. Using metabolomics, Sharma et al showed evidence for mitochondrial dysfunction, which would contribute to cell death and, thus, impairment of kidney function (10). Putative early stage non-invasive biomarkers for diabetic kidney disease have also been identified through urinary metabolomics (11). However, **no such study has been reported for non-diabetic kidney disease in patients with T2DM. Surprisingly, the patient recruitment criteria of most of these aforementioned studies were not based on biopsy confirmation of diabetic kidney disease. Evidently, there has also been no comparative analysis of metabolomic signature associated with biopsy-confirmed diabetic and non-diabetic kidney disease**.

Pathogenesis of diabetes is associated with distinct sets of dysregulation of metabolic machinery involving multiple organs primarily involving liver, pancreas, muscle and adipose tissue. Diabetic kidney disease is a result of this systemic derangement at a later stage. Since kidney plays a major role in filtration and re-absorption of small molecules, impairment of kidney function is expected to affect the metabolic signature in urine as well as serum. The absolute and relative abundance of different metabolites in biofluids will depend not only on kidney function but also on function of other organs involved in their homeostasis. Thus, in case of diabetic kidney disease, change in urinary metabolic signature is expected to have contribution from impaired kidney function as well as aberration in metabolic machinery of other organs such as liver, pancreas, muscle and adipose tissue. On the other hand, non-diabetic kidney disease may not be a result or lead to extensive dysregulation of metabolic machinery in aforementioned organs. Thus, the urinary metabolomic signaturein non-diabetic kidney disease may be distinct from that associated with diabetic kidney disease.

Peptidomics It has long being postulated that uremia is associated with change in accumulation and excretion of 'middle molecules' (12). Although earlier the molecular weight range of these molecules was suggested to be 500-2000 Da, recently it has been revised to be 500-60000 Da (13). A number of studies have, in fact, looked into changes in urinary proteome to find biomarkers for chronic kidney diseases including diabetic nephropathy (14-16). However, it should be noted that, abnormal protein excretion in kidney disease is typically expected to occur after significant glomerular damage has taken place, i.e., in advanced stages. On the other hand, peptides are filtered from blood even at normal physiological condition. Therefore, any changes in circulating or kidney-derived peptides are likely to appear in urine early during pathogenesis of chronic kidney disease and act as early biomarkers. Peptides, being degradation products of protein, are also expected to provide more robust signature compared to proteins themselves, which may undergo further modification and/or degradation during storage in bladder. In addition, emerging evidence also suggest an important role of ubiquitin-mediated proteosomal degradation in renal dysfunction (17-18). Put together, these suggest a change in proteolytic products, i.e., peptides, associated with renal dysfunction. In fact, an earlier study analyzed human urine samples to identify peptides that may serve as putative biomarkers for chronic kidney disease (19). Two recent studies have also revealed changes in urine peptidome in rodent models of diabetic nephropathy (20-21). Given the distinct nature distinct nature of kidney damage in DKD and NDKD, the resulting peptidomic signature may be different between these two diseases. However, till date **there has been no report of comparative analysis between human urinary peptidome associated with biopsy-confirmed DKD and NDKD**. This study proposes to carry out global metabolomic and peptidomic profiling to identify signatures that could be exploited for differential diagnosis of diabetic and non-diabetic kidney disease.

Based on the brief background and literatures mentioned above, we hypothesize that
"**Diabetic kidney disease (DKD) and non-diabetic kidney disease (NDKD) bears *distinct* metabolomic and peptidomic signatures**".

Methodology

Study design

Cross-sectional, Hospital based.

Study settings

OPD of Department of Endocrinology and Metabolism and Department of Nephrology, IPGME&R and SSKM hospital

Study population

Patients who are attending OPD of Department of Endocrinology and Metabolism and Department of Nephrology, IPGME&R and SSKM hospital. We shall recruit 50 biopsy confirmed DKD and 50 NDKD patients

Sampling methods

Among considered study population, who gave consent would be screened by clinical standard criteria of DKD and NDKD as described (1) and grouped as true DKD and true NDKD (confirmed by biopsy).

Sampling techniques Purposive sampling

Inclusion criteria

Age >18 years, both male and female type2 DM patients with proteinuria and /or renal dysfunction (30-90 ml/min or 1.73m^2) GFR

Exclusion criteria

Type-1 diabetic patients, patient unwilling for kidney biopsy and sampling of studies and end stage renal disease (GFR <15ml/min/1.73ml for more than 3 months)

Reference

1. Thijs W et.al, JASN (Journal of American Society of Nephrology) April 1, 2010; 21, 4556-563

Study Techniques

After obtaining the certificate of clearance from ethical committee study population is selected randomly among the patients who are attending OPD and diabetic clinic of SSKM hospital, West Bengal. Morning first-pass urine samples will be collected. Three 1 ml aliquot of the sample will be flash-frozen immediately upon arrival and stored at -80°C until further analysis. 3.6 ml urine will be centrifuged (1.8 ml each in two micro-centrifuge tubes) and three aliquots of 1 ml supernatant will be flash-frozen and stored at -80°C. On basis of reported biopsy confirmation, samples will be grouped into DKD and NDKD. All the data of routine biochemical tests for diabetes profile and CKD profile will be kept for future use. The food and xenobiotic exposure data (particularly, drugs) of the patient the preceding day and on the day of the sample collection will be recorded. Metabolomic and peptidomic analyses will be carried out in collaboration with Dr. Soumen Kanti Manna, Saha Institute of Nuclear Physics, Kolkata as described below.