Association between Genetic Polymorphism of Catalase (CAT) C-262T, Cu/Zn Superoxide Dismutase (SOD1) A251G and Risk of Age-related Macular Degeneration

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Abstract

**Background:** Cells have complex network of antioxidant enzymes that protect cells from induced damages by reactive oxygen species (ROS). Catalase and superoxide dismutase are known for their role as primary protection against oxidative stress. Oxidative damage is an important risk factor in age-related macular degeneration disease (AMD). For the first time in this study the impact of genetic polymorphisms of SOD1 and CAT with AMD has been examined. Hence, the association between genetic polymorphisms of catalase (CAT) C-262T, Cu/Zn superoxide dismutase (SOD1) A251G and risk of exudative AMD has been investigated.

**Methods:** This study was carried out on blood samples collected from 112 exudative AMD patients and 112 healthy individuals. Genotyping of CAT C-262T and SOD1A251G was done by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. Differences in the frequencies were estimated using the χ² test and risk was estimated with a logistic regression after adjusting for smoking, working place and age status.

**Results:** There was significant difference between CAT CT+TT genotype and AMD disease (P=0.009, OR=0.38, 95%CI=0.18-0.78). Also T-allele has a significant association with risk of AMD and decreases risk of disease (P=0.036, OR=0.59, 95%CI=0.36-0.96), but there was no significant differences between SOD1A251G and variant homozygous and heterozygous frequencies in patients compared to controls (P=0.589, OR=0.77, 95%CI=0.3-1.96).

**Conclusions:** The data presented suggest that the T-allele in CAT genotypes can increase catalase expression and activity, as a result of which generation of reactive oxygen species (ROS) can be decreased. Therefore it is suggested that increased expression of CAT as a result of T-allele in CAT genotypes and existence of T-allele in CAT genotypes is associated with decreased risk of AMD.

**Keywords:** Age-related macular degeneration (AMD); Catalase (CAT); Cu/Zn Superoxide dismutase (SOD1); Polymorphism

**Introduction**

Age-related macular degeneration (AMD) is one of the reasons of losing vision in patients over the age of 60 years because of decayed retina. In some people, AMD develops slowly with ageing that gradually can cause losing vision in one or two eyes but in the others, this process is faster (Gehrs et al.,2006). Smoking and exposing by sunlight are some environmental risk factors that have been associated with an increased risk of AMD (El Matri et al.,2012). These factors can cause reactive oxygen species (ROS)-induced...
oxidative stress in retinal pigment epithelium (RPE) cells (Coleman et al., 2008). The high level of ROS causes macromolecular (DNA, Protein, lipid) damages (Dufour et al., 2000). The other risk factors are family history (Smith et al., 1998), sex, race (Clemons et al., 2005), high blood pressure and cholesterol. Genetic factors especially genes coding complement system proteins have been identified to be associated with AMD disease (Hughes et al., 2006). Also there is a Mendelian inherited kind of disease that occurs in the early ages and can be either autosomal dominant or recessive (El Matri et al., 2012). Because of retina’s high metabolic activity, high oxygen pressure and concentration of oxidized poly unsaturated fatty acids (PUFAs), the retina is susceptible to oxidative damage. Also retinal pigments generate ROS while exposing by sunlight (Beatty et al., 2000). Ageing induces retinal changes caused by ROS and oxidative stress in RPE cell shape and size (Gao et al., 1992).

There are two forms of advanced AMD; the "dry form" known as ‘geographic atrophic’ and the "wet form" or neovascular/exudative form. In dry form, ruined cells called drosen are accumulated between the retina and the choroid and the retina removed. In wet form, new blood vessels are formed from the choroid which known as neovascularization (CNV). This form of AMD is called exudative or neovascular AMD (Coleman et al., 2008; Spraul et al., 1997).

CAT is one of the most important antioxidant enzymes that protects organisms from oxidative damages induced by ROS (Chandrasena et al., 2006). This enzyme consists of four identical subunits; its gene is located on chromosome 11P13. CAT is mainly located in peroxisome of tissues such as liver, kidney and in the mature red blood cell in its cytoplasmic form. CAT, like superoxide dismutase and glutathione peroxidase contribute to the cell protection against oxidative stress. CAT catalyzes the hydrogen peroxide into water and oxygen (Halliwell et al., 1989). Many mutation and polymorphism in CAT gene has been associated with thalassemia (Halliwell et al., 1989; Quan et al., 1989; Ogata, 1991). Genetic polymorphisms in CAT have been associated with cancer and type 1 diabetes (Tang et al., 2010; Cebrian et al., 2006, Chistiakov et al., 2004).

One of the most important enzymes of superoxide dismutase (SOD) family is Cu/Zn SOD (SOD1) enzyme in cytoplasm. Along with CAT enzyme, SOD1 protects macromolecules from ROS induced damages that are generated by environmental factors such as, UV and X ray exposure, pressure and heat. SOD1 which is leaked from mitochondria into cytoplasm (Tilak et al., 2004) is a
homodimer protein and its gene is located on chromosome 21q22. Many mutations in SOD1 gene has been described with familial amyotrophic lateral sclerosis (FALS). The level of enzyme activity decreased significantly in most of the mutations (McCord et al., 1969; Forsberg et al., 2001b).

Previously, the association between the CAT and SOD1 gene polymorphism and other age-related diseases such as breast cancer (Oestergaard et al., 2006), glioma (Rajaraman et al., 2010), cataract (Zhang et al., 2011), Alzheimer (Capurso et al., 2008), pseudoxanthoma elasticum (Zarbock et al., 2007), colorectal cancer (Funke et al., 2009), skin cancer (He et al., 2010), pancreatic cancer (Tang et al., 2010) and type 2 diabetes (Hong Chen et al., 2011) has been reported. However, to our knowledge there are no reports on the association of polymorphisms in antioxidant genes such as CAT and SOD1 with AMD disorder. Based on this, the present study is aimed to investigate the possible association between genetic polymorphisms of CAT C-262T, SOD1 A251G and risk of AMD development.

Materials and Methods
Subjects
This case–control study consisted of 112 subjects (44 female and 68 male) diagnosed with exudative AMD in one or two eyes, referred by vitreoretinal surgeon. All the patients were admitted to the Ophthalmic Clinic at the Khalili hospital in Shiraz, Iran and blood sample was collected from each subject. Besides, blood samples from 112 sex-matched controls were obtained from volunteers in the same clinic and considered as control group. The study excluded subjects with diseases such as cataract, asthma, past history of malignancy, cardiovascular and glaucoma. Because these diseases have been associated with CAT and other antioxidant enzymes polymorphisms (Zhang et al., 2011; Fan et al., 2010; Islam et al., 2007; Rajič et al., 2009).

The subjects with exudative AMD in one or both eyes had severe visual disturbance and their corrected visual acuity were under 0.1. Exudative AMD (which is associated with hemorrhage) will lead to sudden severe decrease in vision. All of the patients were referred to ophthalmologist due to severe visual deterioration. After fundus examination by vitreoretinal surgeon, the event is confirmed by fluorescine angiography. General characteristics of the patients and controls have been given in Table 1. Average age of patients and controls were 69.5±8.8 (range: 42 to 87) and 63.2±9.9 years (range: 40 to 85), respectively. Iranian population is a heterogeneous population with variety ethnics (Rafiee et al., 2010). Therefore to minimize the genetic differences among the ethnic groups
both the patients and controls were selected from the same ethnical group (Persian living in Fars province, southern Iran). Information about working place and smoking status was gathered from patient and control groups. This study was approved by the local Ethics Committee. Informed written consent was obtained from all participants.

DNA extraction and genotyping analysis

The collected blood samples from both of groups were stored at -80°C until use. Genomic DNA was extracted from whole blood for polymerase chain reaction (PCR) using standard procedure (Newton,1995). Briefly, rotating 200µl blood sample and 800µl NH4Cl for 20 min, washing created sediment after centrifugation in 400µl NaCl-EDTA, adding 500µl NaOH and placing in boiling water for 5 min, adding 100µl Tris-HCl to above solution, extracting of DNA from supernatant solution.

Genotyping of CAT C-262T: CAT genotyping was performed by using PCR–RFLP method. The C>T conversion in the promoter region in -262 was amplified to form undigested fragment of 190 bp by using primers 5′-CTGATAAACCGGAGCCCCCGCCCTGGGTT and 5′-CTAGGCAGGCCAAGATTGGAAGCCCAATGG-3′ as forward and reverse primers, respectively (Sinagene Company) (Zarbock et al.,2007). The PCR mixture was 13.95µl water, 2.5µl buffer10X, 0.75µl MgCl2, 0.5µl dNTP, 1µl forward and reverse primers and 0.3µl Taq DNA polymerase for every 5µl DNA sample. The PCR condition was; 95°C for 15 min, followed by 35 cycles of 94°C for 1 min, 68°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 15 min. The 190-bp fragment as a PCR product was digested by 5U EcoRV restriction enzyme at 37 °C overnight and separated on a 1.5% agarose gel.

Genotyping of SOD1 A251G: SOD1 genotyping was performed by using a PCR–RFLP method. An A>G in intron was amplified to form undigested fragments of 570 bp using primers 5′-AGTACTGTCAACCACATAGCA-3′ and 5′-CCAGTGTGGCGGCAATGATG-3′ as forward and reverse primers, respectively (synthesized by Sinagene Company) (Zhang et al.,2011). The PCR mixture comprised of 13.95µl water, 2.5µl buffer10X, 0.75µl MgCl2, 0.5µl dNTP, 1µl forward and reverse primers and 0.3µl Taq DNA polymerase for every 5µl DNA sample. PCR conditions were 94°C for 4 min, followed by 33 cycles of 94°C for 50 s, 63°C for 50 s, 72°C for 50 s, and a final extension step at 72°C for 7 min. The 570 bp PCR products were digested at 37 °C overnight with 5U MspI and separated on a 2% agarose gel.

Statistical analysis

Statistical analysis was performed by SPSS for
Windows (version 16.0; SPSS Inc., Chicago, IL). Logistic regression model was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for risk of AMD disease associated with \textit{CAT} and \textit{SOD1} polymorphisms and $\chi^2$ test was used to estimate Hardy–Weinberg equilibrium for studied population, ($\chi^2=0.03$, $P>0.05$) for \textit{CAT} genotypes and ($\chi^2=0.015$, $P>0.05$) for \textit{SOD1} genotypes. Also t-test was employed to evaluate statistical differences for age between case and control. $P$ values less than 0.05 were considered as statistical significant differences. There were significant differences for age, working place and smoking between cases and controls. Therefore ORs were adjusted for these three factors. Variants of homozygotes and heterozygotes were combined to evaluate the dominant effect.

\textbf{Results}

As shown in figure 1A, the EcoRV digestion resulted in one fragment of 190 bp for homozygous (TT); two fragments of 157bp and 33bp for wild-type (CC); and three fragments of 190bp, 157bp and 33bp for heterozygous (CT). MspI digestion also resulted in one fragment of 570 bp for wild-type (AA); two fragments of 369 and 201 bp for homozygous (GG); and three fragments of 570, 369, and 201 bp for heterozygous (AG) (Fig. 1, section B).

In this study both patients and controls were divided into sub-groups, according to sex (male/female), working place (indoor/outdoor), smoking (yes/no) and refractive status (hyperopic, myopic or normal). The subjects were further divided into other illness groups (fatness, hypertension and diabetes mellitus). The patient group was divided into two sub-groups of patient with AMD in one and two eyes. Based on statistical analysis, the frequency of smoking patients was significantly higher than control group (OR=2.66, 95% CI=1.35-5.25, $P=0.004$). Also the number of outdoor patients was significantly higher than control group (OR=1.96, 95% CI=1.08-3.55, $P=0.025$). There was a significant difference in terms of age between cases and controls ($t=5.12$, df = 222, $P<0.001$). Whereas, 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 among the patients diagnosed with other diseases (obese, hypertension and diabetes mellitus) and AMD (Table-1).

To reduce the influence of environmental factors on the genotypes, the ORs were adjusted for age, working place and smoking. After adjusting, binary logistic regression showed significant association between CT genotype of \textit{CAT} C-262T polymorphism and AMD disease (OR=0.37, 95% CI=0.17-0.81,
P=0.012), because of few numbers of TT genotypes, CT and TT genotypes of \textit{CAT} C-262T polymorphism were considered together to evaluate the dominant effect. Data analysis showed significant association between CT+TT genotypes in contrast with CC genotype of \textit{CAT} C-262T polymorphism and AMD (OR=0.38, 95\%CI=0.18-0.78, P=0.009) (Table-2). Likewise, the T-allele showed significant association with risk of AMD and decreases risk of disease (OR=0.59, 95\%CI=0.36-0.96, P=0.036). But there was no significant association between polymorphism of \textit{SOD1} A251G (OR=0.77, 95\%CI=0.3-1.96, P=0.589) and AMD disease. Also data analysis showed no significant association between polymorphisms of \textit{CAT} C-262T (OR=1, 95\%CI=0.41-2.44, P=0.9), \textit{SOD1} A251G (OR=1.06, 95\%CI=0.38-2.95, P=0.91) and bilateralities of the eyes related to AMD.

### Table 1
Demographic data of the AMD patients and controls.

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>AMD group</th>
<th>Control group</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participation</td>
<td>112</td>
<td>112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>42 (37.5%)</td>
<td>16 (14.3%)</td>
<td>2.66 (1.35-5.25)</td>
<td>0.004</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>60 (53.6%)</td>
<td>61 (54.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>10 (8.9%)</td>
<td>35 (31.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work place</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor, n (%)</td>
<td>60 (53.6%)</td>
<td>59 (52.7%)</td>
<td>1.96 (1.08-3.55)</td>
<td>0.025</td>
</tr>
<tr>
<td>Outdoor, n (%)</td>
<td>52 (46.4%)</td>
<td>26 (23.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>27 (24.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refractive status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>15 (13.4%)</td>
<td>26 (23.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperopic</td>
<td>54 (48.2%)</td>
<td>52 (46.4%)</td>
<td>1.67 (0.8-3.49)</td>
<td>0.172</td>
</tr>
<tr>
<td>Myopic</td>
<td>43 (38.4%)</td>
<td>34 (30.4%)</td>
<td>2.48 (1.13-5.46)</td>
<td>0.074</td>
</tr>
<tr>
<td>Other diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>26 (23.2%)</td>
<td>32 (28.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatness*</td>
<td>4 (3.6%)</td>
<td>5 (4.5%)</td>
<td>0.98 (0.24-4.04)</td>
<td>0.983</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (6.3%)</td>
<td>3 (2.7%)</td>
<td>2.87 (0.67-12.22)</td>
<td>0.153</td>
</tr>
<tr>
<td>DM**</td>
<td>10 (8.9%)</td>
<td>4 (3.5%)</td>
<td>3.07 (0.86-10.95)</td>
<td>0.083</td>
</tr>
<tr>
<td>Fatness*+DM**</td>
<td>37 (33%)</td>
<td>30 (26.8%)</td>
<td>1.52 (0.75-3.07)</td>
<td>0.247</td>
</tr>
<tr>
<td>Fatness*+Hypertension</td>
<td>18 (16.1%)</td>
<td>23 (20.5%)</td>
<td>0.96 (0.43-2.15)</td>
<td>0.927</td>
</tr>
<tr>
<td>Hypertension+DM**</td>
<td>10 (8.9%)</td>
<td>15 (13.4%)</td>
<td>0.82 (0.32-2.13)</td>
<td>0.684</td>
</tr>
</tbody>
</table>

*Based on Body Mass Index (BMI), BMI=(Weight(Kg)/Height(m))^2, BMI >30 means Fatness
**DM = Diabetes Mellitus
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Figure 1. A: PCR-RFLP data after enzyme digestion by EcoRV on electrophoresis gel for CAT gene. B: PCR-RFLP data after digestion by MspI on electrophoresis gel for SOD1 gene.

Table 2 Polymorphism in antioxidant genes; CAT C-262T, SOD1 A251G and risk factor of AMD development.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT C-262T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>84 (75%)</td>
<td>69 (61.6%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>25(22.4%)</td>
<td>38(34%)</td>
<td>0.37(0.17-0.81)</td>
<td>0.012</td>
</tr>
<tr>
<td>TT</td>
<td>3(2.6%)</td>
<td>5(4.4%)</td>
<td>0.39(0.06-2.52)</td>
<td>0.323</td>
</tr>
<tr>
<td>CT+TT</td>
<td>28 (25%)</td>
<td>43 (38.4%)</td>
<td>0.38(0.18-0.78)</td>
<td>0.009</td>
</tr>
<tr>
<td>SOD1 A251G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>93(83%)</td>
<td>91(81.3%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>19(17%)</td>
<td>20(17.8%)</td>
<td>0.83(0.32-2.15)</td>
<td>0.697</td>
</tr>
<tr>
<td>GG</td>
<td>0</td>
<td>1(0.9%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>AG+GG</td>
<td>19(17%)</td>
<td>21(18.7%)</td>
<td>0.77(0.3-1.96)</td>
<td>0.589</td>
</tr>
</tbody>
</table>

Discussion

In this study we used a genetic polymorphism of C-262T in promoter region of CAT gene because the impact of this genotype on the level of gene expression and enzyme activity is well established. Also we selected a genetic polymorphism A251G in intron of SOD1 gene because a genetic polymorphism in intron of SOD1 gene can make SOD1 mRNA unstable. CAT is one of the most important antioxidant enzymes that protects organisms from oxidative stress and modulate ROS generation which is responsible for cellular damages by converting H2O2 to H2O and O2 (Halliwell et al., 1989). Every change in detoxification genes such as CAT causes changes in enzyme activity, quantity and ROS detoxification (Forsberg et al., 2001(a)). Also every change in the antioxidant enzymes such as CAT and SOD can interfere in age at onset disease (Muller et al., 2007; Arsova-Sarafinovska1y et al., 2008). AMD is a kind of age of onset disease (Gehrs et al., 2006) and the pathogenic role of ROS in AMD has been reported by
others (Coleman et al., 2008). The retina is a main region of eye that is damaged in AMD, which is susceptible to the deleterious effects of oxidative stress (Beatty et al., 2000). Hence it was assumed that the polymorphisms CAT and SOD1 can play a role in modulation of ROS production in AMD disorder. This study showed a good association of smoking (OR=2.66, 95% CI=1.35-5.25, P=0.004) and working place (OR=1.96, 95% CI=1.08-3.55, P=0.025) status and AMD disease. This finding is in agreement with other reports showing that smoking habit and the amount of leisure time spent outdoors are significantly associated with AMD (Newton, 1995; Muller et al., 2007). Also the information about the role of work place agrees with reports from other laboratories.

Hyperopia has been reported to be associated with wet AMD in several case–control studies (Sandberg et al., 1993; Boker et al., 1993), but 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 there was a significant association between CAT CT+TT genotype (P=0.009, OR=0.38, 95% CI=0.18-0.78) and AMD. Our data showed that the T-allele decreases the risk of AMD (P=0.036, OR=0.59, 95% CI=0.36-0.96). A recent study performed in Sweden, showed that the CAT C-262T gene polymorphism leads to increased expression of CAT in erythrocytes (Forsberg et al., 2001a). According to this study, existence of T-allele can improve antioxidant status and lower the rate of ROS, suggesting that the T-allele can decrease risk of susceptible diseases such as AMD to oxidant. Other reports showed an association between CAT polymorphism and other age onset disease was performed in Germany (Zarbock et al., 2007), by showing that CAT C-262T gene polymorphism is associated with Pseudoxanthoma Elasticum (an early onset disease). The C allele in CAT C-262T has also been reported to be linked to the risk of type 2 diabetes (Hong Chen et al., 2011). The CAT C-262T and some age-related diseases is probably differs in different diseases and the ethnic groups. In this connection Cristiano Capurso and co-workers (2008) reported no significant association between CAT C-262T and Alzheimer in an Italian population. Lack of association between CAT C-262T and other diseases has been reported from other countries (Funke et al., 2009; He et al., 2010). Studies focused on SOD1 A251G polymorphism and age-related diseases also failed to show a major association In Studies by Oestergaard and et al. (2006) in Britain showed no significant association between SOD1 A251G and breast cancer. Similar
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results were reported by Rajaraman et al., 2010 by reporting no significant association between SOD1 A251G and glioma. Studies on a Chinese population, however showed a significant association between SOD1 A251G and risk of age-related cataract and G allele increases risk of cataract but showed no significant association with CAT A-21T polymorphism (Zhang et al.,2011).

In conclusion, the results presented in this study in favor of the reports showing no significant association between SOD1 A251G and AMD. There are several factors which may influence the relationship between the clinical manifestation of the disease and polymorphism in one or more antioxidant enzyme genotypes. Further studies with a larger population with different isoforms of SOD such as SOD2 and SOD3 and other polymorphisms in these genes may help understanding the contribution of these antioxidant enzymes during different stages of AMD development.

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Conflict of Interest
None declared.

References:


[19] He C, Qureshi AA, Han J. 2010. Polymorphisms in genes involved in oxidative stress and their interactions with


