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Investigation of DNA repair genes XPD and hOGG1 in type 2 Diabetes mellitus

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Abstract

Increased oxidative stress in type 2 diabetes cause to the accumulation of DNA damage and results diabetic complications. Xeroderma pigmentosum complementation group D (XPD) and human oxoguanine glycosylase 1 (hOGG1) are genes involved in the repair of oxidative DNA damage. In this study, we aimed to evaluate association between XPD Lys751Gln and hOGG1 Ser326Cys polymorphisms with type 2 diabetes mellitus (T2DM) in the Turkish population. Seventy-one T2DM patients and 54 healthy individuals were incorporated into this study. DNA was extracted from whole blood. The Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) techniques were used. There was statistically significant difference between patient and control groups in the genotype distribution of XPD Lys751Gln polymorphism ($p<0.05$). While the Lys/Lys genotype was found significantly higher in control group, the Lys/Gln was higher in patients. ($p<0.05$). There was no significant difference between groups in the genotype distribution of hOGG1 Ser326Cys polymorphism. Despite the small number of subjects included in the study, it could be interpreted that the Lys/Gln genotype of XPD Lys751Gln polymorphism may be contributing to the development of diabetes.

Keywords: Type 2 diabetes mellitus, XPD, hOGG1 polymorphism, PCR-RFLP

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases with late complications, which severely affect the quality of life, including cardiovascular disease, nephropathy, neuropathy, and retinopathy. Several reports indicated that free radicals have significant roles in the pathogenesis of diabetes. Oxidative stress in diabetes is also related to decreased antioxidant defense [1,2]. DNA damage may be related with type 2 diabetes mellitus (T2DM) and complications of T2DM appear primarily due to oxidative stress. It has been shown that T2DM may be associated with increased oxidative DNA damage and sensitivity to mutagens, and inefficient DNA repair [2].

Xeroderma pigmentosum complementation group D (XPD) gene is located on chromosome 19q 13.3 and it is a DNA repair gene that repairs damages such as thymidine dimers using nucleotide excision repair (NER) pathway. NER of damaged DNA is regulated by XPD, which functions with a complex network of DNA repair enzymes. Glucose and insulin are substantial regulators of the XPD activity. XPD expression is insulin-dependently regulated on mRNA and protein levels according to the insulin receptor activation status. Therefore, mutations that occur in the tyrosine kinase domain restrain the up-regulation of XPD. Moreover, glucose exposure in a toxic level is predicted to reduce insulin dependent DNA repair regulation [3,4]. A XPD gene polymorphism on exon 23 (Lys751Gln, A→C) has been studied in various cancer types such as liver, lung, skin and breast [3].

Human oxoguanine glycosylase 1 (human oxoguanine glycosylase 1; hOGG1) gene is located on chromosome 3 and plays a key role in base excision repair mechanisms. The 8-oxoguanine glycosylase 1 (hOGG1), an enzyme encoded by the hOGG1 gene, directly removes 8-hydroxy-2-deoxyguanine (8-OHDG) from the damaged DNA using the base excision repair mechanism. The most common lesion formed as a result of oxidative stress due to surroundings or ordinary metabolism by-products is the 8-Oxodeoxyguanosine, which eventuates in a GC to TA transversion. [5). 8-OHdG is an indicator of DNA damage in diabetes, and polymorphisms in the hOGG1 gene lead to development of diabetes by altering glycosylase activity and the repair of damaged DNA [6, 7].

T2DM develops due to accumulation of oxidative DNA damage from reactive oxygen species ROS, resulting in pancreatic β-cell dysfunction and loss [8]. DNA repair genes protect DNA against increased free radicals in the case of oxidative stress. The aim of our study was to investigate the relationship between XPD and hOGG1 gene polymorphisms and T2DM development.

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Material and Methods

Study group

The study group consisted of 71 subjects who were diagnosed with T2DM at the Istanbul University Medicine Faculty Internal Medicine Endocrine and Metabolism Department and 54 unrelated healthy control subjects. This study was conducted with the approval of the Istanbul Medical Faculty Ethical Committee, Istanbul University.

DNA isolation and SNP detection

Genomic DNA was isolated from peripheral blood by using salting out method [9]. The specific primers (5'-CCTCTCCCTTTCCTCTGTTC-3' forward and 5′-CAGGTGAGG GGGGACATCT-3′ reverse) were used to identify the XPD gene polymorphism and the other primers (5'-ACTGTCACTAGTCTCACCAG-3' forward and 5′-GGAAGGTGCTTGGGGAAT-3′ reverse) were applied for hOGG1 gene polymorphism.

The PCR reaction volume was set as 25 µl with 1 µl DNA sample of 50-100ng, 2.5 µl dNTP (100 µg/ml), 2 µl MgCl2 (25 mM/ml), 1 µl each primer and 0.5 µl Taq polimerase (5U/µl), 1.5 µl 10X DNA Taq PCR buffer and 16.5 µl apyrogenic water. The PCR reaction for XPD Lys751Gln was performed as 95 ºC for 1 min, 60 ºC for 1 min and 72ºC for 1 min for 35 cycles following the 95ºC for 2 min initial denaturation and a final elongation step as 72 ºC for 10 min. For the hOGG1 Ser326Cys polymorphism analysis we used the protocol; 95 ºC for 1 min, 60 ºC for 1 min and 72 ºC for 1 min for 35 cycles following the 95ºC for 5 min initial denaturation and a final elongation step as 72 ºC for 7 min. PCR products were analyzed with agarose gel electrophoresis.

The amplified PCR products for XPD Lys751Gln and hOGG1 Ser326Cys polymorphisms were cut with PstI and Fnu 4 HI Restriction Endonuclease enzymes, respectively.

Statistical analysis

Chi-square and Fisher analysis of the distribution of XPD Lys751Gln and hOGG1 Ser326Cys genotypes in our populations indicated that all the alleles were in Hardy–Weinberg equilibrium. Statistical analyses were performed with SPSS for Windows version 17.0. Categorical data were reported as number (n) and percent (%). The demographical data was compared if significant difference between the groups in terms of allele and genotype distribution of XPD Lys751Gln and hOGG1 Ser326Cys. Allele frequencies were determined using the gene counting method. Values of $p<0.05$ were regarded statistically significant.

Results

Seventy-one subjects with T2DM were included in the study as the patient group. The control group included 54 age-matched healthy individuals. Sex distribution, serum HDL-cholesterol, LDL and glucose levels were significantly higher in the patient group than in the control group ($p<0.05$) (Table 1). There were no significant differences between the patient and control groups in terms of other parameters ($p>0.05$) (Table 1).

Table 1. Demographic details of the patient and control groups

Parameters	Control (n=54)	Patient (n=71)	P
Sex (Female/Male)	11/43	40/31	0.000
Mean Age (year)	46.46±11.20	51.04±13.31	0.079
BMI (kg/m2)	26.33±3.77	28.32±8.47	0.213
Triglyceride (mg/dl)	174.60±112.47	178.44±206.82	0.911
Total-cholesterol (mg/dl)	198.66±46.62	186.41±69.11	0.316
HDL-cholesterol (mg/dl)	41.47±8.05	33.45±12.30	0.000
LDL-cholesterol (mg/dl)	129.73±42.39	112.95±39.59	0.044
VLDL-cholesterol (mg/dl)	29.78±18.65	30.41±15.05	0.862
Smoking (%) (yes/no)	44.4	21.1	0.027
Glucose (mg/dl)	85.12±10.15	219.35±114.43	0.000
HbA1c	0	23.92±86.48	
Insulin	0	13.44±10.43	
C-peptide	0	5.32±5.71	
Folate	0	7.52±3.54	
B12	0	515.19±398.31	

The distribution of XPD Lys751Gln genotypes between patient and control groups were analyzed and a statistically significant difference was observed ($p=0.031$).

According to our results, the XPD Lys / Lys genotype was significantly higher in the control group and showed a 2.56-fold preventive effect ($p=0.017$, X2: 5.66 OR: 0.390 95% CI: 0.177-0.856). The Lys/Gln genotype was significantly higher in the patient group and was associated with a 2.37-fold risk of disease (p: 0.019 X2: 5.50 OR: 2.37: 95% CI: 1.146-4.929) (Table 2).

It was observed that when individuals carrying the glycine allele (Gln/Gln + Lys/Gln) were combined, Gln + individuals increased statistically significantly in the patient group. Gln+ allele showed a 2.5-fold increased risk of developing diabetes ($p=0.017$ X2:5.66 OR: 2.56 %95 CI: 1.16-5.63).

When we examined the genotype and allele distributions of hOGG1 Ser326Cys gene polymorphism in the patient and control groups, no statistically significant difference was observed (P=0.199). However, it was determined that the Ser/Ser genotype was higher in the patient group and the Ser/Cys genotype was slightly higher in the control group, but it was not statistically significant. (p=0.142 OR: 1.71 %95 CI: 0,834-3,50; p: 0,073 OR: 0,521 %95 CI: 0,254-1,067) (Table 3).

It was found that numbers of individuals carrying the Cys allele were higher in the control group. According to our findings, Cys allele has a 1.7-fold preventive effect for developing diabetes.

Table 2. Distribution of XPD genotype and allele frequencies in patient and control groups

Polymorphism	Control		Patient		P value
	N	%	N	%	
XPD Lys751Gln					
Lys/Lys	22	40.7	15	21.1	0.017
Lys/Gln	19	35.2	40	56.3	0.019
Gln/Gln	13	24.1	16	22.5	0.840
Lys	63	58.33	70	49.29	
Gln	45	41.66	72	50.70	0.156

Table 3. Distribution of hOGG1 genotype and allele frequencies in patient and control groups

Polymorphism	Control		Patient		P value
	N	%	N	%	
hOGG1 Ser326Cys					
Ser/Ser	21	38.9	37	52.1	0.142
Ser/Cys	30	55.6	28	39.4	0.073
Cys/Cys	3	5,6	6	8.5	0.535
Ser	72	66.66	102	71.83	
Cys	36	33.33	40	28.16	0.379

Discussion

Diabetes mellitus is a disease characterized by a complete or partial insulin deficiency and high blood sugar (hyperglycemia). Chronic hyperglycemia causes oxidative stress [10], which is also associated with progression of T2DM and several diabetic complications [11]. The accumulation of ROS lead to modifications that cause DNA damage on nuclear and mitochondrial DNA [12]. ROS plays an important role in the pathogenesis of diabetes by causing cellular dysfunction and cell death. Hyperglycemia, an important feature of diabetes, and insulin resistance are thought to promote free radical production [11]. Several studies have shown that the accumulation of free radicals and the reduction in the amount of antioxidants result in cellular oxidative damage, which are associated with DM [13].

Many sub-variations were found when the XPD Lys751Gln polymorphism was scanned in Arab, Mexican and Indian populations [14-16]. In two meta-analyses, the XPD Lys751Gln and hOGG1 Ser326Cys polymorphisms were found associated with prostate cancer but not with hepatocellular carcinoma [17, 18]. Zhang et al., in their study on diabetic patients reported that XPD Lys751Gln was not, however, the hOGG1 Ser326Cys polymorphism was associated with age related cataract [19]. The Gln allele of the XPD Lys751Gln was found to be significantly different in the mixed type cataract group in the Turkish population [20]. In the Turkish population, the hOGG1 Ser326Cys polymorphism might be associated with increasing risk of colorectal cancer, yet XPD Lys751Gln was not found related [21]. Moreover, they were both found not associated with gastric cancer [22], yet associated with an elevated risk for the development of migraines [23]. In addition, with oxidative stress in reperfusion, the hOGG1 Ser326Cys gene polymorphism was not found related, on the other hand, it was related with the Gln allele of the XPD Lys751Gln polymorphism [24].

In this study, the XPD Lys751Gln Lys/Gln genotype was found significantly higher in the patient group, while the Lys/Lys genotype was significantly higher in the control group. Furthermore, Gln+ individuals were statistically significantly higher in the patient group and the risk of development of diabetes was 2.56-fold higher. Consequently, in accordance with studies on various cancer types, in this study, the Gln allele was found associated with diabetes development. We suggest that presence of the Gln allele may be involved in type 2 diabetes development by conducing to low or insufficient DNA repair.

In a study conducted on the Japanese population, the Cys allele was found to be associated with increased body mass index, fasting blood glucose and total cholesterol. In the Cys/Cys genotyped individuals, body mass index was found to be higher [25]. In another study on the Japanese population, patients carrying the Cys/Cys or the Cys/Ser genotypes, HOMA- beta, an indicator of beta cell function, was decreased with improved glucose tolerance, however, among patients with the Ser/Ser genotype, it was elevated in individuals with glucose intolerance but decreased in ones with diabetes [26]. The hOGG1 Ser326Cys polymorphism was also associated with type 2 diabetes in the Chinese population

[27], on the other hand, it was reported not associated in the Polish population [13]. Furthermore, this polymorphism has been suggested as an indicator of a disposition and the severity of coronary artery disease [28]. Moreover, a relationship was found between coronary artery lesions in diabetic patients with the Cys/Cys genotype having higher effect on the severity [29]. In the Turkish population, it has also been suggested as a possible marker for T2DM development [30].

The genetic variations of Ser326Cys in the hOGG1 gene are affected by oxidative stress and the hOGG1 enzymatic activity changes. Although the Ser allele was associated with high enzymatic activity and the Cys allele was associated with low enzymatic activity, in our study, Ser was found high in the patient group while Cys was high in the control group. Due to high oxidative stress present in diabetic patients high-activity Ser allele does not function, and therefore, we believe diabetes develops.

In conclusion, it is thought that more accurate results can be obtained when the number of cases is increased for both polymorphisms.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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