INTERLEUKIN-10 GENE POLYMORPHISMS IN TYPE-2 DIABETIC PATIENTS WITH NEPHROPATHY

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LIST OF ABBREVIATIONS

ACE	Angiotensin-converting-enzyme
ACR	Albumin creatinine ratio
ADA	American diabetes association
AER	Albumin excretion rate
AGE	Advanced glycation end product
ALT	Alanine transaminase
ANA	Against nuclear antigen
AP	Activator protein
APCs	Antigen presenting cells
ARBs	Angiotensin receptor blockers
AT	Angiotensin
bp	Base pair
Bp	Blood pressure
CHD	Coronary heart disease
CKD	Chronic kidney disease
C-MAF	C-musculoaponeurotic fibrosarcoma factor
CNDP1	Carnosine dipeptidase 1 gene
CREB	cAMP response element binding protein
CRF	Chronic renal failure
CRP	C-reactive protein
CSIF	Cytokine synthesis inhibitory factor
DCs	Dendritic cells
DM	Diabetes mellitus
DN	Diabetic nephropathy
EB	Ethidium Bromide
EBP	Enhancer binding protein
ECM	Extracellular matrix

EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ELMO1	Engulfment and cell motility 1 gene
ESRD	End-stage renal disease
FBG	Fasting blood glucose
GFR	Glomerular filtration rate
GSH	Reduced glutathione
GVHD	Graft-versus-host disease
HbA_{1c}	Glycosylated hemoglobin
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HMCN -1	Hemicentin-1 gene
HRP	Horse raddish peroxidase
ICOS	Inducible T cell co-stimulator
IgG	Immunoglobulin G
IL	Interleukin
IRF	Interferon regulatory factor
Jak	Janus kinase
MAPK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
NF	Nuclear factor
NK	Natural killer
NKF	National kidney foundation
PARP-1	Poly [ADP-ribose] polymerase 1
PBMCs	Peripheral blood monocyte cells
PCR	Polymerase chain reaction
PKC	Protein kinase C
RA	Rheumatoid arthritis

RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
ROS	Reactive oxygen species
Rsa1	Rhodopseudomonas sphaeroides
SH2	Sarcoma homology 2
SHP	Src(sarcoma) homology 2-domain containing tyrosine phosphatase
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SP	Specificity protein
STAT	Signal transducer and activator of transcription
T2DM	Type 2 diabetes mellitus
TAE	Tris-Acetate EDTA buffer
Taq	Thermus aquaticus
TFh	T-helper follicular
TGF-β	Transforming growth factor -beta
Th	T-helper
TMB	Tetramethyl benzidine
TNF	Tumor necrosis factor
TYK	Tyrosine kinase
UAE	Urinary albumin excretion
VEGF	Vascular endothelial growth factor

Abstract

Background: Diabetic nephropathy is the most prevalent cause for end-stage renal disease in the United States and the Western world.
In this disease, tubular injury and progressive interstitial fibrosis contribute significantly to renal failure and predict progression to end-stage renal disease. In early diabetic renal injury, there is podocyte drop-out which is thought to cause glomerular proteinuria and subsequent diabetic glomerular injury.
Different inflammatory molecules including pro-inflammatory cytokines have been proposed as critical factors in the development of microvascular diabetic complications including nephropathy.

Interleukin 10 (IL-10) polymorphic variants are linked with cytokine production and are involved in many chronic inflammatory diseases, including type 2 diabetes mellitus .

Aim of work: Analysis of IL-10 gene polymorphisms and study their possible association with IL-10 gene expression involved in the progression of diabetic nephropathy in type 2 diabetes mellitus .
Methods: For all subjects, IL-10 plasma levels (by ELISA), IL-10 – (-592) gene polymorphism (by PCR) were investigated.

Results: we found that the highest levels of IL-10 were in diabetic patients with nephropathy followed by diabetic patients without nephropathy with the lowest levels in normal healthy subjects. Also we found that there is an increase in the frequency of IL-10-(-592) AA genotype in diabetic nephropathy patients with a significant increase in A allele distribution in them compared to diabetic patients without nephropathy and healthy control subjects.

Conclusion: IL-10 and its gene polymorphism play an important role in the progression of diabetic nephropathy.

Keywords: Type 2 diabetes, diabetic nephropathy, interleukin-10, polymorphism.

Introduction

The prevalence of diabetes mellitus and its clinical complications are increasing rapidly worldwide. Diabetic nephropathy is a major cause of sickness and death in persons with diabetes; it is the leading cause of renal failure ***(American diabetic association, 2009)***.

Type 2 diabetes mellitus (T2DM) is recognized as one of the most common causes of end-stage renal disease ***(Freedman et al., 1998)***. However, factors initiating progressive renal failure in T2DM patients remain largely unknown. In T2DM patients, a certain cytokine genotype is associated with an increased susceptibility to diabetic nephropathy (DN) in different countries ***(Ezzidi et al., 2009)***. Research has shown that ethnicity greatly influences the distribution of genotypes and allele frequencies of cytokine polymorphisms ***(Wan-Ju et al., 2010).***

In Egypt, the prevalence of diabetic nephropathy as a cause of end-stage renal disease (ESRD) increased from 8.9% of patients in 1996 to 14.5% in 2001. The mean age of diabetic nephropathy patients was higher than that of patients with ESRD due to other causes. In addition, mortality among diabetic patients with ESRD in Egypt is higher than mortality for all other causes of ESRD which is probably related to the well known cardiovascular complications of diabetes ***(Afifi et al., 2004).***

Hyperglycemia and glomerular hypertension are the two main initiating and progression factors thus far identified in the development of nephropathy. Hyperglycemia probably acts via direct glucose toxicity, glycation and increased flux through the polyol and hexosamine pathways. It has been suggested that hyperglycemia-induced overproduction of superoxide by the mitochondrial electron transport chain may be the

fundamental abnormality stimulating these individual pathways ***(Brownlee, 2001).***

DN is the leading cause of ESRD. The pathogenesis of DN is multifactorial and is aggravated by hyperglycaemia ***(Gross et al., 2005)***. Previous studies indicated genetic predisposition to DN, evidenced by the clustering of high urine albumin:creatinine ratio (ACR) cases among diabetic families ***(Agius et al., 2006)***. While the exact mechanisms involved in the development and progression of DN remain to be seen, recent studies suggested the contribution of a chronic inflammatory state, related in part to diabetic complications, to T2DM pathogenesis ***(Pickup, 2004)***. It was suggested that local inflammatory events, oxidative stress, and excretion of proinflammatory cytokines mediated, at least in part, the metabolic and haemodynamic abnormalities linked with diabetic nephropathy.

DN is viewed as a state of low-grade chronic inflammation and role for interleukin 10 (IL-10) in its pathogenesis was proposed ***(Mora and Navarro, 2005)***. This was evidenced by the elevation in IL-10 levels in the sera of T2DM ***(Wong et al., 2007)*** and type 1 diabetes ***(Mysliwska et al., 2005)***, patients with nephropathy, and as IL-10 levels correlated with the extent of renal damage in DN ***(Wong et al., 2007)***. This suggests that IL-10 promoter gene variants of chromosome 1q31-1q32 influence DN development and progression by regulating IL-10 production ***(Mysliwska et al., 2005)***.

Currently, reports on the association of cytokine genotypes with cytokine phenotypic expression patterns of DN patients are still very limited.

Aim of the work

Analyses of IL-10 gene polymorphisms and their possible association with IL-10 gene expression to understand the role of genetics on pathogenesis and clinical expression of diabetic nephropathy in type 2 diabetes mellitus.

Diabetes Mellitus

Definition & Description:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both ***(Wold, Ceylan-Isik and Ren, 2005)***. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart, and blood vessels ***(American Diabetes Association, 2009).***

The International Diabetes Federation estimates that the prevalence of diabetes is higher in developed than in developing countries, but the major increase in people with diabetes will occur in developing countries. Type 2 DM is most common among the elderly people in the developed countries, while most people with diabetes in developing countries are middle-aged (45-64 years) ***(World Health Organization, 2009)***. Furthermore, a sedentary lifestyle and a high-carbohydrate diet are important risk factors ***(International Diabetes Federation, 2009).***

Chronic Complications of Diabetes Mellitus:

Chronic complications of diabetes mellitus are broadly divided into two groups: namely macrovascular and microvascular complications ***(Tooke, 2000).***

A- Macrovascular Complications of Diabetes Mellitus:

Cardiovascular disease is the most important cause of mortality and morbidity among patients with type II diabetes ***(Mitsuhashi et al., 2002).*** Several of the known cardiovascular risk factors are