

Role of Epithelial-to-Mesenchymal Cells Transition in Type 2 Diabetic Patients with Nephropathy

Thesis

Submitted For Partial Fulfillment of Master Degree
in **Pharmaceutical Sciences (Biochemistry)**

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2019

Acknowledgments

*First and foremost, I feel always indebted to **GOD** the
Most Beneficent and Merciful.*

*I wish to express my deepest thanks, gratitude and
appreciation to **Prof. Dr. Hala Osman El- Mesallamy**,
Professor of Biochemistry, Dean of faculty of pharmacy, Sinai
university, kantara branch, for her meticulous supervision,
kind guidance, valuable instructions and generous help.*

*Special thanks are due to **Prof. Dr. Mohamed
Hesham El-Hefnawy**, Dean of the National Institute of
Diabetes and Endocrinology, for his sincere efforts, fruitful
encouragement.*

*I am deeply thankful to **Dr. Al Aliaa Mohammed
Sallam**, Lecturer of Biochemistry, Faculty of Pharmacy-
Badr University, for her great help, outstanding support,
active participation and guidance.*

*I would like to express my hearty thanks to all my
family for their support till this work was completed.*

Nada Mostafa Mohamed Qamar El Dawla

List of Contents

Title	Page No.
List of Abbreviations	1
List of Tables	6
List of figures.....	6
Introduction and Aim of the Work.....	1
Literature Review.....	12
2.1 Diabetes mellitus	12
2.2 Complications of Diabetes Mellitus.....	7
2.3 Pathways triggered by hyperglycemia	20
2.4 Periostin.....	20
2.5 E-cadherin	32
Subjects and Methods	36
3.1 Subjects.....	36
3.2 Methods	40
Results	60
4.1 Clinical data and biochemical measurements	60
4.2 Serum periostin levels for studied groups.....	65
4.3 Serum E-cadherin levels in the studied groups.....	58
4.4 Spearman's correlation	59
4.5 Regression analysis	62
4.6 Receiver operating characteristic curve (ROC Curve).....	63
Discussion	67
Summary and Conclusion	76
Recommendations.....	81
References.....	82
Arabic Summary.....	1ص

List of Abbreviations

Abb.	Full term
ACEIs	Angiotensein converting enzyme inhibitors
Ag II	Angiotensin II
AGEs	Advanced glycation products
ANOVA	Analysis of variance
BM.....	Basement membrane
BMP-1.....	Bone morphogenetic protein-1
BRB	Blood retinal barrier
CAD	Coronary artery disease
CAN.....	Chronic allograft nephropathy
CCBs	Calcium channel blockers
CKD.....	Chronic kidney disease
CVD	Cardiovascular disease
DCCT	Diabetes control and complications trial
DM.....	Diabetes mellitus
DN	Diabetic nephropathy
DPN.....	Diabetic peripheral neuropathy
DR	Diabetic retinopathy
ECM	Extracellular matrix
EMT	Epithelial- mesenchymal transition
ESKD	End stage kidney disease
ESRD	End Stage Renal disease
FFA	Free fatty acid
FPG	Fasting plasma glucose
GFR.....	Glomerular filtration rate
GOD	glucose oxidase
HRP.....	Horseradish peroxidase
HTN	Hypertension

IL-1- β Interleukin1 beta

List of Abbreviations

Abb.	Full term
IDF.....	International Diabetes Federation
IL-6.....	Interleukin 6
LADA	Latent autoimmune diabetes in adults
LDL-C	Low-density lipoprotein cholesterol
LDL-C.....	Low-density lipoprotein cholesterol
Met	Metformin
MET	Mesenchymal-epithelial transition
MI.....	Myocardial infarction
MMPs	Matrix metalloproteinases
POD	peroxidase
RAAS.....	Renin angiotensin aldosterone system
RT.....	Room temperature
SMA- α	Alfa smooth muscle actin
Su	Sulfonylurea
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBM	Tubular basement membrane
TC.....	Total cholesterol
TG.....	Triglyceride
TGF- β	Transforming growth factor beta
TNF alpha.....	Tumor necrosis factor alpha
UACR	Urinary albumin to creatinine ratio
UAE.....	Urinary albumin excretion

List of Tables

Table No.	Title	Page No.
Table (1):	General characteristics between control group and the DN group.....	60
Table (2):	General characteristics of studied groups classified according to their UACR.....	61
Table (3):	Metabolic parameters between control group and the DN group.....	62
Table (4):	Kidney parameters for studied groups.....	64
Table (5):	Serum periostin between control group and the DN group.....	65
Table (6):	Serum E-cadherin between control group and the DN group	66
Table (7):	Multivariate regression between E-cadherin, periostin and some parameters	72

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Number of people with diabetes worldwide and per region years in 2017 and 2045.....	5
Figure (2):	Top ten countries with diabetes mellitus prevalence in world	6
Figure (3):	Major Complications of diabetes mellitus	7
Figure (4):	Pathological lesions of diabetic nephropathy.....	19
Figure (5):	Cellular modifications associated with Epithelial-mesenchymal transition program.....	23
Figure (6):	Three types of Epithelial-mesenchymal transition are recognized depending on the phenotype of the output cells	24
Figure (7):	Epithelial-mesenchymal transition and fibrosis.....	25
Figure (8):	Contribution of Epithelial-mesenchymal transition to cancer progression.....	19
Figure (9):	Properties of periostin protein.....	29
Figure (10):	Scaffold model for the cross-linking of type I collagen	31
Figure (11):	Periostin function in collagen cross-link formation.....	32
Figure (12):	Schematic representation of E-cadherin mediated cell-cell adhesion in epithelial cell.....	34
Figure (13):	Changes in the molecular markers during Epithelial-mesenchymal transition.....	27
Figure (14):	Dilution process for periostin.....	52
Figure (15):	Standard curve of periostin.	46
Figure (16):	Dilution process for E-cadherin.	48
Figure (17):	Standard curve of E-cadherin.....	49
Figure (18):	FPG levels in all studied groups.....	63
Figure (19):	HbA1c % levels in all studied groups	63
Figure (20):	UACR levels in all studied group	56
Figure (21):	Serum periostin levels for all the studied groups.....	66
Figure (22):	E-cadherin levels for all the studied groups.	67

List of Figures cont...

Fig. No.	Title	Page No.
Figure (23):	Correlation between periostin and other parameters	69
Figure (24):	Correlation between E-cadherin and other parameters.....	61
Figure (25):	Correlation between E-cadherin and periostin.....	71
Figure (26):	ROC curve regarding periostin.....	73
Figure (27):	ROC curve regarding E-cadherin.	75

Introduction and Aim of the Work

Diabetes mellitus (DM) has routinely been described as a metabolic disorder characterized by hyperglycemia that develops as a consequence of defects in insulin secretion, insulin action, or both. People with diabetes have an increased risk of developing a number of serious life-threatening health problems resulting in higher medical care costs and reduced quality of life (International Diabetes Federation) (**IDF, 2017**).

Diabetes is justly recognized as a developing global epidemic, representing one of the chief reasons of morbidity and mortality worldwide. It has the potential to cause serious complications due to its insidious and chronic nature (**Papatheodorou et al., 2015**).

Diabetic nephropathy (DN) is a major microvascular complication and a foremost cause of chronic kidney failure in individuals with both type1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) (**Tziomalos and Athyros, 2015**).

The risk is preciously elevated with poor blood glucose control and the greatest rate of progression occurs with elevated blood pressure, confirming that hemodynamic and metabolic factors participate in the development and progression of this disorder (**Rizvi and Rukban, 2016**).

The primary location of initial renal injury is the glomerulus, but increasing evidence points to the tubulointerstitium also playing a critical role via the process of epithelial-mesenchymal transition (EMT) (**Loeffler and Wolf, 2015**). During EMT, epithelial cells acquire features of mesenchymal

cells such as myofibroblasts, resulting in loss of E-cadherin expression, the acquisition of mesenchymal markers such as α -smooth muscle actin (SMA - α), and the increased deposition of extracellular matrix (ECM) (**Fragiadaki and Mason, 2011**).

Periostin is a 90 kDa secreted protein that is generated by many tissues (**Li et al., 2006**). It acts as an adhesion molecule during bone formation, supports osteoblastic cell line attachment (**Chijimatsu et al., 2015**). It may be associated with many diseases, such as tumor respiratory diseases and bone diseases (**Li et al., 2006**).

It is induced during nephrogenesis, but it is not observed in normal adult kidney (**Satirapoj et al., 2012**). Previous studies proved that periostin was prominently expressed in tubulointerstitial areas during renal injury and urinary periostin was a measure of the loss of renal tubular cells that had adopted a mesenchymal phenotype in response to diverse renal injuries (**Satirapoj et al., 2015**).

E-cadherin is a cell adhesion molecule that plays an important role in maintaining renal epithelial polarity and integrity (**Lee et al., 2013**). E-cadherin has two domains, the ectodomain and the cytoplasmic domain. The ectodomains of E-cadherin proteins of adjacent cells bind to each other in adherens junctions. The cytoplasmic domain of E-cadherin forms a complex with β -catenin and other proteins at the plasma membrane, which interacts with the cytoskeleton (**Fragiadaki and Mason, 2011**). E-cadherin mainly located in the tubular epithelial cells (**Hongjuan et al., 2009**). E-cadherin is abundant in the distal tubules (**Gaballa and Farag, 2013**) An early event happen during EMT involves

downregulation of expression of E-cadherin, that are induced by transforming growth factor beta (TGF- β) (*Frangiadaki and Mason, 2011*).

Now, early diagnosis of DN remains a main challenge. Therefore, more investigations to find new DN-related biomarkers are needed.

Therefore, the ultimate goals of our study are to:

1. Investigate the circulating levels of E-cadherin and periostin in T2DM with nephropathy.
2. Examine the possible relation between periostin and E-cadherin in order to address the role of EMT in the pathophysiology of DN.
3. Study the association between E-cadherin and periostin with the degree of renal impairment in DN patient.
4. Explore the possibility for using both E-cadherin and periostin as early biomarkers for DN.

Literature Review

2.1 Diabetes mellitus

Diabetes mellitus (DM) is one of the chronic noncommunicable diseases which have emerged as a leading global health problem. It is also a known risk factor for blindness, vascular brain diseases, renal failure, and limb amputations (*Papatheodorou et al., 2015*).

The disorder is rapidly increasing out of proportion in both developed and developing countries, especially T2DM, which is associated with modern lifestyle behaviors such as reduced physical activity, diet, obesity and genetic factors. If left unprocessed, DM can lead to a number of diseases and long-term complications leading subsequently to death (*Aynalem and Zeleke, 2018*).

The total number of estimated diabetics worldwide was about 382 million in 2013 (*Leon and Maddox, 2015*). In 2017, the number increased to reach about 425 million people (20–79 years of age) suffered from DM, and is expected to rise to 629 million by 2045 according to the International Diabetes Federation (IDF) Atlas guideline report as shown in figure (1) (*IDF, 2017*).

Diabetes is one of the most global health emergencies of the 21st century as it is the fifth leading cause of death worldwide, accounting for 5.2% of all deaths. The costs directly related to diabetes range from 2.5% to 15% of the annual health budget, depending on their prevalence and the sophistication of the treatment available which emphasizes that DM not only a financial problem but it also a threatening social burden (*Domingueti et al., 2016*).

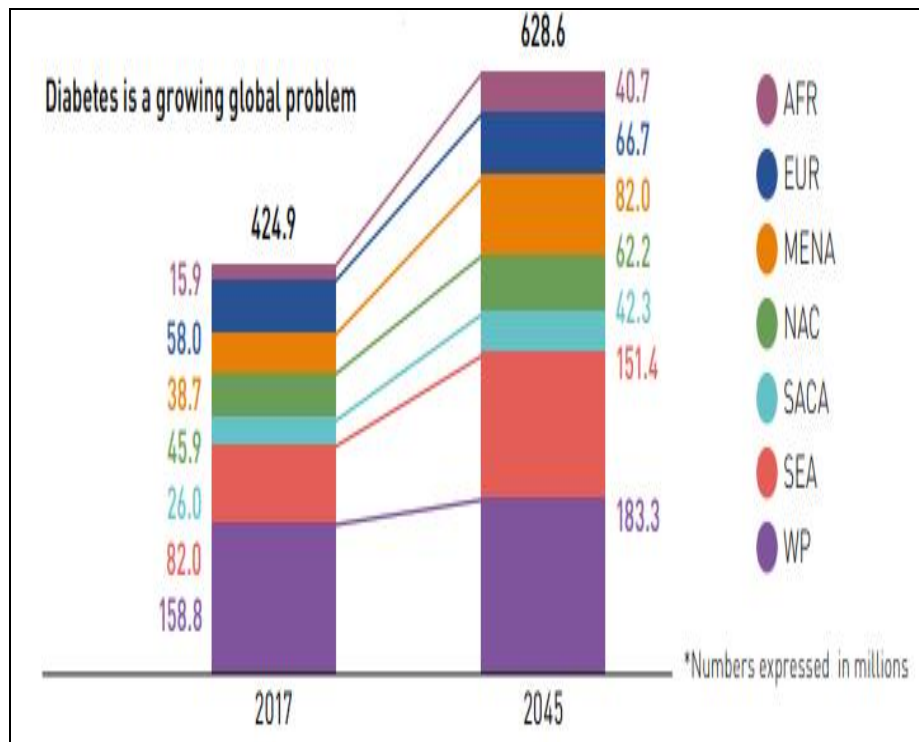


Figure (1): Number of people with diabetes worldwide and per region years in 2017 and 2045 (*IDF, 2017*).

The onset of DM often occurs years before the actual diagnosis. Globally, 45.8% (or 174.8 million cases) of all DM cases in adults were estimated to be undiagnosed (*Beagley et al., 2013*) and the number increased to reach 49.7% in 2017, counting over 224 million adults. The uppermost percentage was found in Africa region as it estimated to be 69.2% of all undiagnosed cases worldwide. (*IDF, 2017*). People with undiagnosed and untreated DM are at a greater risk of complications than those who are receiving treatment. Furthermore, medical costs for patients with DM is up to three times greater than for the general population without DM (*Zimmet, 2017*).

Interestingly, in 2017, Egypt was one of the top ten countries regarding both the number of undiagnosed diabetes coming in the 9th rank as it represented around 53.1% and the 8th rank in number of patients suffered from diabetes reaching about 8.2 million people in 2017 and the number is projected to reach 16.7 million by the year 2045 as shown in figure (2) (IDF, 2017).

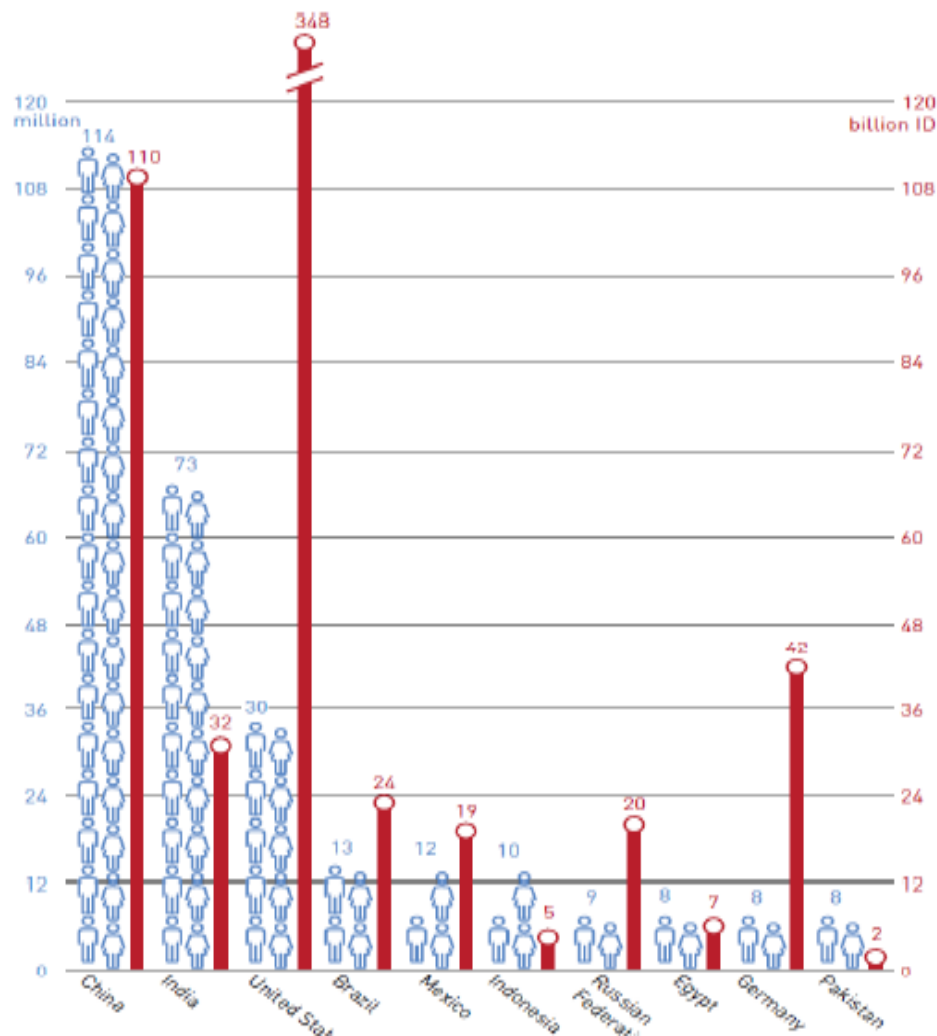


Figure (2): Top ten countries with diabetes mellitus prevalence in world (IDF, 2017).

2.2 Complications of Diabetes Mellitus

Complications of diabetes variety from acute, life-threatening conditions like ketoacidosis or severe hypoglycemia leads to coma to chronic, devastating complications affecting multiple organ systems (*Dutta and Nickerson, 2012*).

The chronic complications are long-term vascular complications, the resulting complications are grouped as microvascular disease and macrovascular disease. Microvascular complications involve retinopathy, nephropathy, and neuropathy. The macrovascular complications include accelerated cardiovascular disease resulting in myocardial infarction (MI) and cerebrovascular disease manifesting as strokes as shown in figure (3). Other chronic complications of diabetes include depression, dementia, and sexual dysfunction (*Fobries and Cooper, 2013*).

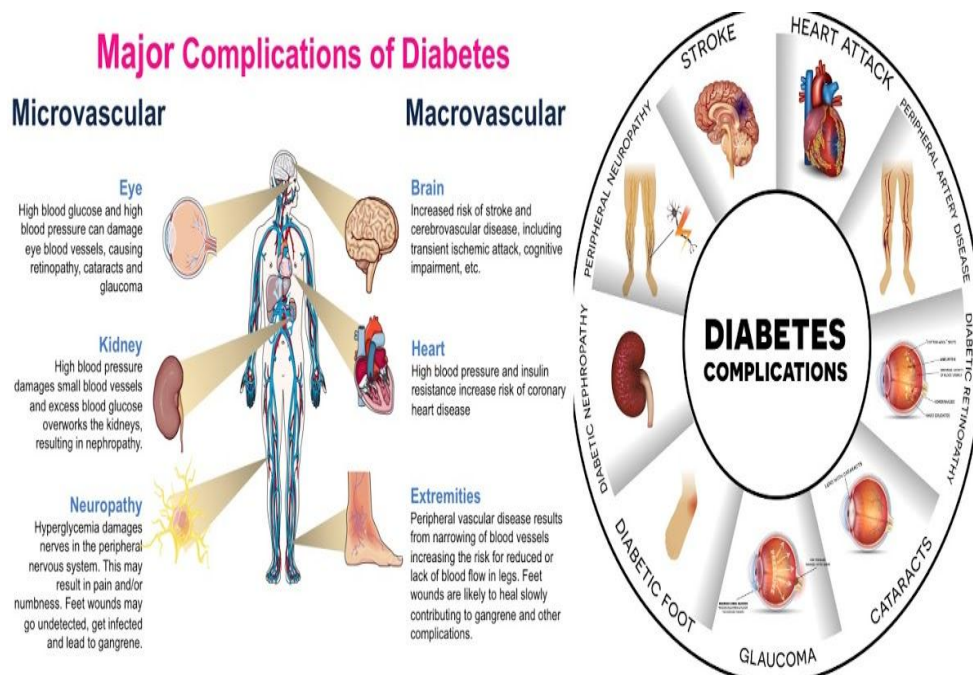


Figure (3): Major complications of diabetes mellitus (*Dayyal, 2018*).