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Polymorphism in Metallothionein 1A Gene in Nepalese Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Polymorphism in metallothionein (MT) 1A gene (rs8052394) has been found to be associated with diabetes mellitus and its complications. We conducted the present study to find prevalence of polymorphism in MT1A gene (rs8052394) in Nepalese type 2 diabetes mellitus patients.

Method: A study was carried out among type 2 diabetes mellitus patients (n = 82: 44 males and 38 females, 54.7 ± 11.7 years) selected from Samjhana Laboratory Clinic, Lalitpur, in Kathmandu, Nepal to investigate single nucleotide polymorphism in MT1 A gene (rs8052394) in Nepalese patients with type 2 diabetes mellitus. Demographic parameters were noted and blood samples were collected, and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis was performed to detect rs8052394 polymorphism.

Results: The mean age of study patients was 54.7 ± 11.7 years. Mean fasting blood glucose and HbA1c were 168.7 ± 42.4 mg/dL and 7.4 ± 2.1% respectively. AA, AG and GG genotype of MTA1 gene rs8052394 was observed in 60.9% (n=50), 35.3% (n=29) and 3.6% (n=3) patients respectively. Allele frequency of A and G allele was 0.786 and 0.213 respectively.

Conclusion: Our findings suggest that polymorphism in metallothionein 1A gene (rs8052394) is common in Nepalese Type 2 diabetes patients.

Keywords: Metallothionein 1A; Polymorphism; Type 2 diabetes mellitus

Introduction

Diabetes mellitus is being one of the greatest health threats for the 21st century [1]. It is rising rapidly throughout the world, and the latest report from International Diabetes Federation (IDF) suggests that about 415 million adults have diabetes worldwide, and by the year 2040, 642 million will have diabetes [1,2]. Type 2 diabetes mellitus (T2DM) causes important morbidity, disability and early mortality, and is associated with a huge economic burden [3].

A number of risk factors like obesity, low physical activity, sedentary behavior, ethnicity etc. have been implicated for the development of Type 2 diabetes [4]. A strong heritable component for T2DM with genetic factors explaining around 25% of the variation in disease risk has been observed. Genome wide association studies (GWAS) have identified many single nucleotide polymorphisms (SNPs) consistently associated with an increased risk for T2DM [5].

Polymorphism in metallothionein (MT) gene has been found to have linked with T2DM. Three different SNPs (rs11640851, rs8052394 and rs1610216) in MT genes were shown to be associated with diabetes and its complications [6]. Polymorphisms of these genes have been associated with reduced intracellular zinc ion availability. +1245 A/G MT1A polymorphism (rs8052394) has also been implicated in T2DM onset [7]. Experimental data suggested that MT exerts cellular protection effects not only against metal toxicity, but also against a variety of oxidative stimuli including the protective effects in animal models of diabetes [8].

Diabetes and the disorders associated with it have been rising in Nepal [9]. Besides other factors, genetic components may also have contribution for the recent rapid rise in diabetes in Nepalese population. One of the predominantly associated genetic factors for diabetes risk is polymorphism in MT1A gene (rs8052394). Thus, we conducted the present study to detect polymorphism in MT1A gene (rs8052394) in Nepalese population with T2DM and to determine if rs8052394 has significant role for diabetes epidemic in Nepal.

Methods

We conducted a study among 82 T2DM patients selected from Samjhana Laboratory Clinic, Lalitpur, Nepal in 2013. Diabetes mellitus was diagnosed on the basis of American Diabetes Association (ADA) criteria [10]. Consents were taken from each patient and the study protocol was approved by the review board of Nobel College, Kathmandu, Nepal. Each patient age, sex, height and weight was noted, and blood samples were analyzed for biochemical parameters (fasting blood glucose and glycated hemoglobin A 1c (HbA1c)), and rs8052394 variation in metallothionein gene was investigated. Blood glucose and HbA1c was estimated by using enzymatic method and immuno chromatographic principle respectively. Genomic DNA was extracted using protocol of Lahiri et al. [11]. After extracting DNA, polymerase chain reaction (PCR) amplification of MT1A gene was done using the forward primer (5'-ACTAAGTGTCCTCTGGGGCTG 3') and reverse primer (5'-AATGGGTCACGGTTGTATGG 3') by using XP thermal cycler.

After restriction digestion of each DNA sample obtained by PCR by PstI (Fermentas), agarose gel electrophoresis (3% agarose gel in 1XTAE buffer) of 15 µl of digested products (mixed with 3 µl of loading dye) along with 100 bp DNA ladder was done for 1 hour at 90 volts. The results were viewed under UV transilluminator for number of DNA fragments (bands) obtained. Individual bands resulting from enzyme digested product were compared with molecular sized marker (100 bp ladder from Fermentas).

The data generated from the study were entered into MS excel and analyzed using SPSS version 11.0. Continuous variables were expressed as mean ± SD and categorical variables as percentage (number). Student *t* test and *chi* square test were applied for continuous variables and categorical variables at 95% confidence interval respectively.

Results

The study population comprised 44 diabetic males and 38 diabetic females, with mean age of 54.7 ± 11.7 years. The mean blood glucose and HbA1c was 168.7 ± 42.4 mg/dL and 7.4 ± 2.1% respectively. General characteristics of study population are shown in table 1. Agarose gel electrophoresis of the amplified MT gene after digestion with PstI showed: 3 bands of 118 bp, 165 bp and 283 bp in heterozygous patients with SNP (rs8052394), 2 bands of 118 bp and 165 bp in homozygous patients with SNP (rs8052394), and single band of 283 bp for patients with no polymorphism (wild type). SNP was observed in 32 patients. AA, AG and GG genotype of MTA1 gene rs8052394 was observed in 60.9% (n=50), 35.3% (n=29) and 3.6% (n=3) patients respectively. Allele frequency of A and G allele was 0.786 and 0.213 respectively. Body mass index (BMI), fasting blood glucose and HbA1c in patients with and without polymorphism was 25.3 ± 2.6 Kg/m^2 versus 24.7 ± 2.7 Kg/m^2; p=0.33, 169.7 ± 39.5 mg/dL versus 168.1 ± 44.5 mg/dL; p=0.867, and 7.4 ± 2% versus 7.4 ± 2.2%; p=0.956 respectively.

Discussion

Type 2 diabetes develops as consequence of interplay of various genetic and environmental factors [5]. Among different MT genes, the isoform MT1A with SNP rs8052394 is found to have a significant relationship with T2DM [7]. In the present study, we found that 39% Nepalese diabetic patients have rs8052394 polymorphism. This polymorphism is the result of a nucleotide change from A to G at position 55231329 of chromosome 16, and it results in the substitution of lysine by arginine at 51 position of the MT1A protein. In the wild type, codon AAA codes for lysine but in mutated type, codon AAA changes to AGA hence it codes for amino acid arginine [12].

Variables	Total population N=82	Male N=44	Female N=38	P value
Age (years)	54.7 ± 11.7	55.9 ± 11.4	53.3 ± 12	0.336
BMI (Kg/m^2)	25 ± 2.7	24.4 ± 2.7	25.6 ± 2.5	0.063
BG (mg/dL)	168.7 ± 42.4	168 ± 47.1	169.5 ± 36.7	0.872
HbA1c (%)	7.4 ± 2.1	7.4 ± 2.1	7.4 ± 2.1	0.91
Genotypes				
AA	60.9% (n=50)	32.9% (n=27)	28% (n=23)	0.938
AG	35.3% (n=29)	18.2% (n=15)	17% (n=14)	0.795
GG	3.6% (n=3)	2.4% (n=2)	1.2% (n=1)	0.645
AG+GG	39% (n=32)	20.7% (n=17)	18.2% (n=15)	0.938
Alleles				
A	0.786 (n=129)	0.42 (n=69)	0.365 (n=60)	0.645
G	0.213(n=35)	0.115 (n=19)	0.097 (n=16)	0.938

Table 1: General characteristics of study population
Data are presented as mean ± SD, percentage (number) and as frequency (number). P value was calculated between diabetic males and females at 95% confidence interval. BG, blood glucose; BMI, body mass index; HbA1c, glycated hemoglobin A 1c.

Metallothioneins are cysteine rich metal binding proteins which, by means of their antioxidant and zinc-buffering properties, might prevent the development of diabetic cardiovascular complications [13]. Polymorphism in MT1A gene may lead to formation of nonfunctional metallothionein, thus affecting diabetic patients. In the present study, we observed three genotypes of MT1 gene polymorphism; AA, AG and GG. AA, AG and GG genotypes were observed in 60.9% (n=50), 35.3% (n=29) and 3.6% (n=3) patients respectively. Similarly, A and G allele frequency was 0.786 and 0.213 respectively. In a study in Bulgaria, AA, AG and GG genotype at MT1A (A+1245G) rs8052394 was observed in 63.4%, 32.7% and 4.0% of diabetic patients (101 patients) respectively. Frequency of A and G allele was 0.798 and 0.204 respectively [6]. In a study in china, a significant difference in the frequency distributions of the allele A and G for SNP rs8052394 in the MT1A gene between the T2DM (397 cases) and non-diabetic control (454 cases) groups was found. Although no significant difference in the frequency distributions of the genotype for MT1A gene rs8052394 between T2DM and non-diabetic control groups was observed, but if combined GG and GA together, there was a significant difference between the T2DM group and the control group [12].

Diabetes mellitus is a chronic disease characterized by an overproduction of reactive oxygen species (ROS), which perturbs zinc metabolism and promotes the onset of CVD [13]. In the present study, we however, did not assess status of CVD, kidney disease, and diabetes complications which may have association with the polymorphism in MT1A gene. In a study in Bulgaria, it was found that the single nucleotide polymorphisms +1245 A/G MT1A and -209 A/G MT2A are related with diabetic complications and coronary artery disease (CAD) developing [6]. In a study in Greece, +1245 MT1A G+ genotype significantly increased the risk of CVD in Greece (34.4% versus 23.2%; odds ratio=1.88, 95% CI=1.14-3.08; p=0.013) but not in Italy [14]. A study in China revealed that rs8052334 and rs10636 SNPs are significantly associated with hyperlipidemia, and both SNPs rs11076161 and rs10636 are found to be significantly associated with the incidence of T2DM with neuropathy [12].

Studies have reported increase in oxidative stress in diabetic patients with rs8052394. In one study, +1245 MT1A AG+GG genotype showed higher plasma advanced glycation end products (AGEs) and ROS production in peripheral blood mononuclear cells (PBMCs) than AA genotype at the baseline. Thus, +1245 MT1A A/G polymorphism has a significant influence on baseline AGEs and ROS production [7]. In China, a significant decrease in serum superoxide dismutase (SOD) activity was observed in both GG carriers and GA carriers compared with AA carriers [12].

In conclusion, 39% of Nepalese diabetic patients had polymorphism in MT1A gene (rs8052394). The magnitude of polymorphism in MT1A gene (rs8052394) is important to estimate the burden of diabetes and its contribution to epidemic in Nepal. Further studies are required with larger sample size to find the association of polymorphism in MT1A gene with complications of diabetes.

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Author's contribution

SK, PT, and NDP designed the study. SK, PT, RS, PLD and NDP performed laboratory analysis. SK and MKT performed statistical analysis. SK wrote the manuscript and all authors reviewed manuscript. All authors read and approved the final version of the manuscript.

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