Chemokine Receptor 5 (CCR5) gene Polymorphism in patients with diabetic Nephropathy in Type II Diabetes

Thesis

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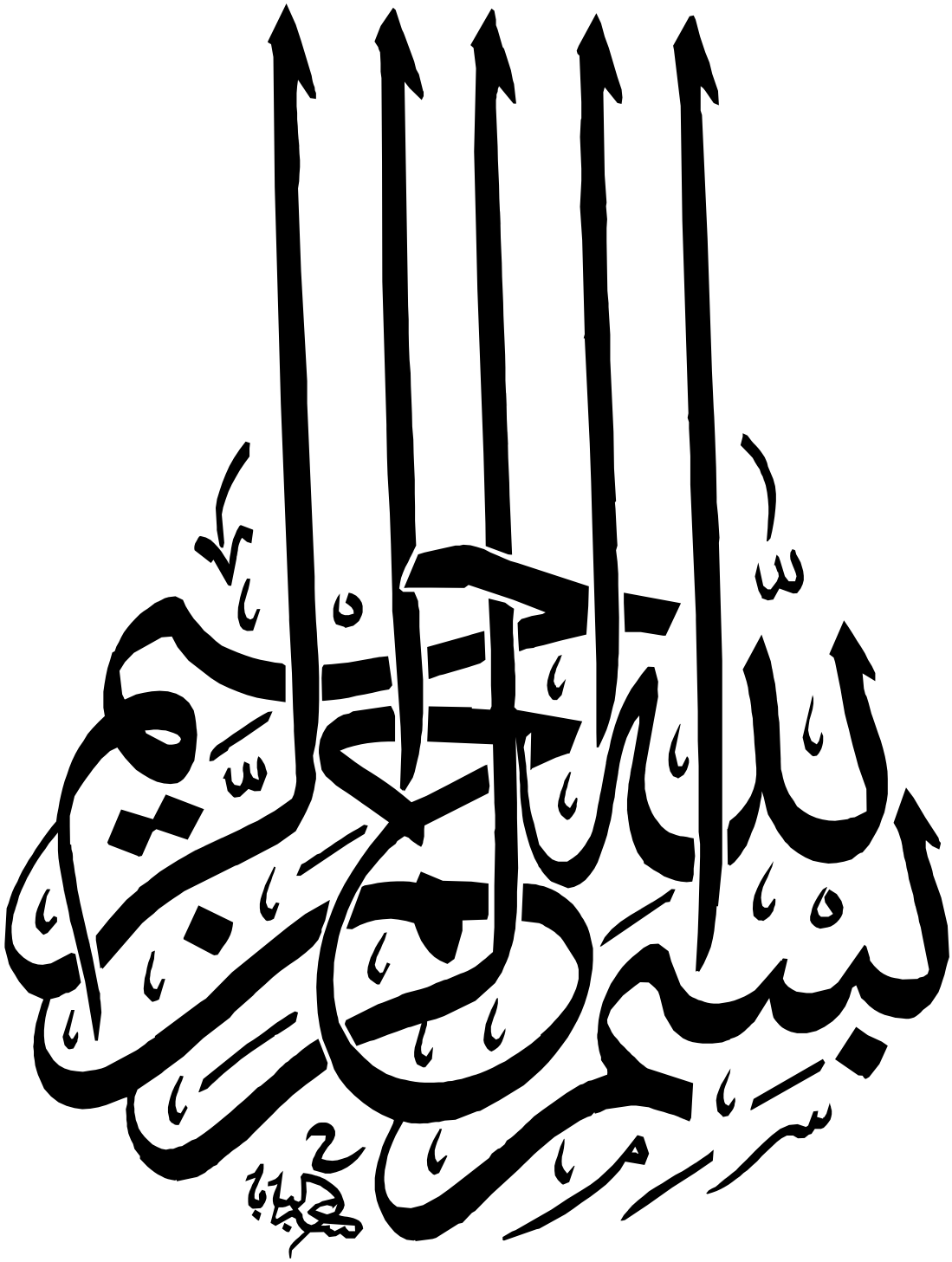
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Abstract

Diabetic nephropathy is a serious complication in individuals with type II diabetes. Chemokines & their receptors have been implicated in the development of diabetic nephropathy. The aim of this work is to investigate the possible association of chemokine receptor CCR5 G59029 A gene polymorphism with diabetic nephropathy in patients with type II diabetes in order to find out the pathogenesis, risk of development & progression of nephropathy in type II diabetic patients. This study was conducted on 55 patients with type II DM including 15 patients with no nephropathy. (Control group) & 40 patients with nephropathy (Microalbuminuria group, Macroalbumiuria group & ESRD group). CCR5 59029 genotyping was performed using PCR followed by digestion with Bsp1286I restriction enzyme. *Conclusion:* There was no significant association of CCR5 G59029A gene polymorphism with diabetic nephropathy.

Keywords: chemokine receptor 5 (CCR5), Type II diabetes, Diabetic nephropathy, gene polymorphism, nephropathy groups (Microalbuminuria group, macroalbuminuria group, ESRD group).

List Of Abbreviations

A	Adenine
ADA	American Diabetes Association
ACE	Angiotension-converting enzyme
AGEs	Advanced glycation end products
ARB	Angiotension receptor blocker
ANOVA	Analysis of variance
AR	Aldose reductase
ATP	Adenosine triphosphate
Bp	Base pair
C	Cytosines
DAG	Diacylglycerol
DCCT	Diabetes Control and Complications Trial
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DTPA	Diethylenetriaminepentaacetic acid
ESRD	End stage renal disease
G	Guanine
GDP	Guanosine di-phosphate
GTP	Guanosine tri-phosphate
GDM	Gestational diabetes mellitus
GLUT	Glucose Transporter
GFR	Glomerular filtration rate
HbA_{1c}	Hemoglobin A_{1c}
HDL	High-density lipoproteins
HMG-CoA	Hydroxymethylglutaryl coenzyme A
IL-1β	Interleukin-1 beta
KD	Kilo Dalton
LDL	Low-density lipoproteins
MAPK	Mitogen-activated protein Kinase
$MgCl_2.6H_2O$	Magnesium Chloride Hexahydrate
MCP	Monocyte Chemoattractant protein
MDRD	Modified Diet in Renal Disease

mRNA	Messenger RNA
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NaCl	Sodium chloride
NaOH..	Sodium hydroxide
NHANES	National Health & Nutrition Examination Survey
NOS	Nitric oxide synthetase
P value	Probability value
PCR	Polymerase chain reaction
PKC	Protein kinase C
R	Regression coefficient
RAGE	Receptor for Advanced Glycation End Products
RANTES	Regulated upon Activation Normal T-Cell Expressed & Secreted.
RIA	Radioimmunoassay
RID	Radialimmundiffusion
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
SDS	Sodium Dodecyl Sulfate
SOD	Super oxide dismutase
T	Thymine
TBE	Tris-borate EDTA
TGF	Transforming growth factor
TNF-α	Tumor necrosis factor-alpha
Tris-Hcl	Tris-Hydrochloric Acid
UAE	Urinary albumin excretion
UDP	Uridine diphosphate.
U	Uracil
UAE	Urinary albumin excretion
USA	United States of America
UV	Ultraviolet.
UKPDS	United Kingdom Prospective Diabetes Study
VEGF	Vascular endothelial growth factor
VLDL	Very low density lipoproteins

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Introduction

Diabetic nephropathy, or diabetic kidney disease, affects 20 to 30 percent of patients with diabetes. It is a common cause of kidney failure. Diabetic nephropathy presents in its earliest stage with low levels of albumin (microalbuminuria) in the urine. The most practical method of screening for microalbuminuria is to assess the albumin-to-creatinine ratio with a spot urine test ***(Micah, 2005)***.

Because of the large prevalence of diabetes in the general population, diabetes has become the leading cause of end-stage renal disease in the United States.& world wide. There is good evidence that early treatment delays or prevents the onset of diabetic nephropathy, or diabetic kidney disease ***(United States Renal Data Systems, 2002).***

The hyperglycemic state causes increase in the production of proinflamatory cytokines such as TNFα & IL1β. These cytokines in turn, stimulate the expression of monocyte chemoattractant RANTES (Regulated upon Activation Normal T-cell Expressed & Secreted) by mesangial cell in renal tissue ***(Schlodorff et al ., 1997).***

The major receptor for RANTES is CCR5 .So CCR5 & CCR5-mediated recruitment of monocytes & differentiation of these cells into macrophages in the glomeruli may play a key role in the etiology of diabetic nephropathy. Single nucleotide polymorphism, G-to-A substitution at nucleotide position 59029 in the promoter region of CCR5 has been identified ***(McDermott et al., 1998).***

Aim of the Work

To find out any association between CCR5 G59029 A gene polymorphism and nephropathy in patients with type 2 diabetes.

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 endstage 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 DM. Patients with microalbuminuria who progress to macroalbuminuria are at increased risk of progression to renal failure ***(ADA, 2007).***

Definition & Epidemiology:

Definition:

Diabetic nephropathy is typically defined by either macroalbuminuria that is, a urinary albumin excretion of greater than 300 mg in a 24-hour urine collection or by abnormal renal function as represented by an increase in serum creatinine, decrease in calculated creatinine clearance, or glomerular filtration rate (GFR). The common progression from microalbuminuria to overt nephropathy has led many to consider microalbuminuria to define early or incipient nephropathy. Renal disease is suspected to be secondary to diabetes in the clinical setting of long-standing diabetes. This is supported

by the history of diabetic retinopathy, particularly in type I diabetics, where there is a strong correlation. Clinically, diabetic nephropathy also is usually associated with hypertension, and a high risk of cardiovascular morbidity and mortality ***(Vidit et. al., 2005).***

Prevalence of diabetic nephropathy:

Diabetic nephropathy is more prevalent among African Americans Asians and Native Americans than Caucasians ***(Bethesda et. al., 2003).***

The incidence of diabetic nephropathy doubled from the years 1991-2001 ***(Craig et al 2003).***

Fortunately, the rate of increase of diabetic nephropathy has slowed down .This is due to several measures that contribute to the early diagnosis and prevention of diabetic nephropathy, which thereby decreases the progression of established renal disease. However, the implementation of these measures is far below the desirable goals***.*** In Egypt, a six-year study revealed that the prevalence of diabetic nephropathy among ERDS patients increased from 8.9% in 1996 to 14.5% in 2001. This increase in prevalence is caused by an actual increase in occurrence of diabetes mellitus, increasing age of the dialysis population & better survival rates for patients with diabetes, allowing more time for diabetic nephropathy to develop ***(Ossman et al., 2006).***

Mortality is probably related to the well known cardiovascular complications of diabetes **(*Afifi et. al., 2004).***

Stages, Clinical Features, and Clinical Course:

Stages:

Diabetic nephropathy has several distinct phases or stages of development. Functional changes occur in the nephron at the level of glomerulous including:

- Glomerular hyperfiltration
- Glomerular hyperperfusion

These 2 stages occur before onset of any measurable clinical changes. Subsequently,

- Thickening of the glomerular basement membrane
- Glomerular hypertrophy
- Messangial expansion ***(Dronavalli et al., 2008)***

Table (1) shows the Cutoff values of albuminuria, as well as clinical features of micro & macroalbumiuria groups in patients with diabetic nephropathy**.**

Table (1): Cutoff values of albuminuria & Clinical characteristics (Quoted from Gross et al., 2005)

Stages	Albuminuria cutoff values	Clinical characteristics
Microalbuminuria	20–199 µg/min Or	Abnormal nocturnal decrease of blood pressure and increased blood pressure levels
	30–299 mg/24 h Or	Increased triglycerides, total and LDL cholesterol, and saturated fatty acids
	30–299 mg/g creatinine	Increased frequency of metabolic syndrome components
		Endothelial dysfunction
		Association with diabetic retinopathy, amputation, and cardiovascular disease
		Increased cardiovascular mortality
		Stable GFR
Macroalbuminuria	≥200 µg/min Or	Hypertension
	≥300 mg/24 h Or	Increased triglycerides and total and LDL cholesterol
	>300 mg/g creatinine	Asymptomatic myocardial ischemia
		Progressive GFR decline