ASSOCIATION OF AGT (M268T) GENE POLYMORPHISM IN DIABETES AND NEPHROPATHY IN PAKISTAN

[1]SYED M. SHAHID, [2]ROZEENA SHAIKH, [3]SYEDA N. NAWAB, [3]ABID AZHAR

[1]The Karachi Institute of Biotechnology & Genetic Engineering (KIBGE), University of Karachi, Karachi, Pakistan
[2]Department of Biotechnology, Faculty of Life Sciences, BIUTEMS, Quetta, Pakistan
E-mail: [1]smshahid@uok.edu.pk

Abstract- Diabetes mellitus is considered as a group of metabolic disorders which have multiple etiologies. Pathogenesis of nephropathy in diabetes is presented by variations in genes encoding the significant components of renin angiotensin aldosterone system (RAAS) including angiotensin converting enzyme (ACE), angiotensin receptor, angiotensinogen (AGT) genes. The present study was conducted to explore the possible association of AG (M268T) polymorphism in diabetic patients with nephropathy in Pakistan. Study subjects included 100 controls, 260 diabetic patients without renal insufficiency and 190 diabetic nephropathy patients with persistent albuminuria. Fasting blood samples were collected from all the subjects after getting institutional ethical approval and informed consent. The biochemical estimations, PCR amplification and direct sequencing for the specific region of AGT gene was carried out. A significantly high frequency of genotype (TT) and allele (T) of AGT (M268T) was observed in diabetic nephropathy as compared to normal control subjects and diabetic patients without any known renal impairment. The genotype (TT) and allele (T) of AGT (M268T) polymorphism may be measured as a risk factor for the expansion and progression of renal impairment in diabetes. Further cross sectional population studies would be of help to establish and confirm the observed possible association of AGT gene variations with development of nephropathy in diabetes.

Keywords- RAAS, AGT (M268T), Diabetes, Nephropathy.

I. INTRODUCTION

Diabetes mellitus (DM) is considered as a group of metabolic disorders which have multiple etiologies. Chronic hyperglycemia is the characteristic of DM and is related with impaired carbohydrate, protein and fat metabolism. Pakistan is among the top ten countries of DM prevalence list. It ranks 7th and about 7.1 million people are affected by DM. It is predicted that, by the year 2030 around 13.8 million Pakistani populations will be diabetic and Pakistan will move to 4th position from 7th. According to IDF update for the year 2012, Pakistan is not among the top ten countries of DM prevalence list in 2011, but it is estimated that it will move to the 10th position among the top 10 countries and about 11.4 million people will be affected by DM in the year 2030 (IDF 2015).

DM is associated with higher risk for a various serious and sometimes life-threatening macro-and microvascular complications (Ragucci et al, 2003). Significant morbidity and mortality is caused by the micro and macrovascular pathological lesions, which involve several tissues and organs. There are various factors which are involved in the onset and progression of chronic complications of DM, These factors include glycemic control (Shahid et al, 2012), increased age (Islam et al, 2009), longer duration of diabetes, (Hashim et al, 1999), less physical activity (Christie et al, 2007), hypertension (Vijan et al, 2003), history of smoking (Sairenchi et al, 2004) and obesity (Hussain et al, 2007). Vascular complications in type 2 DM are highly prevalent in Indian studies. Pakistani DM patients are also showing high figure of complications. Increased prevalence of DM in the country stresses the need of adoption of strict measures of prevention of DM complications (Shera et al, 2004).

The etiology of DM is a complex interaction of genetics, environmental factors and lifestyle choices (Pickup & William, 2003). DM is highly prevalent among Pakistanis and it is ranging from 7.6 to 11%. Genetics is an important factor for the predisposition of South Asians to DM (Mathias et al, 2009; Shen et al, 2006). Genetic variants and type 2 DM associations have been observed in immigrant Pakistani population (Rees et al, 2009). Nephropathy in diabetes has been the main cause of chronic renal insufficiencies. Cardiovascular mortality becomes increased in DN patients (Bruno et al, 2000; Valmadrid et al, 2000). Approximately 40% of type 1 DM as well as and type 2 DM are affected by DN. The causes of ESRD are 50% DM, 27% hypertension, 13% glomerulonephritis and 10% other causes. The low glomerular filtration rate (GFR) without micro or macroalbuminuria is observed in 10% of DM, and also in type 1 DM and microalbuminuria (Perkins et al, 2009). A 24-hours urine collection or spot urine measurement of microalbumin may screen microalbuminuria or DN (ADA, 2007).

Pathogenesis of DN depends on genetic factors as well and genetically susceptible individuals can develop DN after environmental interaction DN is a complex, polygenic disease. For the identification of associated genes for the development of DN two main strategies have been used which include: analysis of candidate genes and more recently genome- wide scan (Carpena et al, 2010). Genetic polymorphisms also play an important role in

progression of DM and its complication. The angiotensin (AGT) gene, situated on chromosome 1 at 1q42-43, included five exons (Gillard et al, 1989). It is established that about 23 different variants of this gene has associations with high blood pressures and cardiovascular disorders in the form of AGT M268T (rs699) gene polymorphisms (Say, 2005). A number of studies identified the possible association between diabetes and cardiovascular events (Rieder et al, 1999; Pilbrow et al, 2007). This association also causes disequilibrium with T207M and promoter region A-6 G polymorphisms in AGT gene.

The present study was conducted by keeping in mind the significance of RAAS as well as the role of genetic variations in AGT in various metabolic and systemic mechanisms. This study thus evaluates the possible involvement of genetic variants of in AGT gene in development of nephropathy in diabetes in Pakistani patients.

II. DETAILS EXPERIMENTAL

The fasting blood samples of patients and controls following a 12-hours fasting were collected after the approval of Institutional ethical board. Total 5mL of blood was obtained in ACD vacutainers containing Acid Citrate Dextrose for extraction of deoxyribo nucleic Acid (DNA). All the participants were divided in the following groups:

Group-1: 100 healthy individuals as control,
Group-2: 260 DM patients
Group-3: 190 diabetics with persistent renal insufficiency.

Biochemical and clinical topographies of all the participants were observed which included Age, BMI, systolic and diastolic blood pressures and duration of diabetes. Fasting blood glucose, HDL-Cholesterol, LDL-Cholesterol, triglycerides, total cholesterol, creatinine and urea were evaluated by enzymatic colorimetric methods. The GFR was estimated using mathematical prediction equation.

Genomic DNA was extraction by whole blood using phenol chloroform methods. The M68T polymorphism of AGT gene was detected by using ARMS-PCR in a multiblock system. The specific primers (in pairs) were constructed using online software Primer-3 to amplify the regions of interest. A total volume of 20 µL was used to carry out the PCR reaction in standard Buffer which contained 50mM $MgCl_2$, 2mM of dNTPs, 20µM primers (each), 1.25 units Taq Polymerase and 250ng DNA. Agarose gel electrophoresis (2%) was used to visualize the amplified PCR products.

III. RESULTS AND DISCUSSION

The biochemical and clinical parameters observed during the study revealed that the duration of diabetes was raised in nephropathy patients to diabetics without renal impairment. Similarly the FBS levels also observed to be raised in diabetics and diabetic nephropathy patients in comparison to control where as HbA1c levels were comparatively high ($p<0.05$) in diabetic nephropathy patients as compared to control and diabetic patients without nephropathy. Lipid profile estimations showed triglyceride levels in diabetic patients without nephropathy.

The significance of AGT (M268T) gene variants has been recently established in many studies in various population studies which turned out to be inconsistent (Borah et al, 2012). A significant and strong association of TT genotype with patients with hypertension and vascular problems have been identified in many studies including conducted in Brazile (Pereira et al, 2003), Romania (Procopciuc et al, 2005), Spain (Glotov et al, 2007), Russia (Avogaro et al, 2007), Turky (Agachan et al, 2003), Mongolia, as well as in Taiwan (Xu et al, 2004). On the other hand, no and/or significantly negative association of similar genetic variant of AGT gene has also been detected with hypertensive patients in Dutch (Schmidt et al, 1993) and Brazilian population (Freitas et al, 2007). In this context, the given study observed a significant difference in allele and genotypic frequencies regarding M268T in diabetes with and without persistent albuminuria in comparison to normal healthy individuals. The genotype (TT) and allele (T) were found to be raised in diabetic patients with nephropathy as compared to normal controls and diabetic patients without nephropathy.

CONCLUSIONS

The presented work may conclude a possible association of genetic variations of polymorphism in (M268T) AGT gene. In the given study population, the allele (T) of AGT variant are thought to be linked with the progression of renal impairment in diabetes. However, the given association of AGT variants were analyzed in a cross section of the population based on less number of patients. To validate and verification of these findings more elaborative and extended studies may be of great help get rid the diabetic patients undergoing the renal insufficiency in diabetes mellitus.

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