Faculty of science
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Study of Urinary RNA Profiling as a Potential Biomarker in Type 2 Diabetic Nephropathy Patients

Thesis

Submitted for partial fulfillment of master degree in biochemistry

By

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Declaration

I declare that this **thesis** has been composed by myself and that this work, which has been recorded here in after has been done by myself. It has not been submitted for a **degree** at this or any other university.

Mona mostafa

Dedication

I would like to dedicate this thesis with all my love to my husband, my family and for all my friends and those from whom I have learned, whenever and wherever they are.

Mona mostafa

Biography

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Mona Mostafa

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Abstract

Study of Urinary RNA Profiling as a Potential Biomarker in Type 2 Diabetic Nephropathy Patients. *Mona Mostafa Abd El-Rahman Ahmed, Biochemistry Department, Faculty of Science, Ain Shams University.*

Diabetic nephropathy (DN) is the most common cause of end-stage kidney disease worldwide, and is associated with increased morbidity and mortality in patients with both type 1 and type 2 diabetes. Autophagy is a highly conserved 'self-eating' pathway by which cells degrade and recycle macromolecules and organelles. Impairment of autophagy is implicated in the pathogenesis of DN. Emerging body of evidence suggests that targeting the autophagic pathway to activate and restore autophagy activity may be renoprotective. Microtubule-associated protein 1 light-chain 3 (LC3) was employed in monitoring the cellular autophagy.

Bioinformatic analysis was done to retrieve promising autophagy biomarker relevant to diabetic nephropathy based on previous microarray studies. A total of 65 diabetic patients type II provided a single voided urine samples for quantifying urinary expression of *MAP1LC3B* RNA by real-time quantitative polymeras. Of the 65 cases 22 were normoalbuminuria, 24 were microalbuminuria whereas the

remaining 19 were macroalbuminuria. A group of 15 healthy volunteers was also enrolled in this study. Sera of all subjects were collected for detecting glucose level, glycosylated haemoglobin, serum creatinine, albumin/creatinine ratio and lipid profile.

The optimal threshold value for urinary *MAP1LC3B* expression level was calculated by receiver operator characteristic curve (ROC) as 0.866. The overall sensitivity and specificity of *MAP1LC3B* were 83.7% and 78.4% respectively. The positivity rate was significantly higher in macroalbuminuric group (89.5%) and microalbuminuric group (79.2%) than in normoalbuminuric group (36.4%) and normal healthy group (0%). There was significant negative correlation among the diabetic group regarding fold change of *MAP1LC3B* with systolic blood pressure, duration of diabetes mellitus and HbA1c and positive correlation with eGFR.

In conclusion the autophagy gene *MAP1LC3B* may have great clinical value as accurately promising biomarker in diabetic nephropathy assessment

Key words: Diabetic Nephropathy – Autophagy – Microtubule-Associated Protein 1 Light Chain3

List of Abbreviations

ACE : Angiotensin converting enzyme

ACR : Albumin-to-creatinine ratio

AER : Albumin excretion rate

AGEs : Advanced Glycation End products.

Ang II : Renal angiotensin II

AR : Aldose reductase

AUC : Area under the curve

BAMBI : Bone morphogenetic protein and activing Receptormembrane- bound inhibitor

BMI : Body Mass Index

CI : Confidence interval

CKD : Chronic kidney disease

CMA : Chaperone-mediated autophagy

Ct : Threshold cycle

DAG : Diacylglycerol

DBP : Diastolic blood pressure.

DEPC : Diethylpyrocarbonate

DKD : Diabetic kidney disease

DM : Diabetic mellitus

DN : Diabetic nephropathy

dUTP :Deoxyuridine triphosphate

dTTP :Deoxy thymidine triphosphate

ECM : Extracellular matrix

eGFR : Estimated Glomerular Filtration Rate.

eNOS : Endothelialnitric oxide synthase

ER : Endoplasmic reticulum

ESRD : End-stage renal disease

ET-1 : Endothelin-1

FGF21 : Fibroblast growth factor 21

FGF23 : Fibroblast growth factor 23

FN : False negatives

FOXO1 : Forkhead Box O1

FP : False positives.

GAPDH **:** Glyceraldehyde 3 phosphate dehydrogenase.

GBM **:** Glomerular basement membrane

GDM **:** Gestational diabetes mellitus

GFAT **:** Fructose-6-phosphate amidotransferase

GFR **:**The glomerular filtration rate

GBM **:** Glomerular basement membrane

GLUT2 **:** Glucose transporter 2

GSR **:** Glutathione Reductase.

HDL **:** High density lipoprotein.

IL-1β **:** Interleukin-1β

KIM-1 **:** Kidney injury marker-1

LAMP-2A : Lysosome-associated membrane protein-2A

LDL **:** Low density lipoprotein.

MAP1LC3B : Microtubule-associated protein 1 light chain 3 beta

MC **:** Mesangial cells

MiRNA **:** Micro RNA.

mTORC1: **:** Mammalian target of rapamycin complex

NADPH : Nicotinamide Adenine Dinucleotide Phosphate.

NAG : N-acetylglucosaminidase

NFK : The National Kidney Foundation

NGAL : Neutrophil gelatinase-assiociated lipocalin

NPV : Negative predictive value

PAI-1 : Plasminogen activator inhibitor-1

PBS : Phosphate buffer saline

PEDF : Pigment epithelium-derived factor

PKC : Protein Kinase C

PPV : Positive predictive value

pr./cr. : Protein/creatinine ratio

PRR : Pro Renin Receptor.

Qpcr : Quantitative real-time polymerase chain reaction

qRT-PCR : Quantitative Reverse-Transcription Polymerase Chain Reaction.

RAAS : Renin Angiotensin Aldosterone System

RAGE : Receptor for advanced glycation end product

RAGE : Receptor for Advanced Glycation End products.

RBS **:** Random blood Sugar,

RNA **:** Ribonucleic acid.

ROC curve : Receiver Operating Characteristics curve

ROS **:** reactive oxygen species

RPM **:** Round per minute.

RT-PCR **:** Real time -Polymerase Chain Reaction.

SBP **:** Systolic blood pressure

SD **:** Standerd deviation

SDS **:** Sodium dodecyl sulfate

SGLT2 **:** Sodium-glucose cotransporter 2

shRNA **:** Small hairpin ribonucleic acid

STAT1 **:** Signal transducer and activator of transcription 1

STZ **:** Streptozotocin

T1DM **:** Type 1 Diabetes Mellitus

T2DM **:**Type 2 diabetes mellitus

TGF ß1 **:** Transforming Growth Factor beta1

TGF-α1 **:** Transforming growth factor-α1

TIMP3 **:** Tissue inhibitor of metalloproteinase-3

TN **:** True negatives

TNF-α **:** Tumor necrosis factor-α

TP **:** True positives

UNG **:** Uracil -N- glycosylase

VLDL **:** very low density lipoprotein

π-GST **:** π-Glutathione-S-Transferase