Association of Heat-Shock Protein-70 Gene Polymorphisms with Type 2 Diabetic Nephropathy

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Editorial

HSP (heat-shock proteins) are molecular chaperones and its production begins under stress environment and these contribute in kidney cell growth as well as change the physiology of the kidney in acute and chronic kidney disease. Many studies have revealed the relationship of genetic polymorphisms HSP70-2+1267 A/G gene of heat-shock protein (HSP) with diabetic nephropathy (DN). Current study determined that the HSP70 gene polymorphisms influence the progression to DN (diabetic nephropathy) in patients with T2DM (Type 2 diabetes mellitus). The study participants having T2DM were divided into case and control groups on the basis of elevated proteinuria (>150 mg/24h). Study contained 140 individual in which 70 patients of type 2 diabetes mellitus without nephropathy that is the control group and 70 patients of Type 2 diabetes mellitus with nephropathy that is the case group and duration of diabetes is more than 10 years in case and control groups. All clinical analytes such as Fasting blood glucose level (FBG), Random blood glucose level (RBG), Diastolic blood pressure (DBP), Systolic blood pressure (SBP), Glycated hemoglobin (HbA1C), Body mass index (BMI), Urea, Creatinine and Proteinuria were analyzed in study subjects by immunoassay through BECKMAN COULTER AU480. The comparative analysis of clinical parameters including FBG, RBG, BMI, creatinine, and proteinuria between cases and control were found significant with ($p<0.05$). Non significant association ($p>0.05$) were observed between cases and controls with mean value of parameters DBP, SBP, Urea and HbA1c respectively. Samples were genotyped for the HSP70-2+1267 A/G polymorphisms by PCR-RFLP. The frequencies of AA, AG, and GG genotypes were 45.8%, 50.0% and 52.8% in the T2DM control and 54.2%, 50.0% and 47.2% in DN cases, correspondingly. No significant difference ($p>0.05$) was found using chi-square among genotype distribution of patients with T2DM with DN and controls for the HSP70-2+1267 A/G gene polymorphism within Pakistani community.

Diabetes mellitus (DM) is a metabolic disease that shows the elevation of blood glucose level due to the lack of insulin. Insufficient level of insulin production or defect in action of the insulin produced by the Beta cells of Langerhans of pancreas is a major cause of DM. The permanent high level of blood glucose causes the macro or micro problems including cardiac disease, liver disease and kidney disorders. DM is mainly divided into three main categories, first; type 1 diabetes mellitus (T1DM) is also called insulin dependent diabetes mellitus (IDDM), second; type 2 diabetes mellitus (T2DM) is also called Noninsulin-dependent diabetes mellitus (NIDDM) and third is called gestational diabetes mellitus (GDM) during the pregnancy of many women. The kidney function is disrupted due to DM is called DN. In DN the amount of albumin in urine is increased from threshold level of albumin in urine is called albuminuria. The diabetic nephropathy divides on the basis of albuminuria such as narmoalbuminuria in which albumin excretion in urine is less than 30 mg/ 24h, micro albuminuria in which albumin excretion in urine is equal to or in between 30-300 mg/24h, and macro albuminuria in which albumin excretion in urine is greater than 300 mg/24h. The chaperones (HSP) are actually protein in nature that helps in the folding and unfolding of others macromolecule. The chaperones are classified according to their size into the following classes such as 90, 70, 40, 60, 100 and small heat shock protein (sHSP). The HSP70 genes are further classified into HSP70-hom, HSP70-1 and HSP70-2. HSPs are intracellular tissue protective molecules and they are also present in cell organelles e.g. mitochondria, endoplasmic reticulum, peroxisome, cytoplasm, nucleolus and on cell membrane. But play role in connection of cell to cell and also body immune system

and inflammatory mechanism. The HSP70 have been observed with the following genes (HSPA1L, HSPA1B, and HSPA1A) in the mammalian cell and their concerned coding proteins are HSP70-hom, HSP70-1, and HSP70-2. These genes are situated in class III major histocompatibility complex (MHC) at chromosome 6 and many single nucleotide polymorphisms (SNPs) have been identified in HSP70 genes. The main study regions are situated at location +1267 of HSP70-2 (rs1061581), +2437 of HSP70-hom (rs2227956), and +190 of HSP70-1 (rs1043618). These SNPs have been reported for the influence of HSP70 protein that associated with multiples disorders susceptibility and stress resistance. The HSPA1A and HSPA1B genes encode a similar protein that expressed the heat inducible HSP70 protein and the HSPA1L gene encodes a non-heat inducible protein. The HSP are used for the diagnosis of different types of disease and HSP-70 elucidate the liver resection, post-operative organ dysfunction, post-operative infection, hepatic ischemic time, arterial calcification, renal and hepatic vascular disorders. The present study aims to investigate the association of heat- shock protein 70-2 (HSP70 2) +1267 A/G Single nucleotide polymorphism with type 2 diabetic nephropathy.

Materials and methods

Both male and female one hundred forty patients between the ages 35 years to 70 years, having type 2 diabetes mellitus, Seventy patients will be controlled group having type 2 diabetes mellitus but without nephropathy and remaining seventy will be the patients with DN and Patients of more than 10 years' history of diabetes mellitus'. Patients without type 2 diabetes mellitus, Patients of less than 35 years or more than 70 years of age, Patients having less than 10 year's history of diabetes mellitus and Patients of consistent hematuria and history of proteinuria before renal function deterioration. Patients of reduced kidney size congenitally or any systemic and inflammatory kidney disease. The 70 patients were diagnosed of T2DM and 70 patients diagnosed with DN in the Diabetic clinic OPD of Sheikh Zayed Hospital Lahore. The consent form was signed from each participant for the permission of giving a blood specimen for research purposes analysis. After collection of blood sample moved to the University of Lahore and saved at -20°C until assayed. The study was approved by the Ethics Committee of the University of Lahore Pakistan.

When the PCR (Veriti 96 well thermal cycler by AB Applied Bioscience Company) procedure was completed then run the 1% Agarose gel for the confirmation of amplified region of genomic DNA. Different Annealing temperatures were applied for the amplication of genomic DNA but 55°C the best optimization temperature for annealing at which the target region of genomic DNA was amplified completely. At 55°C the primer binds with the target region exactly. The 1 Kb DNA ladder was also used as a standard for the reading of our desired bands on gel.

HSPs are family of well conserved molecules that make up the major part of renal defense system and on the basis of their molecular size these HSPs have been classified into different groups i.e. HSP70, HSP90, HSP27, HSP65 and HSPs. The intracellular HSP participate in the maintenance of cellular integrity and also act as intracellular chaperones, preserving protein shapes and structures. Different HSPs are present in normal kidneys including HSP70 that play key role in the development of chronic kidney disease under stress condition and also provide the anti-inflammatory and immune regulatory assistance. Present study observed there are no significant association between HSP70-2 +1267 gene in DN patients with T2DM. The genotype investigation of cases and controls groups in the present study found that the heterozygous "AG" genotypes were more prevalent than homozygous genotype groups "AA" and "GG" in both case and control group patients. But the basic clinical analyte proteinuria has been significant association between HSP70-2+1267 gene polymorphism in DN patient with T2DM ($p=0.01$). The study also observed that the urea and creatinine level were greater in DN patients as compared to T2DM control groups. Previous study by Khanam et al reported the significantly ($p<0.01$) high "G" allele frequency of HSP70-2+1538 A/G polymorphism in diabetic patients of high albuminuria when compared with non diabetic patients. However there was no significant association observed with microalbuminuria in complete "G" allele of HSP70-2+1538 A/G polymorphism was linked with narmoalbuminuria and found a significant risk for macroalbuminuria samples. Some studies also observed that in advanced age, absence of physical activities, poor glycemic control and high blood pressure were significantly associated with increased prevalence of diabetic nephropathy. High blood pressure is well-known risk factors for diabetic patients to develop nephropathy and poor glycemic control was significantly found associated with high

prevalence of DN. The narmoalbuminuria, microalbuminuria, and macroalbuminuria subjects in association to AA, GG and AG genotypes of HSP70-2+1538 A/G were analyzed and searched that the homozygous GG genotype in normal subjects had very high levels of fasting plasma glucose (FPG) and HbA1C when associate with homozygous AA genotype. The patients with metabolic syndrome (MS) observed high risk factors for CKD patients. These patients with MS have been associated significantly with rapidly decrease of GFR. This decrease in GFR remained statistically significant between MS and CKD patients considering age and sex in the population. In comparative study the patients with MS and without MS considering baseline GFR, smoking, alcohol intake, high protein diet, hyperlipidemia, CRP, age and sex were at high risk for CKD in MS patients. In cases and controls groups the microRNA-29a (miR-29a) gene level was elucidated that there was no significant variation. While serologically miR-192 levels were different in case and control groups with proteinuric patients ($p=0.0138$). When patients with different GFR were studied the miR-21 was found significant ($p=0.036$) in patients with decrease GFR<60 ml/min as compared to patients with high GFR>60 ml/min in MS patients. Another study reported that the population with T2DM of HSP70-hom+2437 T/C gene polymorphism contained CT genotypes were significantly associated with the risk factors of T2DM ($p=0.015$) but in female the results were more significant ($p=0.002$). The male group of the study population have been found no significant association ($p=0.958$). In the entire group of the study population with T2DM the C allele was linked significantly ($p=0.016$) but typically with the female gender ($p=0.001$). The GG genotype and G allele of HSP70-2+1267 gene polymorphism were strongly associated with T2DM patients with DN and these patients have been greater values of lipid profile such as LDL (low-density lipoprotein) and cholesterol but low values of LDL and cholesterol in those patients that were contained AA genotype and A allele. Shortly the HSP70-1 and HSP70-2 have been significantly associated with the risk of DN in T2DM.

In the current study, HSP-70 gene polymorphism was evaluated in T2DN patients with (case) and without (control group) diabetic nephropathy. There were no statistically significant differences ($P>0.05$) were found among genotype distribution of patients with T2DM with DN and controls for the HSP70-2 +1267 A/G gene polymorphism within Pakistani community.