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Research Article

Association between Genetic Polymorphism of NQO1 C609T and Risk of Age-Related Macular Degeneration Disease

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Abstract

Background: Oxidative stress is one of the most important reasons of age-related macular degeneration disease (AMD) that is caused by smoking, U.V. and X ray exposure, pressure and heat which result in increasing of reactive oxygen species (ROS) level. As a result, high level of ROS causes cell structure damage. Cells have complex network of antioxidant enzymes such as NAD(P)H Quinone Dehydrogenase 1 (NQO1) that protects cells from ROS-induced damages. In considering that every change in antioxidant enzymes cause changes in antioxidant capacity of retina, we tried to study genetic polymorphism of NQO1 C609T, antioxidant gene and risk of AMD disease.

Methods: This study was included 112 AMD patients and 112 control subjects. For identifying of NQO1 C609T polymorphism, we used polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. Differences in the frequencies were estimated using the $\chi 2$ test and risk was estimated with a binary logistic regression after adjusting for smoking, working place and age status.

Results: After regression binary logistic analysis, there was no significant association between NQO1 CT+TT genotype and risk of AMD (P=0.618, OR=0.84, 95%CI=0.41-1.69) and there was no significant difference in the homozygous and heterozygous frequencies between patient and control groups. Also T allele has not a significant association with risk of AMD (P=0.735, OR=0.92, 95%CI=0.59-1.44).

Conclusion: The present study may prove that not all polymorphisms in antioxidant genes associate with age at onset disease such as AMD, unlike those of that have been observed in some studies.

Keywords: Macular Degeneration Disease; NQO1; Polymorphism; Age at Onset

Abbreviation

AMD: Age-Related Macular Degeneration; NQO1: NAD(P)H Quinone Dehydrogenase 1; PUFAs: Polyunsaturated Fatty Acids; ROS: Reactive Oxygen Species; RPE: Retinal Pigmented Epithelial.

Introduction

Age-related macular degeneration (AMD) is an eye disease in the elderly population in the developed countries that causes losing vision and blindness with slow progress [1,2]. AMD has two dry and wet forms: In non-neovascular or dry form, drusen accumulate between the retina and the choroid that occurs gradual degeneration of photoreceptors and retinal pigmented epithelial (RPE) cells and neovascular or wet form occurs by developing of new blood vessels from the choroid behind the retina to retina and subretina space, ultimately leading to blood leakage under the macula that causes damage to the photoreceptors and losing vision. Overall inducer factors of Oxidative stress like smoking, sunlight and antioxidant genes deficiency are considered as possible reasons of AMD [3-6]. There are many reasons for development of AMD such as aging, Hypertension, Cardiovascular status, Race [7],

family history, macular degeneration genes [8,9]. Deletion of the complement factor H-related genes CFHR3 and CFHR1 [10,11]. The retina is exposed easily by oxidative stress because the retina has high metabolic activity, oxygen tautness and high oxidized polyunsaturated fatty acids (PUFAs) and it is susceptible to generate reactive oxygen species (ROS) when exposed by oxidative stress factors [12].

ROS are reactive molecules such as oxygen ions and peroxides. ROS are generated among of normal metabolism of oxygen in mitochondria, during of environmental stress and exogenous sources such as ionizing radiation, U.V. or heat exposure [13]. These reactive molecules may result in damage to macromolecules and causes oxidative stress.

NAD(P)H Quinone Dehydrogenase 1 (NQO1) as DT-Diaphorase, is a quinone reduction enzyme that converts reactive quinone to hydroquinone by using NADPH or NADH. NQO1 is one of the cytosolic antioxidant enzymes that protect cells from ROS formation and semiquinone damages [14]. Also NQO1 can cause apoptosis by activating and stabilizing of p53 [15]. There are two forms of quinone, endogenous and exogenous such as vitamin E quinone, ubiquinone and cigarette smoke [16]. NQO1 gene is located on chromosome 16q22 with 6 exon and 5 intron. NQO1 TT genotype has no or decreased activity because of rapid degradation by the ubiquitin/proteasomal system [17]. NQO1 CT genotype has an activity almost half of NQO1 CC genotype activity [18]. NQO1 C609T causes proline to serin substitution in exon 6, codon 187. NQO1 C609T polymorphism has been identified in some cancers such as esophageal and gastric cancer [19], renal [20], breast cancer [21], and bladder cancer [22]. Also NQO1 C609T polymorphism has been correlated with late onset disease such as Parkinson [23]. In considering that NQO1 is as an essential protection against oxidative stress and oxidative stress is as an important risk factor in AMD disease, also every change in antioxidant enzymes cause changes in antioxidant capacity of retina, we tried to investigate the possible association between genetic polymorphism of NQO1 C609T and risk of AMD.

Materials and Methods

Subjects

This study was included 112 cases (44 females, 68 males) with exudative AMD, collected from Khalili Hospital ophthalmic clinic in Shiraz (southern Iran), referred by vitreoretinal surgeon and 112 controls (44 females, 68 males) collected from volunteers in the same clinic. The patients and controls with disease such as cataract, asthma, past history of malignancy, cardiovascular and glaucoma were excluded from this study. Because these mentioned diseases have been associated with NQO1 and other antioxidant genes polymorphisms [19-22,24-27]. The subjects with exudative AMD in one or both eyes had severe visual disturbance and their corrected visual acuity were under 0.1 (<20/200) (range: 8/200 to 19/200). Exudative AMD (which is associated with hemorrhage) will lead to sudden sever decrease in vision. All of the patients were referred to ophthalmologist due to sever visual deterioration. After fundus examination by vitreoretinal surgeon, the event is confirmed by flourescine angiography. General characteristics of the patients and controls have been given in table 1. The age average of patients and controls were 69.5 ± 8.8 (range: 42 to 87) and 63.2 ± 9.9 years (range: 40 to 85), respectively. Iran has a heterogeneous population with different ethnics [28]. So we chose patients and controls from the same ethnical group for decreasing of ethnic differences (Persian living in Fars province, southern Iran). Information about working place and smoking status is gathered by questionnaire. This study was approved by the local ethics committee. Informed written consent was obtained from all participants.

DNA extraction and genotyping analysis

Genomic DNA for PCR was extracted from whole blood using the thawed blood samples by standard procedure [29].

Genotyping of NQO1 C609T: PCR–RFLP method was used for NQO1 genotyping. Used primers to form undigested fragments of 230bp were 5-TCCTCAGAGTGGCATTCTGC-3 (forward) and 5-CCTTCTTTGCGGACCTCTTAT-3 (reverse). PCR conditions were 94°C for 7 min, 35 cycles at 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, and extension period at 72°C for 5 min. PCR products were digested at 37°C overnight with 5U HinfI restriction enzyme and separated on a 3% agarose gel.

Statistical analysis

Statistical analysis was performed with SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) of AMD disease. χ^2 test was used to estimate Hardy–Weinberg equilibrium for studied population (χ^2=0.0076, P>0.05). Also t-test was employed to evaluate statistical differences for age between case and control groups. A P values less than 0.05 were considered as statistical significant differences. There were significant differences for age, working place and smoking distribution between cases and controls. Therefore ORs were adjusted for these three factors. Variants of homozygotes (TT) and heterozygotes (CT) were combined to evaluate the dominant effect.

Demographic data	AMD group	Control group	OR (95%CI)	P-value
Number of participation	112	112		
Smoking status				
Yes, n (%)	42 (37.5%)	16 (14.3%)		
No, n (%)	60 (53.6%)	61 (54.5%)		
Missing	10 (8.9%)	35 (31.3%)	2.66 (1.35-5.25)	0.004
Work place				
Indoor, n (%)	60 (53.65%)	59 (52.7%)		
Outdoor, n (%)		26 (23.2%)	1.96 (1.08-3.55)	0.025
Missing	52 (46.4%)	27 (24.1%)		
Refractive status				
Normal		26(23.2%)		
Hyperopic	15(13.4%)	52(46.4%)		0.172
Myopic	54(48.2%)	34(30.4%)	1.67(0.8-3.49)	0.074
Other diseases	43(38.4%)		2.48(1.13-5.46)	
None		32(28.6%)		
Fatness*	26(23.2%)	5(4.5%)		0.983
Hypertension	4(3.6%)	3(2.7%)	0.98(0.24-4.04)	0.153
DM**	7(6.3%)	4(3.5%)	2.87(0.67-12.22)	0.083
Fatness*+DM**	10(8.9%)	30(26.8%)	3.07(0.86-10.95)	0.247
Fatness*+ Hypertension	37(33%)	23(20.5%)	1.52(0.75-3.07)	0.927
Hypertension + DM**	18(16.1%)	15(13.4%)	0.96(0.43-2.15)	0.684
	10(8.9%)		0.82(0.32-2.13)	

Table 1. Demographic data of the patients and controls

*Based on Basic Mass Index (BMI), BMI= (Weight (Kg)/Height(m))2, BMI >30 means Fatness

**DM= Diabetes Mellitus

Results

As shown in figure 1, the HinfI digestion resulted in one fragment of 200 bp for wild type (CC); two fragments of 151 bp and 49 bp for homozygote (TT); and three fragments of 200 bp, 151 bp and 49 bp for heterozygous (CT). Fragment of 49 bp was not visible in electrophoresis gel (Figure 1).

In this study both case and control groups were divided to working place (indoor and outdoor), smoking (yes and no) groups, sex (male and female) and refractive status (hyperopic, myopic and normal). The subjects were divided to other illness groups (fatness, hypertension and diabetes mellitus). The patient group was divided to two groups of patient with AMD in one and two eyes. With

regard to presence of the retinal lesions, the patient group was divided to two groups (yes and no). In considering to statistical analysis, there was a significant difference for smoking (OR=2.66, 95% CI=1.35-5.25, P=0.004) between case and control groups, frequency of smoking in case group was higher than control group. There was a significant difference for working place (OR=1.96, 95%CI=1.08-3.55, P=0.025), between case and control groups, number of outdoor patients was higher than control group. There was significant difference for age between cases and controls (t = 5.12, df = 222, P<0.001). There was no significant association between hyperopia (OR=1.67, 95%CI=0.8-3.49, P=0.172) and myopia (OR=2.48, 95%CI=1.13-5.46, P=0.074) and exudative AMD. Also there was no significant association between other diseases (fatness, hypertension and diabetes mellitus) and AMD (Table 1). In Considering that there were significant differences for age, smoking and working place distribution between cases and controls, ORs were adjusted for smoking, working place and age. After adjusting, regression logistic analysis showed no significant association between polymorphism of NQO1 C609T and AMD disease (OR=0.84, 95%CI=0.41-1.69, P=0.618,) (Table 2). Also data analysis showed no significant association between polymorphism of NQO1 C609T and bilateralities of the eyes related to AMD (OR=0.9, 95%CI=0.4-2, P=0.792) and between polymorphism of NQO1 C609T and presence of the retinal lesions (OR=1.14, 95%CI=0.48-2.7, P=0.757).

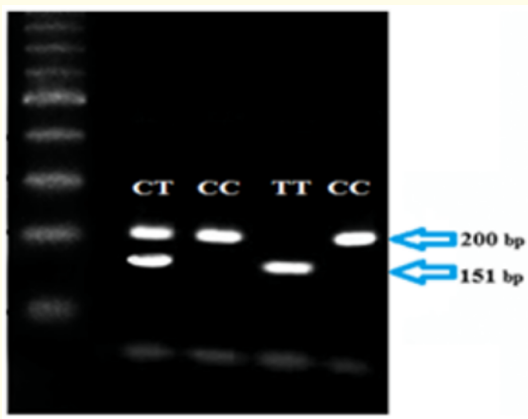

Figure 1: PCR-RFLP data after enzyme digestion by HinfI on electrophoresis gel for NQO1 gene. the HinfI digestion resulted in one fragment of 200 bp for wild type (CC); two fragments of 151 bp and 49 bp for homozygote (TT); and three fragments of 200 bp, 151 bp and 49 bp for heterozygous (CT). Fragment of 49 bp was not visible in electrophoresis gel.

Genotype	Patients (%)	Controls (%)	OR (95%CI)	P-value
***NQO1* C609T**				
CC	71(63.4%)	66(59%)		
CT	33(30%)	40(35.7%)	0.81(0.38-1.72)	0.586
TT	8(6.6%)	6(5.3%)	0.96(0.22-4.19)	0.961
CT+TT vs CC	41(36.6%)	46(41%)	0.84(0.41-1.69)	0.618

Table 2. Polymorphisms in antioxidant gene *NQO1* C609T and risk of AMD development.

Discussion

NQO1 is a part of antioxidant system that causes detoxification of carcinogenic and mutagenic quinone by using NAD(P)H. NQO1 protects cells from quinone-induced damages and active semiquinone production. Also NQO1 protects cells from oxidative stress. Every change in antioxidant genes that causes changes in expression and enzymes function and results in changes in ROS detoxification and increased ROS can increase risk of late onset diseases [30,31]. Pathological role of ROS in increased risk of AMD, a late onset disease has been confirmed by many studies [32], especially the retina where damages in AMD, is susceptible to oxidative stress. So we assumed that NQO1 C609T polymorphism can be interfered in increased risk of AMD disease with effect on antioxidant system. So it is necessary to investigate NQO1 C609T polymorphism in some diseases especially age of onset diseases such as AMD. In this article we studied association between NQO1 C609T polymorphism and risk of AMD disease. But there was no significant association between NQO1 C609T polymorphism and risk of AMD. It might be because of small sample size and thus the limited statistical power to detect difference. In our study, significant associations were seen between either history of outdoor working place or history of smoking and AMD. History of outdoor working place significantly increased the risk of AMD (OR = 1.96, 95% CI=1.09–3.55, P = 0.025). History of smoking was associated with increased risk of AMD (OR = 2.66, 95% CI=1.35–5.25, P = 0.004). Many studies have demonstrated that smoking habit and outdoor working place were significantly associated with AMD [6,31]. Hyperopia has been reported to be associated with wet AMD in several case– control studies [33,34], however current study showed no significant association between hyperopia (OR=1.67, 95%CI=0.8-3.49, P=0.172) and myopia (OR=2.48, 95%CI=1.13-5.46, P=0.074) and wet AMD.

After adjusting smoking, working place and age status was seen no significant association between NQO1 gene polymorphism and AMD (P=0.618, OR=0.84, 95% CI=0.41-1.69). Some studies have been shown association between this polymorphism and some cancer such as esophageal and gastric cancer [19], renal [20], breast cancer [21]. In the study performed by Arshad A Pandith., *et al* in 2011 showed that there was a significant association between NQO1 C609T polymorphism and bladder cancer in Kashmiri population as T allele increases risk of bladder cancer [22]. Also NQO1 C609T polymorphism has been correlated with late onset diseases such as Parkinson [23]. But there was no association between this polymorphism and AMD. In a conclusion, results suggest that T allele in NQO1 genotypes may not be associated with an increased risk of AMD (P=0.735, OR=0.92, 95%CI=0.59-1.44). We suggest studying more in a large-scale study, studying on other genetic polymorphisms in NQO1 gene and other classes of NQO gene such as NQO2 and NQO3. We suggest studying on another form of AMD (dry AMD) in different stages of disease from early to advance.

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Conflict of Interest

None declared.

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