Use of Urinary Neutrophil Gelatinase Associated Lipocalin (uNGAL) as Early Predictor of Diabetic Nephropathy in Children and Adolescents with Type 1 Diabetes Mellitus

Thesis
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Abstract

Background and aims: Diabetic nephropathy is a major cause of morbidity and mortality among young adults with type 1 diabetes. Clinical management and therapeutic intervention from early stage of DN is of major importance to prevent progression to end-stage renal disease. Renal tubulointerstitium plays an important role in the development and progression of diabetic nephropathy.

Methods: In the present study, we aimed at evaluating the levels of urinary neutrophil gelatinase-associated lipocalin (uNGAL) - a tubular stress protein - from a cross sectional study of 50 patients with type 1 diabetes mellitus at DEMPU categorized into two groups (normoalbuminuria and microalbuminuria) and 18 healthy controls.

Results: Patients with type 1 diabetes showed increased mean uNGAL values with respect to controls; interestingly, increased NGAL levels were already found in diabetic patients without early signs of glomerular damage (normoalbuminuric). uNGAL increased in parallel with the severity of renal disease, poor glycemic control and duration of diabetes

Conclusions: NGAL might play an important role in the pathophysiology of renal adaptation to diabetes, probably as a defensive mechanism aiming to mitigate tubular suffering. Furthermore, NGAL measurement might become a useful and noninvasive tool for the evaluation of renal involvement in diabetic patients as well as for the early diagnosis of incipient nephropathy.

Key words:

(Type 1 diabetes mellitus - Diabetic nephropathy - Normoalbuminuria - Microalbuminuria – Urinary neutrophils gelatinase associated lipocalin).

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List of Abbreviations

125I……	125 iothalamate.
51Cr-EDTA……..	51 Cr-Ethylene-Diamine-Tetra-Acetic acid
AAT……..	Alpha-1 antitrypsin.
AC……..	Albumin concentration
ACE……..	Angiotensin Converting Enzyme.
ACORD…….	The Anemia CORrection in Diabetes.
ACR……	Albumin/Creatinine ratio
ADPKD…….	Autosomal dominat polycystic kidney disease.
AER……	Albumin excretion rate
AGEs…….	Advanced glycation end products.
AKI……	Acute kidney injury.
ARBs…….	Angiotensin Receptor Blockers.
BCG……..	Bacillus, Calmette-Guerin.
BG……..	Blood glucose
BM……..	Basement membrane
BMI…….	Body mass index
BUN……	Blood urea nitrogen
CHF…….	Chronic heart failure
CKD……	Chronic kidney disease
CSII…….	Continuous Subcutaneous Insulin Infusion.
CTLA4……	Cytotoxic T lymphocyte-associated antigen-4.
DCCT……..	The diabetes control and complications trial.
DKA……	Diabetic ketoacidosis.
DM……..	Diabetes mellitus.
DN……..	Diabetic nephropathy.
DPC…….	Diagnostic products corporation.
DPT-1…….	The National Institutes of Health Diabetes Prevention Trial.
ECM……	Extracellular matrix .
EDIC……..	Epidemiology of Diabetes Interventions and Complications.
eGFR……..	Estimated Glomerular filtration rate

EIA …….	ELISA
ELISA……	Enzyme Linked Immuno Sorbent Assay
ESRD……..	End-Stage Renal Disease.
EURODIAB…….....	European Diabetes.
GADA…….	Glutamic acid decaroboxylase auto-antibodies 65
GDM……...	Gestational diabetes mellitus.
GFR…….	Glomerular filtration rate.
Hb……...	Hemoglobin.
HbA1c…….	Glycosylated hemoglobin.
HHS…….....	Hyperglycemic Hyperosmolar state.
HLA……	Human leukocyte antigen
HOT……	Hypertension Optimal Treatment.
IAA…….	Islet autoantigen-insulin
ICA…….	Islet cell auto-antibodies.
INF-alpha……	Interferon-alpha.
IRMA……..	Immunoradiometric assay
KIM-1…….	Kidney Injury Molecule.
LVMI……..	Left ventricular mass index.
MDI……	Multiple Daily Injections
MDRD…….....	Modification of Diet in Renal Disease
MICRO-HOPE……...	Heart Outcomes Prevention Evaluation.
MMP-9……	Metalloproteinase 9.
mRNA…….	Messenger RNA.
NAG……	N-Acetyl-Beta-(D)-Glucosaminidase
PKC…….	Protein Kinase C.
PTPN22……...	Protein tyrosine phosphatase nonreceptor-types 22.
RAS…….	Renin-angiotensin system.
RIA……...	Radioimmunoassay.
SLE…….	Systemic lupus erythematosus.
SMBG…….	Self- monitoring of blood glucose.
sNGAL……	Serum Neutrophils Gelatinase Associated Lipocalin.
T1DM…….	Type 1 diabetes mellitus.
T2DM…….	Type 2 diabetes mellitus.

TBM……	Tubular basement membrane.
UAE……	Urinary albumin excretion.
uNGAL……...	Urinary Neutrophils Gelatinase Associated Lipocalin.
α-GST……	Alpha glutathione s-transferase.
π-GST…….	Pie glutathione s-transferase.

Introduction

Diabetes is a metabolic disorder of multiple causes characterized by chronic hyperglycemia and disorders of carbohydrate, fat, and protein metabolism. It may be classified as autoimmune mediated type 1 diabetes, or as insulin resistance associated type 2 diabetes, or a combination of these factors. Type 1 diabetes mellitus (T1DM) commonly occurs in childhood or adolescence, although the rising prevalence of type 2 diabetes mellitus (T2DM) in these age groups is now being seen worldwide ***(ADA, 2010)***.

Diabetic nephropathy is one of the most common microvascular complications of diabetes mellitus, greatly affecting the life quality and survival of the patients. As global prevalence of type 1 diabetes is steadily increasing, the numbers of patients with diabetic nephropathy is expanding day by day. In adults, diabetic nephropathy is one of the leading causes of end stage renal disease (ESRD), a disease that is described as a medical catastrophe of worldwide dimensions ***(Ritz et al., 1999).***

Therefore, the prevention of the disease or at least the postponement of its progression has emerged as a key issue. Adverse outcomes of renal failure can be prevented or delayed through early detection and treatment ***(Levey et al., 2003).***

At present, albuminuria measurement is used as a standardized, noninvasive test to diagnose early DN. Diabetic kidney disease, however, is not detected by this test in some cases ***(Zachwieja et al, 2010).***

Pathological albuminuria and proteinuria constitute the consequence of diffuse diabetes induced glomerular damage. However, renal tubulointerstitium also seems to play an equally important role in the genesis of diabetic nephropathy, as the consequence of a persistent exposure to a variety of metabolic and hemodynamic injuring factors associated with sustained diabetic disease ***(Bolignano et al, 2009).***

Neutrophil gelatinase associated lipocalin (NGAL) is an acute phase protein that is rapidly released not only from neutrophils, but also a variety of cell types upon inflammation and tissue injury. Its small molecular size and protease resistance could render it an excellent biomarker of renal injury ***(Ding et al., 2007).***

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein first identified in neutrophils but expressed at very low concentration in several tissues, including the lung, the gastrointestinal tract and the kidney. Circulating NGAL is filtered by the glomerulus and captured by the proximal tubule and only a minimal amount is excreted in urine ***(Parravicini, 2010).***

In contrast, urinary NGAL (uNGAL) derives mostly from the thick limbs of Henle and collecting ducts in both the postischemic and postseptic kidney. uNGAL values in children and adults are markedly elevated with acute kidney injury, anticipating the rise of creatinine by 24-48 hrs ***(Parravicini, 2010).***

Increased uNGAL level was already found in diabetic patients without early signs of glomerular damage (normoalbuminuric). The increasing of uNGAL is parallel with the severity of renal disease, reaching higher levels in patients with diabetic nephropathy ***(Bolignano et al., 2009).***

NGAL might play an important role in the pathophysiology of renal adaptation to diabetes, probably as a defensive mechanism aiming to mitigate tubular suffering. Furthermore, uNGAL measurement might become a useful and noninvasive tool for evaluation of renal involvement in diabetic patients as well as for early diagnosis of incipient nephropathy ***(Bolignano et al., 2009).***

Aim of work

We aim to evaluate the level of urinary neutrophil gelatinase-associated lipocalin (uNGAL) in urine as a marker of tubulointerstitial damage in children with type 1 diabetes in relation with the level of albuminuria and renal function in order to explore the potential role of (uNGAL) as an early predictor for the progression of nephropathy in type 1 diabetic patients.

Chapter One

Diabetes mellitus

Definition of diabetes mellitus:

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels ***(ADA, 2010).***

Etiologic Classification of diabetes mellitus:

Diabetes mellitus is classified according to the etiology into various types:

I) Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency).

A. Immune mediated

B. Idiopathic

II) Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance).

III) Other specific types of diabetes:

A. Genetic defects of β-cell function.

B. Genetic defects in insulin action.

C. Diseases of the exocrine pancreas: Pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis.

D. Endocrinopathies: Acromegaly, Cushing syndrome, pheochromocytoma, hyperthyroidism.

E. Drug or chemical induced: Vacor, pentamide, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, thiazide, phenytoin, and clozapine.