ESTIMATION OF PLASMA MICRORNA-192 IN PATIENTS WITH DIABETIC NEPHROPATHY

Thesis

Submitted for the partial fulfillment of the requirement of M.D. degree of internal medicine

Presented by

Ghada Rabie Abdel Aziz Ahmed

Supervised by

Prof. Dr. Mohamed Gamal El-Din Saadi
Professor of Internal Medicine
Faculty of Medicine- Cairo University

Prof. Dr. Amr Abdel Hady Elmeligi
Professor of Internal Medicine
Faculty of Medicine- Cairo University

Prof. Dr. Mervat Saad El-Ansary
Professor of Clinical Pathology
Faculty of Medicine- Cairo University

Dr. Alkhatib Kadry Alkemary
Lecturer of Internal Medicine
Faculty of Medicine- Cairo University

Faculty of Medicine
Cairo University
2015

بسم الله الرحمن الرحيم

"سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم"

صدق الله العظيم

الآية (32) سورة البقرة

Acknowledgement

First of all, thanks to "**Allah**" who granted me the ability to accomplish this work.

I would like to express my deepest gratitude and highest appreciation to **Dr. Mohamed Gamal El-Din Saadi,** Professor of internal medicine for his continuous encouragement, generous support and unlimited help.

I am extremely grateful to **Dr. Amr Abdel Hady Elmeligi,** Professor of internal medicine, for his continuous guidance and suggestions, saving no effort or time to make this work better.

I am greatly honored to express my deepest appreciation to **Dr. Mervat Saad El-Ansary**, Professor of clinical pathology for her outstanding guidance and kind support throughout the work.

I express my deep sense of gratitude to **Dr. Alkhatib Kadry Alkemary,** lecturer of internal medicine who had to bear a heavy load of responsibility and concern in bringing this work to a successful end.

Ghada Rabie Abdelaziz

Dedications

I dedicate this work to my beloved family especially my parents whose affection, love, encouragement and prays of day and night make me able to get such success and honor, my husband and my Sweet daughters who provided me with strong love shield that always surrounded me and never lets any sadness enter inside.

Abstract

Diabetic nephropathy (DN) is the leading cause of kidney failure. This study was designed to evaluate the blood level of microRNA-192 and its relation to the disease severity in patients with type2 diabetes mellitus with and without evidence of diabetic nephropathy evidenced by presence of albuminuria expressed by urinary Albumin/Creatinine ratio. We found that microRNA-192 levels were significantly higher in patients with lower eGFR and higher Albumin/Creatinine ratios. Our findings may help to find a new marker for detection of diabetic nephropathy and this could be used in the future as a novel therapeutic approach for treatment of diabetic nephropathy.

Key Words:

1. **Type 2 diabetes mellitus (T2DM)**
2. **Diabetic nephropathy (DN)**
3. **Albumin/Creatinine ratio**
4. **microRNA-192**

List of Contents:

List Of Abbreviations

AACE	**American Association of Clinical Endocrinologists**
ACCORD	**Action to Control Cardiovascular Risk in Diabetes**
ACE	**Angiotensin Converting Enzyme**
ACR	**Albumin-to-Creatinine Ratio**
ADA	**The American Diabetes Association**
ADAR1	**Adenosine deaminase acting on RNA1**
AER	**Albumin excretion rate**
AGEs	**Advanced glycation end products**
Apo1A-1	**Apolipoprotein 1A-1**
ARB	**Angiotensin receptor blocker**
AUC	**Area under the curve**
BDNF	**Brain-derived neurotrophic factor**
BMI	**Body mass index**
BMPs	**Bone morphogenetic proteins**
BP	**Blood pressure**
BUN	**Blood urea nitrogen**
CBC	**Complete blood count**
CDC	**Centers for Disease Control and Prevention**
CI	**Confidence interval**
CKD	**Chronic kidney disease**
Col1a2	**Collagen 1a2**
COX-2	**Cyclooxygenase-2**
CRP	**C-reactive protein**
CSA	**Cross-sectional area**
CSII	**Continuous subcutaneous insulin infusion**

CT	The threshold cycle
Cut	Cut-off point
CVD	Cerebrovascular disease
DCCT	Diabetes Control and Complications Trial
DME	Diabetic macular oedema
DNA	Deoxyribonucleic acid
DPN	Diabetic polyneuropathy
DPP-4	Dipeptidyl peptidase IV
DR	Diabetic retinopathy
DSPN	Distal symmetric polyneuropathy
DN	Diabetic nephropathy
EASD	European Association for the Study of Diabetes
EC	Endothelial Cell
ECM	Extracellular matrix
eGFR	Estimated glomerular filtration rate
ER	Endoplasmic reticulum
ESRD	End-stage renal disease
FC	Fold change
FFA	Free fatty acid
FPG	Fasting plasma glucose
GAD	Glutamic acid decarboxylase
GDM	Gestational diabetes
GFR	Glomerular filtration rate
GIPR	Gastric inhibitory polypeptide receptor
GLP-1	Glucagon like peptide 1
GLUT-4	Glucose transporter 4
GWAS	Genome-wide association studies

HbA1c	**Glycohemoglobin**
HbF	**Fetal hemoglobin**
HDL	**High density lipoprotein**
HG	**High glucose**
HMGA1	**High mobility group A1**
HMGA2	**High Mobility Group AT-Hook 2**
HOMA-IR	**Homeostatic model assessment for insulin resistance**
HUVECs	**Human Umbilical Vein Endothelial Cells**
IA	**Islet autoantibodies**
ICA	**Islet cell antibodies**
ICAM-1	**Intercellular adhesion molecule 1**
IDNT	**Irbesartan Diabetic Nephropathy Trial**
IFG	**Impaired fasting glucose**
IGF-1	**Insulin like growth factor–1**
IGT	**Impaired glucose tolerance**
INSR	**Insulin receptor gene**
LADA	**Latent autoimmune diabetes of the adult**
LDL	**Low-density lipoprotein**
LVEF	**Left ventricular ejection fraction**
MC	**Mesangial Cells**
MCP-1	**Monocyte Chemoattractant Protein 1**
MCPIP1	**Monocyte Chemo attractant Protein-1-induced Protein 1**
MDRD	**Modification of Diet in Renal Disease**
MGB	**Minor groove binder**
MI	**Myocardial infarction**
micRNAs	**MicroRNAs**
MMVEC	**Myocardial microvascular endothelial cells**

MODY	**Maturity onset diabetes of the young**
NCV	**Nerve conduction velocity**
NFQ	**Nonfluorescent quencher**
NO	**Nitric oxide**
NPDR	**Nonproliferative diabetic retinopathy**
NPH	**Neutral Protamine Hagedorn**
NPV	**Negative predictive value**
OGTT	**Oral glucose tolerance test**
PAI-1	**Plasminogen activator inhibitor 1**
PCOS	**Polycystic ovary syndrome**
PCR	**Polymerase chain reaction**
PDGF	**Platelet-derived growth factor**
PDR	**Proliferative diabetic retinopathy**
PPV	**positive predictive value**
PTCs	**Proximal tubular cells**
QAFT	**Quantitative autonomic function tests**
QST	**Quantitative sensory tests**
QUICKI	**Quantitative insulin sensitivity check index**
RAGE	**Advanced glycation end products**
RECs	**Retinal endothelial cells**
RISC	**RNA-induced silencing complex**
ROC	**Receiver Operating Characteristics**
ROS	**Reactive oxygen species**
Rq	**Relative quantification**
RT	**Reverse Transcription**
RT-PCR	**Reverse Transcription – Polymerase Chain Reaction**
SGLT-2	**Sodium-Glucose Transporter-2**

siRNA	**Small (or short) interfering RNA**
SMBG	**Self-monitoring of blood glucose**
SNPs	**Single-nucleotide polymorphisms**
SRF	**Serum response factor**
STAT	**Signal transducer and activator of transcription**
STZ	**Streptozotocin**
T1DM	**Type 1 diabetes mellitus**
T2DM	**Type 2 diabetes mellitus**
TGF-β1	**Transforming growth factor-β1**
TZDs	**Thiazolidinediones**
UKPDS	**United Kingdom Prospective Diabetes Study**
VCAM-1	**Vascular cell adhesion molecule 1**
VEGF	**Vascular endothelial growth factor**
VLDL	**Very-low-density lipoprotein**
VSMC	**Vascular smooth muscle cells**
WHO	**World Health Organization**

List Of Tables

list of figures and Diagrams

INTRODUCTION

With 285 million individuals affected worldwide in 2010 and more than 400 million expected in 2030, Diabetes mellitus is a major public health concern and a huge economic burden **(Romano, 2010)**.

There remains a critical need to better understand the underlying disease mechanism responsible for diabetes complications in order to develop new and improved therapeutic strategies for these chronic conditions **(Kantharidis et al., 2011)**.

New insights have come from unlikely alley, the worm C.elegans, in which research has identified a novel family of endogenous, small (~ 22 nucleotides), single stranded, noncoding RNA molecules known as microRNAs (micRNAs) as developmental regulators **(Lee et al., 1993)**. These molecules, only identified in humans in the last decade, modulate physiological and pathological processes by the post transcriptional inhibition of gene expression **(Van Rooij, 2011)**.

Whether these micRNAs are involved in the damage that occurs in diabetes is yet to be established. The association between altered micRNA expression and the development and progression of the various diabetes complications implicates certain micRNAs in the development of diabetes-related injury in the heart, kidney, peripheral nerves, and retina **(Kantharidis et al., 2011)**.