https://doi.org/10.36570/jduhs.2022.1.1609

JDUHS

ORIGINAL ARTICLE

Superoxide Dismutase (SOD1) Gene Polymorphism in Cataract Patients of Karachi, Pakistan

Atif Mahmood,[1] Qamer Aziz,[2] Ruqaya Nangrejo,[2] Iftikhar Ahmed Siddiqui,[3] Mohammad Saleh Soomro,[2] Iffat Ara Aziz[3]

1. Department of Physiology, Bhitai Dental and Medical College, Mirpurkhas, Pakistan.
2. Department of Physiology, Baqai Medical University, Karachi, Pakistan.
3. Department of Biochemistry, Baqai Medical University, Karachi, Pakistan.

Correspondence to: Dr. Atif Mahmood, Email: atif_mahmood20@yahoo.com, ORCiD: 0000-0002-3399-5820

ABSTRACT

Objective: To investigate the polymorphism superoxide dismutase (SOD1) gene and its relationship with the development of cataracts in patients of Karachi.

Methods: This was a case-control study carried out at different centers in Karachi, Pakistan between September 2019 and 2020. A single nucleotide polymorphism (SNP) at rs2070424(SOD1) locus was examined with polymerase chain reaction (PCR) using high resolution melting curve (HRM) technique in 250 cataract patients and 250 healthy control groups of similar age and gender.

Results: Of 500 subjects, there were 250 cases and 250 controls. The mean age of the cataract patients (cases) was 43.97± 13.36 years. Among cases, 150 (60.0%) were males and 100 (40.0%) were females whereas in controls, 127 were males (50.8%) and 123 (49.2%) were females. A significant association of cataract was found with age (p value = 0.005), and smoking (p-value = 0.001). AG genotype has a smaller risk (OR=1.77, 95% CI 1.11 – 2.66, p-value= 0.006) associated with cataract while GG has a higher risk (OR = 2.83, 95% CI 1.71 – 4.68, p-value < 0.001). Further, calculation of allelic distribution revealed that the G allele was more prevalent in the cataract group 200 (61.7%) as compared to controls 124 (38.3%) (p-value=0.001).

Conclusion: The significantly higher G allele frequency in the SOD1 gene at the rs2070424(SOD1) locus in the cataract group indicates that potential risk factors contribute to cataracts' development. This study contributes to the genetic profiling of cataract patients in the Pakistani population.

Keywords: Cataract, Polymerase Chain Reaction, Polymorphism, Reactive Oxygen Species, Superoxide Dismutase, Single Nucleotide.

INTRODUCTION

Among all the causes of visual impairment and blindness, the cataract has been reported to be the most important and most common as it is responsible for blindness in 94 million (47.8%) individuals out of a total of 1 billion blinds present worldwide.[1,2] The genetic susceptibility of the cataract has an established role in congenital cataracts but also considered a contributor for the rapid progression of other forms of cataracts including senile cataracts.[3]

Though many factors are involved in the initiation of cataract formation, deficiency of antioxidants are most frequently reported.[2] In cases of antioxidants depletion, there is an overall decrease in the antioxidants' levels compared to pro-oxidants, which disturbs the redox homeostasis. Therefore, decreased production or regulation of antioxidant enzymes can adversely affect the reactive oxygen species (ROS) detoxification process and can be caused an initial event in the progression of some cataracts.[4,5]

Among other antioxidant genes for ROS detoxification, the superoxide dismutase (SOD1) gene seems to have a greater impact. SOD1 enzyme is also found in the ocular lens and its relatively compromised activity has also been observed in cataract patients.[6] Protective effects of SOD1 against oxidative stress have been demonstrated in whole rat lenses and human lens epithelial cells when these enzymes were overexpressed.[7] On the contrary, lenses from SOD1-

knockout mice developed lens opacities earlier than wild-type mice.[8] Further, genetic variations in the genes that code for these enzymes may modify the risk of disease as they protect the cell against ROS.[9] Many studies reveal that single nucleotide polymorphisms (SNP) in the SOD1 gene are associated with different diseases like diabetes.[10] Similarly, one study found SOD1 gene variant rs2070424(SOD1) link to cataract.[11] However, this SNP in SOD1 gene was not studied in the Pakistani population yet. Therefore, present study aims to find out the association of polymorphisms of SOD1 gene rs2070424(SOD1) with cataract in Pakistani people.

METHODS

This was a case-control study carried on cataract patients who attended outpatient departments of Fatima Hospital, Baqai Medical University, and LRBT Hospital between September 2019 and 2020. The study was approved by the ERC/BASR of Baqai Medical University and written informed consent was obtained from all the participants.

The total sample size was 500 (cataract patients; 250, control; 250) and it was calculated using Rao Soft sample size calculator.[12]

$$x = Z(^{c}/_{100})^{2}r(100-r)$$
$$n = {}^{Nx}/_{((N-1)E^{2}+x)}$$
$$E = \mathrm{Sqrt}[{}^{(N-n)x}/_{n(N-1)}]$$

Where *N* is the population size, *r* is the fraction of responses that you are interested in, and *Z*(*c*/100) is the critical value for the confidence level *c*.

All the subjects with cataract with severe visual disturbances, and their best-corrected visual acuities in the better eye under 0.3 (3/60) and cataract in at least one eye and no other eye abnormalities that could explain the vision impairment were included in the study. Whereas, all the subjects having cataracts due to secondary diseases like diabetes, hypertension, and trauma and those due to administration of steroids were excluded from the study.

Each consenting participant had to undergo a detailed medical history with the help of a questionnaire and an ocular examination on a slit lamp performed by experts. Socio-demographic data, family history, and brief medical history were also obtained from each patient.

Whole blood samples were collected from all cases and controls. A total of 5ml of blood specimen was collected by venipuncture in an anticoagulant (EDTA) containing tube (purple top). The entire blood collection process was performed by an experienced phlebotomist. Once the blood sample was collected, it was immediately stored at -80 °C till further use.

The blood samples were thawed and centrifuged at 800X g for 10-15 minutes. The buffy coat was carefully removed into a separate 1.5ml DNase and RNase-free Eppendorf tube. The genomic DNA extract was performed according to the guidelines provided by the kit manufacturing company (Thermo Fisher, K022). For detection of DNA sequence variations, we used a ready-to-use master mix (Thermo Scientific, cat no K1031) for the High-Resolution Melt (HRM) analysis method.

Briefly, all samples were vortexed and centrifuged after thawing. Master Mix (2X), primers, and water were added in a tube for each PCR reaction at room temperature and dispensed at appropriate volumes into PCR tubes followed by the addition of DNA template (≤ 20 ng/reaction). The thermal cycler was run according to the following program. Initial temp 95°C for 10 minutes, denaturation temperature 95°C for 10 seconds, and annealing temperature were 60°C for 60 seconds run 40 cycles.

For the HRM curve, the temperature range was 65-90 °C with an increment of 0.2 °C. For detection of possible SNPs, data were included in the graph from 80-90 °C. At least three samples were run on the gel for confirmation of SNP for each gene.

For statistical analyses, we utilized IBM-SPSS (version 21.0; SPSS Inc., Chicago, IL). We performed a 2-tail Chi-square test to determine the association of alleles with genotype while comparing test sample results to the controls. Binary logistic regression analysis was done to see the relationship of polymorphisms within genotype allele of distinct genes among the test and control groups with an estimation of odds ratio (OR) along with 95% confidence interval (CIs). The p-value of ≤ 0.05 was considered statistically significant.

The following genes were amplified with the specific set of primers given:

S. No	SNP and gene symbol	Primer Sequence
1.	rs2070424(SOD1) (SOD-1)	SOD1 Left 5'- CTGAAAACTAGTCGAGACTCCAT -3' SOD1 Right 5' –CAAGGCTTCACGTCTACAC – 3'

RESULTS

Of 500 subjects, there were 250 cases and 250 controls. The mean age of the cataract patients (cases) was 43.97± 13.36 years. Most of the patients had family history of cataract 295 (59.0%) while majority of the patients were nonsmokers 365 (60.0%).

Table 1 shows the distribution of risk factors among cases and controls. Majority of the patients 202 (53.5%) were greater than 50 years of age. Among cases who were included in the study, 150 (60.0%) were males and 100 (40.0%) were females whereas in controls, 127 were males (50.8%) and 123 (49.2%) were females. Overall, most of patients 155 (52.5%) and most of the controls 140 (47.5%) had family history of cataract. A significant association of cataract was found with age (p value = 0.005) and smoking (p-value = 0.001).

Melting curves of different genotypes of gene represented in the Figure 1A. Heterozygous genotype of this gene shifted HRM curve about 1 °C towards lower temperature while homozygous shifted only 0.3 °C as compared to wild type AA (Figure 1A). The statistical analysis revealed a possible risk of the *SOD1* genotype in the development of cataracts (p-value <0.001).

Graphical representation of this data is shown in Figure 1B.The genotypic frequencies of AA, GG, and AG between the control and cataract groups are illustrated in Table 2. We observed significantly increased risk for the development of cataract associated with mutant AG gene (OR=1.77, 95% CI 1.11 – 2.66, p-value= 0.006) and GG genotype polymorphism (OR = 2.83, 95% CI 1.71 – 4.68, p-value < 0.001).

Further, we calculated allelic distribution among cases and control. We observed a higher frequency of the A allele 376 (55.6%) while the G allele was estimated at only 124 (38.3%) among controls. However, the A allele frequency was down to 300 (44.4%) in the cataract group and the G allele became more prevalent 200 (61.7%) in the cataract group (Table 2).

DISCUSSION

The study was conducted to analyze the possible association of cataract with polymorphisms of the SOD1 gene in Pakistani cataract patients. The oxidative

Table 1: Distribution of risk factors of cataract (N=500)

Risk Factors	Total	Samples (N=500)		*p-value*
		Cases (n=250)	Control (n=250)	
Age				
≤50 years	123	48 (39.0)	75 (61.0)	0.005*
>50 years	377	202 (53.5)	175 (46.5)	
Family History of Cataract				
Yes	295	155 (52.5)	140 (47.5)	0.173
No	205	95 (46.3)	110 (53.7)	
Smoking				
Yes	136	52 (38.2)	84 (61.8)	0.001*
No	364	198 (54.4)	166 (45.6)	

Chi-Square test applied, *p-value ≤ 0.05

Table 2: Allelic distribution of SOD1 variant (rs2070424) between control and cataract patients (N=500)

Genotypes	Cases (n=250)	Control (n=250)	OR (CI)	*p*-value
A/A (n=266)	110 (41.4)	156 (58.6)	1	
A/G (n=144)	80 (55.6)	64 (44.4)	1.77 (1.112.66)	0.006
G/G (n=90)	60 (66.7)	30 (33.3)	2.83 (1.71-4.68)	< 0.001
A alleles frequency (n=676)	300 (44.4)	376 (55.6)	1	
G alleles frequency (n=324)	200 (61.7)	124 (38.3)	2.0 (1.54-2.64)	< 0.001

OR: odds ratio, CI: confidence interval

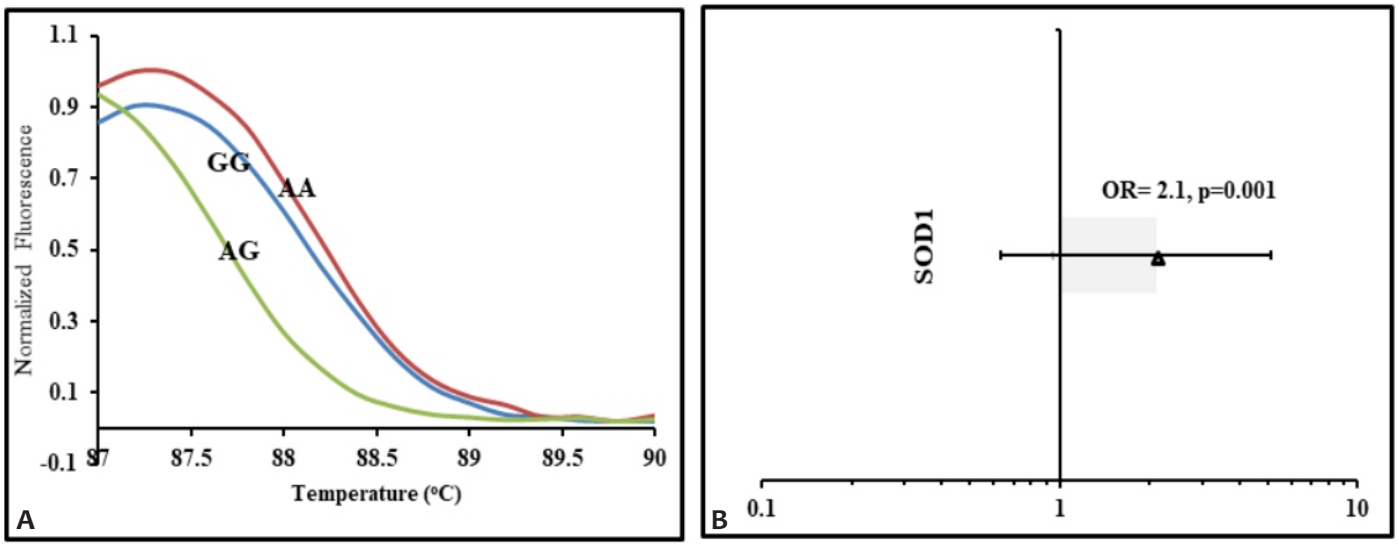

Figure 1: Risk association of SOD1 gene with cataract. A) Figure showing the melting curve of SOD1 gene. B) Plot represented calculated odds ratios for SOD1 genes

damage caused by the altered function of the antioxidant enzyme which in turn is due to the findings polymorphisms in the antioxidant enzyme gene may explain the possible mechanism of the development of cataracts. The present study reported an increased risk of cataracts due to SOD1 gene polymorphisms. These support that increased oxidative stress or decreased antioxidant capacity can increase the risk of the formation of cataracts in individuals with genetic polymorphisms of the SOD1 gene.

Epithelial cells play a very important role in the maintenance of the entire organ and are the first line of defense of the lens against stress. Furthermore, the transport fluid to the lens is also controlled by the epithelial cells which makes them most vulnerable to oxidative damage as they are in direct contact with the aqueous humor.[13] Bulk protein oxidation, inactivation of key enzymes, DNA breaks, and lipid peroxidation are modes of higher oxidative stress that may damage lens epithelial cells and it is similar to the damage caused in other organs.[5]

In the oxidative defense mechanism, SOD1 is considered to be very important among the antioxidant enzymes. Several studies reflect the association of SOD1 activity with the formation of cataracts. It is reported that expression of SOD1 in the lens is higher in H_2O_2-induced oxidative damage[14] and the level of SOD1 activity is reported significantly lower in mature cataractous lenses.[15] Similarly, chromosomal modifications and gene mutation of SOD1 are related to the development of cataracts. For example, reduced histone acetylation at the promoter region of SOD1 was observed in senile cataracts[15] and SOD1-null mice produced more cases of cataract.[16]

Our results of SOD1 gene polymorphism are similar to a Chinese study. They found that the genotype frequency of the GG and AA of SOD1–251A/G was significantly different in cataract patients.[11] On the contrary, there was no significant difference reported in the distribution of SOD1+35 A/C polymorphism in the Iranian population.[17] Another study conducted on the Estonian population reported that SNP in the SOD gene did not show any associations with the risk of cataracts.[18] These results suggested that different populations and polymorphism locations on genes may have variable strength of risk of disease.

The present study is conducted at multiple centres in Karachi. Although Karachi is a cosmopolitan city with multicultural demographics, still there is a need for broader studies to be conducted across the country and thus, this forms the major limitation of the study. There is a need for further exploration of the other loci of superoxide dismutase genes in our population. The study provides a platform for all the researchers to further explore all the antioxidant enzyme gene polymorphism in the Pakistani population.

CONCLUSION

In conclusion, this study is suggestive of a possible association between the SOD1 gene polymorphisms with the formation of cataracts in Pakistani cataract patients. There is, however, a large gap in our understandings of our ethnic complexities and our sociocultural values, and their relationship with other antioxidant enzymes polymorphisms like Glutathione

Peroxidase (GPX) and Catalase (CAT). This data can facilitate us in preventing or slowing the progression of age-related cataract formation by using antioxidant enzyme and their genes as pharmacological targets to reduce ROS production. Nevertheless, there is a need for larger studies such as RCTs that could support the use of targeted pharmacotherapy or gene therapy.

ETHICAL APPROVAL: Ethical approval for the study was granted by the Ethics Committee of the Baqai Medical University Karachi, Pakistan (Reference No. BMU-EC/05-2021).

AUTHORS' CONTRIBUTION: AM: Principal investigator, analysis and interpretation of data. QZ: Revising manuscript critically. MSS: Concept & drafting of the work. IAS: Final approval of the manuscript. RN: Critical interpretation of data. IA: Lab technical support and data analysis.

CONFLICT OF INTEREST: No conflict of interest from any author.

FUNDING: None.

Received: February 07, 2022
Accepted: April 06, 2022

REFERENCES

1. Bourne R, Steinmetz JD, Flaxman S, Briant PS, Taylor HR, Resnikoff S, et al. Trends in prevalence of blindness and distance and near vision impairment over 30 years: an analysis for the Global Burden of Disease Study. Lancet Global Health 2021; 9:e130-43. doi: 10.1016/S2214-109X(20)30425-3
2. Steinmetz JD, Bourne RR, Briant PS. GBD 2019 Blindness and Vision Impairment Collaborators; Vision Loss Expert Group of the Global Burden of Disease Study. Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: the Right to Sight: an analysis for the Global Burden of Disease Study. Lancet Glob Health 2021; 9:e144-60. doi: 10.1016/S2214-109X(20)30489-7
3. Vrensen GF. Early cortical lens opacities: a short overview. Acta Ophthalmol 2009; 87:602-10. doi: 10.1111/j.1755-3768.2009.01674.x
4. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 2015; 30:11-26. doi: 10.1007/s12291-014-0446-0
5. Periyasamy P, Shinohara T. Age-related cataracts: Role of unfolded protein response, Ca^{2+} mobilization, epigenetic DNA modifications, and loss of Nrf2/Keap1 dependent cytoprotection. Prog Retin Eye Res 2017; 6:1-19. doi: 10.1016/j.preteyeres.2017.08.003
6. Rajkumar S, Praveen MR, Gajjar D, Vasavada AR, Alapure B, Patel D, et al. Activity of superoxide dismutase isoenzymes in lens epithelial cells derived from different types of age-related cataract. J Cataract Refract Surg 2008; 34:470-4. doi: 10.1016/j.jcrs.2007.10.044
7. Lin D, Barnett M, Grauer L, Robben J, Jewell A, Takemoto L, et al. Expression of superoxide dismutase in whole lens prevents cataract formation. Mol Vis 2005; 11:853-8.
8. Olofsson EM, Marklund SL, Behndig A. Enhanced age-related cataract in copper-zinc superoxide dismutase null mice. Clin Exp Ophthalmol 2012; 40:813-20. doi: 10.1111/j.1442-9071.2012.02794.x
9. Forsberg L, de Faire U, Morgenstern R. Oxidative stress, human genetic variation, and disease. Arch Biochem Biophys 2001; 389:84-93. doi: 10.1006/abbi.2001.2295
10. Kafeel S, Hashim Z, Fawwad A, Nawab SN. Predisposition of SOD1, GPX1, CAT genetic variants and their haplotypes in cataractogenesis of type 2 diabetes mellitus in Pakistan. Acta Diabetol 2022. [Online Ahead of Print] doi: 10.1007/s00592-021-01832-5
11. Zhang Y, Zhang L, Sun D, Li Z, Wang L, Liu P. Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. Mol Vis 2011; 17:2325-32.
12. Hassan B, Ahmed R, Li B, Noor A, Hassan ZU. A comprehensive study capturing vision loss burden in Pakistan (1990-2025): Findings from the Global Burden of Disease (GBD) 2017 study. PloS One 2019; 14:e0216492. doi: 10.1371/journal.pone.0216492
13. Roberts JE. Screening for ocular phototoxicity. Int J Toxicol 2002; 21:491-500. doi: 10.1080/10915810290169918
14. Lin D, Barnett M, Grauer L, Robben J, Jewell A, Takemoto L, et al. Expression of superoxide dismutase in whole lens prevents cataract formation. Mol Vis 2005; 11:853-8.
15. Rong X, Qiu X, Jiang Y, Li D, Xu J, Zhang Y, et al. Effects of histone acetylation on superoxide dismutase 1 gene expression in the pathogenesis of senile cataract. Sci Rep 2016; 6:34704. doi: 10.1038/srep34704
16. Olofsson EM, Marklund SL, Behndig A. Enhanced age-related cataract in copper-zinc superoxide dismutase null mice. Clin Exp Ophthalmol 2012; 40:813-20. doi: 10.1111/j.1442-9071.2012.02794.x
17. Taherabadi MS, Nakhaee A. Association Between SOD1 Polymorphism (+ 35A/C) and Senile Cataract. Alborz Univ Med J 2017; 6:219-26.
18. Celojevic D, Nilsson S, Behndig A, Tasa G, Juronen E, Karlsson JO, et al. Superoxide dismutase gene polymorphisms in patients with age-related cataract. Ophthalmic Genet 2013; 34:140-5. doi: 10.3109/13816810.2012.746377