Value of Urinary Heme Oxygenase 1 as an Early Biomarker in Diabetic Nephropathy

A Thesis

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in **Internal Medicine**

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بسم الله الرحمن الرحيم
قالوا
سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم الحكيم
صدق الله العظيم
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Aya Mohamed Magdi Abdelalim

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List of Abbreviations

Abbrev.	Full term
ACCORD trial	Action to Control Cardiovascular Risk in Type 2 Diabetes
ACE	Angiotensin converting enzyme
ACR	Albumin creatinine ratio
AD	Alzheimer's disease
ADA	American Diabetes Association
ADVANCE	Action in Diabetes and Vascular Disease Preterax and Diamicron Modified Release Controlled Evaluation
ALS	Amyotrophic lateral sclerosis
Ang II	Angiotensin II
ANP	Atrial natriuretic peptide
ARBs	Angiotensin receptor blockers
AREs	Antioxidant response elements
BENEDICT	Bergamo Nephrologic Diabetes Complications Trial
BMI	Body mass index
BP	Blood pressure
BR	Bilirubin
BTB	Tramtrack and Broad complex
BV	Biliverdin
CANVAS	Canagliflozin Cardiovascular Assessment Study
CKD	Chronic kidney disease
CNC	Cap 'n' collar
CNS	Central nervous system
CO	Carbon monoxide
COX-2	Cyclooxygenase-2

CREDANCE : Evaluation of the Effects of Canagliflozin on Renal and Cardiovascular Outcomes

CVD : Cardiovascular disease

DAPA-CKD : A Study to Evaluate the Effect of Dapagliflozin on Renal Outcomes

DIRECT : Diabetic Retinopathy Candesartan Trial

DM : Diabetes mellitus

DN : Diabetic nephropathy

DPP-4 : Dipeptidyl peptidase 4

DRI : Direct renin inhibitors

EDIC : Epidemiology of Diabetes Interventions and Complications

ELISA : Enzyme-linked immunosorbent assay

EMPAREG : Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes

EPC : Endothelial progenitor cells

ESRD : End-stage renal disease

GBM : Glomerular basement membrane

GFR : Glomerular filtration rate

HD : Huntington's disease

HDL : High-density-lipoprotein

HIF-1 : Hypoxia inducible factor

HMW : High-molecular weight

HOPE : Heart Outcomes Prevention Evaluation

Hos : Heme oxygenases

HRs : Hazard ratios

IDNT : Irbesartan Diabetic Nephropathy Trial

IFN- γ : Interferon gamma

IL-1 : Interleukin-1

iNOS : Inducible nitric oxide synthase

IQR : Interquartile range

IRFs : Interferon response factors

IRMA-2	: Irebesrtan in the Development of Diabetic Nephropathy in Patients with T2DM
IVIG	: Intravenous immunoglobulin G
JNC	: Joint National Committee
JNK	: C-Jun N-terminal kinase
kDa	: Kilodalton
LDL	: Low-density-lipoprotein
LMW	: Low-molecular weight
LPS	: Lipopolysaccharide
MAPKs	: Mitogen-activated protein kinases
Nf- κB	: K nuclear factor
NF-B	: Nuclear factor
PBS	: Phosphate-buffered saline
PD	: Parkinson's disease
PI3K	: Phosphatidylinositol 3-kinase
PMNs	: Polymorphonuclear leukocytes
PRA	: Plasma renin activity
PRC	: Plasma renin concentration
RAS	: Renin-angiotensin system
RASS	: Renin-Angiotensin System Study
RENAAL	: Reduction in End-Points in Non-Insulin Dependent Diabetes Mellitus With the Angiotensin II Antagonist Losartan
REs	: Regulatory elements
RMPs	: Renal mononuclear phagocytes
ROADMAP	: Randomized Olmesartan and Diabetes Microalbuminuria Prevention
ROS	: Reactive oxygen species
RRT	: Renal replacement therapy
SD	: Standard deviation
SER	: Smooth endoplasmic reticulum

SGLT2 : Sodium glucose cotransportor 2
STZ : Streptozotocin
T1/T2DM : Type 1 and type 2 DM
TGF-beta : Transforming growth factor-beta
TLR4 : Toll-like receptor 4
TNF-α : Tumor necrosis factor-α
UAE : Urinary albumin excretion
UKPDS : United Kingdom Prospective Diabetes Study
VADT : VA Diabetes Trial
VCAM-1 : Vascular cell adhesion molecule- 1

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Introduction

Micro albuminuria has been proven as a remarkably useful biomarker used for diagnosis of diabetic nephropathy (DN). Some other biomarkers (i.e., glomerular, tubular, inflammation markers, and biomarkers of oxidative stress) precede albuminuria. However, their usefulness is widely debated in the literature and has not yet led to the validation of a new "gold standard" biomarker for the early diagnosis of DN. Tubular biomarkers in DN seem to be of a paramount importance in the early diagnosis of DN since tubular lesions occur early.

Diabetic nephropathy is a chronic condition that develops over many years, and is typically characterized by a gradual increase in urinary albumin excretion (UAE), blood pressure and risk of cardiovascular disease (CVD), and decreasing glomerular filtration rate (GFR) leading to the development of end-stage renal disease (ESRD), and need for renal replacement therapy (RRT) ***(Tang et al., 2016).***

Diabetic nephropathy can occur in patients with either T1DM or T2DM, and all patients with diabetes mellitus should therefore be examined regularly for renal dysfunction. Although intensive management of diabetes mellitus through concurrent control of glucose, lipids and blood pressure, can slow the progression of diabetic nephropathy, diabetes mellitus

remains the most common cause of ESRD ***(Motlich et al., 2015).***

Good evidence suggests that early treatment delays or prevents the onset of diabetic nephropathy or diabetic kidney disease. This has consistently been shown in both type1 and type 2 diabetes mellitus.

Regular outpatient follow-up is key in managing diabetic nephropathy successfully ***(Andy, 2014).***

Patients in whom proteinuria did not develop have a low and stable relative mortality rate, whereas patients with proteinuria have a 40-fold higher relative mortality rate.

In additional to diabetic glomerular pathology, the lesion progresses in the tubulointerstitial compartment. Among them, tubulointerstitial injury is one of the most important factors in the pathophysiology and progression of diabetic nephropathy ***(Satirapoj et al., 2012).***

Moreover, it has been proposed that tubular injury could precede glomerular injury in diabetic nephropathy, which may explain the early appearance of an increase in several urinary biomarker excretions compared with albumin, which has been used as a diagnostic marker for glomerular injury in diabetic patients ***(White et al., 2013).***

Importantly, one third of patients with diabetic nephropathy lost renal function even during normal albuminuria or without any marker of glomerular injury ***(Tabaei et al., 2001).***

Therefore, there is an urgency to find reliable biomarkers for tubulointerstitial injury, which could be used to monitor the early progression of diabetic nephropathy and facilitate its diagnosis and treatment.

Heme oxygenases (HOs) are essential enzymes in heme catabolism, which catalyze the rate-limiting step in the conversion of heme to biliverdin, carbon monoxide and iron.

HOs act as an antioxidants and potent anti-inflammatory proteins during oxidative injury ***(Nath, 2006).***

They consist of three isoforms, among which HO-1 is induced by oxidative stress ***(Courtney et al., 2008).***

It has also been reported that HO-1 plays a pivotal role in maintaining renal function and protecting renal structure under conditions of oxidative stress such as proteinuria ***(Yang et al., 2003).***

Additionally, uHO-1 is considered a valuable biomarker for studying oxidative damage to assess whether the injury may

progress to chronic kidney disease. The in situ histopathologic expression patterns in the kidney suggest that HO-1 participates in the pathogenesis of renal disease in response to transforming growth factor-beta (TGF-beta), and increases of HO-1 expression protected animals from renal injury ***(Zarajou et al., 2012).***

Recent studies have demonstrated that HO-1 within the damaged cells is dropped off into the cavity of renal tubules in experimental animal models of chronic kidney disease (CKD) and patients with various kidney diseases ***(Yokoyama et al., 2011).***

However, no quantitative information exists on the relationship between uHO-1 and clinical staging of diabetic nephropathy. The aim of the present study is to study the possible role of heme Oxygenase-1 as a potential marker in early diabetic nephropathy.

Aim of the Work

The aim of the work is to study the possible role of heme Oxygenase-1 as a potential marker in early diabetic nephropathy.