SINGLE NUCLEOTIDE POLYMORPHISM OF IL-18 PROMOTER REGION IN PATIENTS WITH DIABETIC NEPHROPATHY

Thesis

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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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Dedication

To My family and my Husband for their great help, encouragement, love and continuous support.

Abstract

Background: Between 20% and 40% of patients with diabetes ultimately develop diabetic nephropathy (DN), which is the most common cause of end stage renal disease requiring dialysis. Interleukin-18 (IL-18) is a pro-inflammatory cytokine and possibly plays an important role in the pathogenesis of diabetic nephropathy. The promoter region of the human IL-18 gene has common single nucleotide polymorphisms at positions -607 and -137**.** These polymorphisms have been shown to regulate the IL-18 production of peripheral blood monocytes and be associated with the transcriptional activity of the IL-18 gene.

Aim: The purpose of this study is to detect IL-18 (-607C/A) polymorphism, and to find its association with IL-18 and its binding protein levels in patients with diabetic nephropathy.

Methods: For all subjects, the (-607C/A) gene polymorphism was detected by PCR/RFLP, while the IL-18 and IL-18BP levels were determined by ELISA.

Results: There was no statistical significant difference among the studied groups as regards the percentage distribution of the genotypes and allele frequency, where the CC genotype was the most frequent genotype in the DN group. Also, there was highly significant increase in IL-18 and IL-18BP levels in DN group compared to diabetic group without nephropathy and to control group.

Key words: Type 2 diabetes mellitus, Diabetic nephropathy, Interleukin-18, IL-18 binding protein, Single nucleotide polymorphisms.

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List of Abbreviations

2h-PPG	2 hours post prandial glucose
ACEI	Angiotensin converting enzyme inhibitor
ADA	American diabetes association
AER	Albumin excretion rate
AGEs	Advanced glycation end products
ALA	Alanine
Ang II	Angiotensin II
APCs	Antigen presenting cells
APOE	Apolipoprotein E
AR	Aldose reductase
BP	Blood pressure
BR	Bradykinin receptor
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary deoxyribonucleic acid
CTGF	Connective tissue growth factor
DC	Dendritic cells
DEPC	Diethylpyrocarbonate
DG	Deoxyglucosone
DM	Diabetes mellitus
DN	Diabetic nephropathy
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EB	Ethidium bromide
EDIC	The epidemiology of diabetes interventions and complications
EDTA	Ethylene diamine tetra acetic acid
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme linked immunosorbent assay

ESRD	End stage renal disease
FBG	Fasting blood glucose
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
GLUT-1	Glucose transporter-1
GM-CSF	Granulocyte / monocyte – colony stimulating factor
H4TF	Histone transcription factor
Hb	Hemoglobin
HbA1c	Glycated hemoglobin
HDLC	High density lipoprotein cholesterol
HL	Hepatic lipase
HMCs	Human mesangial cells
HWE	Hardy Weinberg equilibrium
ICAM-1	Intracellular adhesion molecule-1
ICE	IL-1β-converting enzyme
IFN-γ	Interferon-γ
IGF-1	Insulin like growth factor-1
IGIF	INF-γ inducing factor
IL-18BP	Interleukin-18 binding protein
IL-18R	IL-18 receptor
LDL	Low density lipoprotein
MAFs	Minor allele frequencies
MMPs	Metalloproteinases
MTHFR	Methylene tetrahydrofolate reductase
NO	Nitric oxide
NOD	Non obese diabetic
OPG	Osteoprotegerin
P38 MAPK	P38 mitogen activated protein kinase
PCR	Polymerase chain reaction

PDGF	Platelet derived growth factor
PKC	Protein kinase C
PPAR-γ	Peroxisome proliferators activated receptors –γ
RAGE	Receptors for advanced glycation end products
RAS	Renin-angiotensin system
RBF	Renal blood flow
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
SD	Standard deviation
SNPs	Single nucleotide polymorphisms
T2DM	Type 2 diabetes mellitus
TAFI	Thrombin-activatable fibrinolysis inhibitor
TFh	Follicular T-helper cells
TG	Plasma triglycerides
TGF- **β** 1	Transforming growth factor- **β** 1
Th cells	T-helper cells
TMB	Tetramethylbenzidine
TNF	Tumor necrosis factor
UAE	Urinary albumin excretion
UKPDS	United kingdom prospective diabetes study
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2
VLDLC	Very low density lipoprotein cholesterol

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INTRODUCTION

Diabetic nephropathy (DN) is the leading cause of kidney disease in patients starting renal replacement therapy. DN is defined by increased urinary albumin excretion (UAE) in the absence of other renal diseases. It is categorized into stages: microalbuminuria (UAE >20μg/min and ≤199μg/min) and macroalbuminuria (UAE ≥200μg/min) ***(Gross et al., 2005).***

Diabetic nephropathy occurs in ~30% of people with type 1 diabetes and 25-40% of people with type 2 diabetes. It increases the risk of death, mainly from cardiovascular causes ***(Philip et al., 2006).***

Several potentially modifiable risk factors, such as smoking, poor glycemic control, hypertension, hyperlipidemia, and urinary albumin excretion rate, and genetic factors predict the development of incipient and overt diabetic nephropathy in normoalbuminuric type 2 diabetic patients ***(Rossing et al., 2004).***

Diabetic nephropathy has several distinct phases or stages of development. Functional changes occur in the nephron at the level of the glomerulous including glomerular hyperfiltration and glomerular hyperperfusion. These two stages occur before the onset of any measurable clinical changes. Subsequently, thickening of the glomerular basement membrane, glomerular hypertrophy and mesangial expansion occur ***(Dronavalli et al., 2008).***

The exact cause of diabetic nephropathy is unknown but various mechanisms are considered such as altered renal hemodynamics,

hyperglycemia, advanced glycation end products and activation of cytokines ***(Remuzzi et al., 2002).***

Cytokines are small proteins or peptides that occur naturally in mammalian species and have multiple physiologic functions, including modulation of immune response ***(Bennatt, 1996).***

Interleukin-18 (IL-18) has potent immunomodulatory effects. It is the only cytokine with a unique capacity to induce T-helper cell (Th1) or (Th2) polarization, depending on the immunological condition ***(Cheuk-Chun et al., 2009).*** Serum levels of IL-18 are increased in many pathological conditions, including glucose intolerance and diabetic nephropathy ***(Mahmoud et al., 2004***).

In nephropathy, the activated macrophage infiltrates the glomerulus and produces IL-18 in the course of kidney injury. Therefore, the increase of IL-18 suggests another mechanism of glomerular injury by the infiltrated and activated macrophages in addition to the usual endothelial injury ***(Araki et al., 2007).***

In an IL-18 promoter transcription activity assay, ***Giedraitis et al., (2001)*** demonstrated low promoter activity for both the A and C alleles at positions -607 (C3A) and -137 (G3C) when present on the same haplotype. These results are suggestive of functionality, rendering these promoter single nucleotide polymorphisms (SNPs) attractive candidates in tests for genetic association with immune-mediated diseases. These promoter SNPs have also been implicated as susceptibility loci for various diseases, including type 1diabetes ***(Kretowski et al., 2003),*** rheumatoid arthritis ***(Sivalingam et al., 2003),*** sarcoidosis ***(Takada et al., 2002),*** atopic eczema ***(Novak al., 2005),*** adult-onset Still's disease ***(Sugiura, 2002)*** and seasonal allergic rhinitis ***(Kruse, 2003).***

AIM OF THE WORK

The aim of this study is to detect IL-18 (-607C/A) polymorphism, and to find its association with IL-18 and its binding protein levels in patients with diabetic nephropathy.

DIABETIC NEPHROPATHY

Introduction:

Between 20% and 40% of patients with diabetes ultimately develop diabetic nephropathy, which in the USA is the most common cause of end stage renal disease requiring dialysis. Diabetic nephropathy has several distinct phases of development and multiple mechanisms contribute to the development of the disease and its outcomes **(*Dronavalli et al., 2008).***

Microalbuminuria is the earliest sign of renal affection in diabetes mellitus (DM). Patients with microalbuminuria who progress to macroalbuminuria are at increased risk of progression to renal failure ***(American diabetes association, 2007).***

Therapeutic strategy in patients with microalbuminuric or macroalbuminuric type 2 diabetic nephropathy (DN) usually fails to restore renal function but merely slows the renal disease progression. In contrast, the restoration of renal function as well as renal perfusion can be accomplished in early stage of type 2 DN (normoalbuminuria) by correcting the hemodynamic maladjustment in renal microcirculation with vasodilators ***(Narisa, 2009).***

Definition & Epidemiology:

Definition:

Diabetic nephropathy has been classically defined by the presence of proteinuria >0.5g/24h. This stage has been referred to as overt nephropathy, clinical nephropathy, proteinuria, or macroalbuminuria. In