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EXPERIMENTAL WORKS

Association of Catalase (rs7943316) and Glutathione Peroxidase-1 (rs1050450) Polymorphisms with the Risk of Type 2 Diabetes (T2DM)[1]

S. Saravani[a], H. R. Miri*[, b], R. Saravani[c], D. Yari[c], A. Nakhaee[c], and M. Mahjoubifard[d]

[a] *Department of Biology, Zabol University, Zabol, Iran*

[b] *Deparment of Laboratory science, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran*

[c] *Cellular and Molecular Research Center and Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*

[d] *Children and Adolescent Health Research Center Ali-ebne Abitaleb Hospital and Department Anesthesia, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*

e-mail: samirasar2002@yahoo.com, Hr.miri1354@gmail.com, saravaniramin@yahoo.com, Davidyari.85@gmail.com, alireza_nakhaee@yahoo.com, m_mahgoobifard@yahoo.com

Abstract—Reactive oxygen species are involved in the pathogenesis of type 2 diabetes mellitus (T2DM). Catalase and Glutathione peroxidase-1 are antioxidant enzymes and the activity of them is essential for the protection against damage caused by reactive oxygen species. The aim of this study was to investigate the association between genetic polymorphisms of *CAT* gene (rs7943316) and *GPx-1* gene (rs1050450) in patients with type 2 diabetes. In this case-control study, a total of 120 Iranian patients with T2DM and 120 healthy individuals as control were included. Genotypes were determined by PCR-RFLP. A significant difference was found between genotyping distribution of *CAT* (–21 A/T) polymorphism.The frequency of TT genotype was increased in patients compared to controls and we observed a statistically significant difference (*OR* = 1.797, 95% *CI* = 0.975–3.318, *P* = 0.044). Distribution of genotypes did not differ for *GPx-1* (198 C/T) polymorphism between the cases and controls subjects. This study indicated that *CAT* rs7943316 genotype (TT) was associated with an increased risk of T2DM. No evidence was found to support an association between *GPx-1* (198 C/T) polymorphism and T2DM.

Keywords: *CAT*, gene polymorphism, *GPx-1*, type 2 diabetes (T2DM)

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most common non transmissible diseases in the world and its complications have become a major cause of death and disability [1]. The prevalence of diabetes is on the rise in the recent decades. The global number of diabetes patients will increase to 366 million by 2030 [2]. In Iran 7.7% of adults (n = 2 million) have diabetes and 16.8% (n = 4.4 million) have impaired fasting glucose [3]. Diabetes is a group of metabolic diseases and is caused by environment factors, such as high-caloric fat, carbohydrate enriched diets and reduced physical activity. In addition, as one of the most well-known polygenic diseases [4]. Diabetes is diagnosed by high levels of hyperglycemia. It is the result of the body not creating enough insulin to keep blood glucose levels in the normal range or the cells in the body don't recognize the insulin that is present. The symptoms of T2DM include of increased urination, polydipsia, polyphagia and unusual weight loss [5]. Oxidative stress has been considered to be a pathogenic factor of atherosclerosis, insulin resistance, type 2 diabetes and cancer [6]. Oxidative stress results from increased reactive oxygen species (ROS) or decreased levels of antioxidants. There are numerous enzyme systems that catalyze reactions to neutralize free radical and ROS. These enzymes consist of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR) and catalase (CAT). There is evidence that hyperglycemia can be caused by oxidative stress [7]. Catalase is an enzyme found in all living organisms exposed to oxygen. This enzyme is a tetramer with molecular weight of about 240000. Per tetrameric molecule exists four heme groups [8]. Hydrogen peroxide (H_2O_2) is converted to oxygen and water by catalase. Catalase is usually located in peroxisome. *CAT* is located at position 11p13 and *CAT* polymorphism, rs7943316 (–21 A/T) is located within the promoter region near to the start site [9]. Insufficiency of catalase may cause high concentrations of H_2O_2 and increase the risk of developing T2DM [10]. GPx-1 is

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* Corresponding author.

an intracellular antioxidant enzyme that converts H_2O_2 to water and protects the organism from oxidative damage [11]. Selenium is a structural constituent of the active center of GPx-1. *GPx-1* was mapped on 3p21.3 and it is polymorphic at codon 198 C/T (rs1050450) [12]. The role of this SNP has not been well defined in diabetic and healthy cohorts, thus, the current study aimed to examine the possible associations between *CAT* polymorphism, rs7943316 and *GPx-1* polymorphism, rs1050450 with risk of T2DM in an Iranian population.

MATERIALS AND METHODS

Subjects

In this case-control study, 120 patients with T2DM were enrolled from Zahedan Diabetes Center in Iran and 120 individuals as the control group were recruited from Bouali Hospital, Zahedan, Iran. The mean ages were 56.34 ± 10.8 years. Informed consent from all patients and healthy individuals obtained. Diagnosis of T2DM was based on standards established by the American Diabetes Association and World Health Organization as follows:

Fasting blood glucose (FBG) of greater than or equal to 126 mg/dL;

Oral Glucose Tolerance Test (OGTT) of greater than or equal to 200 mg/dL;

Random Plasma Glucose Test of greater than or equal to 200 mg/dL.

Patients were treated by diet, metformin or insulin. Medical history including gender, age, duration of diabetes, type of medicine were recorded. Fasting blood was investigated for FBG, cholesterol, triglyceride (TG), HDL, LDL, creatinine and urea.

Ethical approvals for recruitment were obtained from Ethics Committee of Zabol University. A blood sample was taken from all patients and healthy individuals and stored in EDTA-containing tubes for DNA extraction.

CAT and GPx-1 Polymorphisms Genotyping

Genomic DNA was extracted from peripheral blood of the participants by salting-out method. After assaying the quality of extracted DNA by electrophoresis on 1% agarose gel and the quantity of DNA by spectrophotometry, the extracted DNA was stored at −20°C until further use. The genotypes of *CAT* and *GPx-1* were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was done in a final volume of 20 μL, using 5 μL of genomic DNA, 3.8 μL DNase-free water, 0.6 μL of each primer and 10 μL master mix (Ampliqon, Denmark).

Genotyping of rs7943316 polymorphism of *CAT* (−21 A/T) gene: PCR was performed for *CAT* by using a forward primer, 5'-CTTCCAATCTTGGCCTGCCTAG-3' and a reverse primer, 5'-CCGCTTTCTAAACGGACCTTCG-3'. PCR product size was 312 bp. The amplification was done with a primary denaturation step at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s with a final extension at 72°C for 5 min. per reaction was assayed on a 2% agarose. Amplified DNA was digested with restriction enzyme HinfI (BIORON, Germany) at 37°C for 3 hours (10 μL PCR product, 2 ul buffer, 1 μL HinfI and 7 μL distilled water). The digestive products were run by 2% agarose. HinfI digestion resulted in two fragments of 109 and 203 bp for AA genotype, three fragments of 312, 203 and 109 bp for AT genotype and one fragment of 312 bp for TT genotype.

Genotyping of rs1050450 Polymorphism of GPx-1 (198 C/T) gene

DNA amplification was carried out by using a forward primer, 5'-TATGACCGACCCCAAGCTC-3' and a reverse primer, 5'-GACACCCGGCACTTTATTAGTG-3'. PCR product size was 390 bp. The cycling conditions were: initial denaturation step at 95°C for 5 min, followed by 25 cycles at 95°C for 30 s, 61°C for 30 s, and 72°C for 25 s with a final extension at 72°C for 5 min. PCR band was detected in 2% agarose. PCR product was digested with restriction enzyme ApaI (Thermo scientific, Lithuania) at 37°C For 3 hours (10 μL PCR product, 2 μL buffer, 1 μL ApaI and 7 μL distilled water) and analyzed with 2% agarose. ApaI digestion resulted in three fragments of 84, 88 and 218 bp for CC genotype, two fragments of 84 and 306 bp for TT genotype and four fragments of 84, 88, 218 and 306 bp for CT genotype.

Statistical Analysis

The results were statistically analyzed using the SPPS software for windows, version 19 (SPSS Inc, Chicago, IL, USA). The association between genotypes and T2DM was assayed by calculating the odds ratio (*OR*) and 95% confidence intervals (95% *CI*) by using the Chi-square (*2*). *P* values less than 0.05 were considered significant. Student's *t*-test was used to compare quantitative data. The Hardy–Weinberg equilibrium (HWE) was tested by using the χ^2 for each of the SNPs.

RESULTS

Frequency of *CAT* (rs7943316) Genetic Polymorphism Frequency of allele and genotype of *CAT*, rs7943316 A>T are mentioned in Table 1 in patients with T2DM and healthy individuals. We found statistically significant differences concerning genotyping distribution of *CAT* polymorphism (rs7943316). The frequency of TT genotype was increased in patients compared to healthy individuals (38.3% vs. 26.6%,

Table 1. Polymorphisms in antioxidant genes of *CAT* –21 A/T and *GPX-1* 198 C/T

Genotype	Patients (%)	Controls (%)	*OR* (95% CI)	*P*-value
CAT –21 A/T				
TT	46 (38.3)	32 (26.6)	1.797 (0.975–3.318)[1]	0.044[1]
AT	56 (46.6)	70 (58.3)		
AA	18 (15)	18 (15)		
T allele frequency	148 (61.6)	134 (55.83)	1.273 (0.870–1.863)	0.194
A allele frequency	92 (38.3)	106 (44.16)		
GPx-1 198 C/T				
CC	0	0		
CT	120 (100)	120 (100)		
TT	0	0		
C allele frequency	120 (50)	120 (50)		
T allele frequency	120 (50)	120 (50)		

[1] *P*-value and Odds Ratio (95% Confidence Interval) were calculated major/major vs. major/minor and minor/minor allele.

Table 2. Comparison of features between diabetics and controls

Features	Mean ± standard deviation		*P*-value
	diabetic ($n = 120$)	control ($n = 120$)	
FBG[a] (mg/dL)	172.51 ± 77.316	75.53 ± 12.04	0.001
Cholesterol (mg/dL)	175.79 ± 38.291	190.09 ± 15.04	0.001
TG[b] (mg/dL)	152.47 ± 73.499	120.25 ± 10.12	0.001
HDL[c] (mg/dL)	59.03 ± 6.743	60.36 ± 4.53	0.116
LDL[d] (mg/dL)	89.82 ± 13.004	90.22 ± 6.03	0.878
Urea (mg/dL)	31.33 ± 10.950	30.23 ± 6.12	0.185
Creatinine (mg/dL)	1.0225 ± 0.263	1.01 ± 0.233	0.352

[a] FBG, fasting blood glucose; [b] TG, triglyceride; [c] HDL, high density lipoprotein; [d] LDL, low density lipoprotein.

respectively) and we observed a significant difference ($OR = 1.797$, 95% $CI = 0.975–3.318$, $P = 0.044$). It revealed carriers of TT genotype had a higher risk for T2DM.

Frequency of GPx-1 (rs1050450) Genetic Polymorphism

No significant difference in distribution of the genotype frequency for *GPx-1*, rs1050450 C>T was observed. All the genotypes were heterozygous (CT) in patients with T2DM and healthy individuals (Table 1).

Furthermore, we observed no statistically significant differences in allele frequencies in all SNPs of the studied genes in patients with T2DM and controls (Table 1). Other variables significantly associated with T2DM were cholesterol, FBS and TG. No significant association has been demonstrated for HDL, LDL, Urea and creatinine between patients with T2DM and controls (Table 2).

DISCUSSION

T2DM develops in individuals as a result of hyperglycemia and it is a metabolic disorder [13]. In diabetic patients, dysfunction occurs in metabolism of carbohydrates, lipids and proteins which leads to long term damages in various organs of the body [7]. Reactive oxygen species are produced under physiological conditions but over production of ROS has pathological outcomes in individuals [14, 15]. If there is a increase in production of free radical, or a decrease in exclusion of free radical, oxidative stress ensues [16]. There is

experimental and clinical evidence that T2DM is closely associated with oxidative stress [17]. Antioxidant enzymes such as GPx-1 and CAT confront against oxidative stress [18]. CAT is expressed in almost all kinds of eukaryotic cells and it is responsible for converting H_2O_2 to H_2O and O_2 [19]. *CAT* gene is 34 kb and divide into 13 exons [20]. *CAT* polymorphism, rs7943316 (–21 A/T) is located in the promoter region [9]. Panduru and colleagues investigated the association of –21 A/T (rs7943316) polymorphism of *CAT* gene with diabetic nephropathy in patients with type 1 diabetes. They reported T allele did not consider a risk factor for diabetic nephropathy ($OR = 0.757$, 95%, $P = 0.381$) and no association was found between –21 A/T (rs7943316) polymorphism with diabetic nephropathy in patients with type 1 diabetes [21]. Vialykh and co-workers showed that –21 AA *CAT* genotype is associated with high risk of cerebral stroke in hypertensive men ($OR = 1.77$, 95% $CI = 1.01 - 3.07$) [22].

GPx-1 is a selenoprotein, reduces H_2O_2 to H_2O. GPx-1 comprises two exons [11]. GPx-1 polymorphism, rs1050450 (198 C/T) is situated in exon 2 [23]. Genetic variations among enzymes that support cell against ROS have been suggested in proneness to T2DM, cancer and other diseases [24]. Tang and colleagues suggested that T allele of *GPx-1* (rs1050450) is associated with decreased enzyme activity and they examined association between *GPx-1* rs1050450 (C>T) gene variant and peripheral neuropathy in patients with diabetes. They observed a significant association between T allele and peripheral neuropathy [23]. Men and co-authors designed a meta-analysis to determine the association of *GPx-1* rs1050450 (C/T) polymorphism with bladder cancer. They found that rs1050450 C>T polymorphism was associated with an increment risk of bladder cancer (T vs. C: $OR = 2.111$, 95% $CI =$ 1.020–4.368; *CT/TT* vs. CC: $OR = 1.876$, 95% $CI =$ 1.011–3.480) [25].

In this study, we observed *CAT* rs7943316 TT genotype act as a risk factor for developing T2DM in our population. Frequently of *CAT* rs7943316 TT genotype was more in patients than in the controls (38.3% vs. 26.6% respectively) and might increase the risk of T2DM by 1.79-fold. Polymorphism of *GPx-1* gene, rs1050450 did not differ between patients with healthy individuals and all the genotypes were the same in patient and control groups. There was also significant statistical difference between patient and healthy individual groups in terms of levels of FBS, TG and cholesterol with a p value of 0.001 in polymorphisms of *CAT* and *GPx-1* genes.

In conclusion, our results suggest that there is a significant association between the *CAT* rs7943316 TT genotype with T2DM in our studied population and also, the levels of FBS, TG and cholesterol show a significant difference between patient and control groups in polymorphisms of *CAT* and *GPx-1* genes. Further researches are essential to clarify the role of these polymorphisms in the pathogenesis of T2DM and also, Further studies with various ethnic groups and large population are required to confirm our findings.

There is no conflict of interest.

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