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Glutathione Peroxidase and Catalase Gene Polymorphism in Pakistani Cataract Patients

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ABSTRACT

Introduction: Cataract is the major cause of blindness and visual impairment. Although there are several reasons for the generation of cataract, however, most of the cases are due to inter-individual genetic variations in antioxidant genes. Many studies have been conducted for the understanding of the role of reactive oxygen species (ROS) in the regulation of cataract. However, the study of single nucleotide polymorphisms (SNP) of the Glutathione peroxidase gene and Catalase gene and its association with cataract is still lacking in the Pakistani population.

Objective: To investigate the relationship between GPX and CAT gene variations and the development of Cataract in Pakistani cataract patients.

Methods: This was a case-control study carried out at different centres in Karachi, Pakistan between September 2019 and 2020. A single nucleotide polymorphism (SNP) at rs1050450 for GPX and rs7943316locus for Catalase gene mutations 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.

Results: We detected the ratio of SNP in GPX gene in cataract patientsis higher than in control (OR: 1.8, 95% CI) while there is no significant difference in CAT gene between cataract and control participants.

Conclusion: Our findings indicate potential genetic variations in antioxidant genes particularly GPX and CAT genes polymorphisms as risk factors for cataract. This is study the first studyto report the antioxidant SNPs associated with the developmentof cataract in the Pakistani population.

Key Words: Cataract, Glutathione peroxidase, Catalase, Polymorphism, SNP, ROS

INTRODUCTION

Cataract refers to the opacification of the crystalline lens in the human eye.[1] It is the opacity of the natural, crystalline lens of the eye and remains the most common cause of visual loss in humans. A cataract is a multifactorial disease that can be interpreted via biochemical, epidemiological, and histopathological findings to unwrap this ailment's complexity.[2,3]

Previously, distinct epidemiological data have shown various risk factors that might be involved in the prevalence of cataract among individuals. The most determinant factor in cataract transmission is heredity in character which means that it is passed on from parents to their offspring.[3] However, oxidative stress, UV exposure and antioxidant depletion could also result in the initiation of cataract formation and contributes towards many cases. Oxidative stress is believed to be an early event in the development of some cataracts due to the excessive production of reactive oxygen species.[4] Protection from oxidative damage and hence maintenance of lens transparency is maintained by antioxidant enzyme systems that consist of non-enzymatic and enzymatic components. [5,6] The enzymatic component includes enzymes called superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). [7]CAT and GPX are present in the eye lens and serve to protect the eye against oxidative stress.[8]

Genetic variations in the antioxidant genes coding for the GPX and CAT enzymes may lead to decreased or impaired regulation of their enzymatic activity and alter ROS de-

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toxification. Therefore, genetic variations in these enzymes may modulate disease risk.[9,10]Several studies explain the correlation between SNPs within the coding region of CAT or GPX genes and the development of cataract.[11] It was suggested that the polymorphisms of CAT–21A/T and GPX1–198C/T reduce their antioxidant capacity; these genes polymorphisms are risk factors for different kinds of diseases.[12,13,14]

This study aims to analyze the association between CAT and GPX gene polymorphisms and cataract development in Pakistani cataract patients.

MATERIAL AND METHODS

This is a case-control study carried out on cataract patients who attended outpatient departments of Fatima Hospital, Baqai Medical Universityand LRBT hospital. Age and sex-matched cases and controls were collected from unrelated volunteers from the same hospitals / OPDs. 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. 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. Total 5ml of blood specimen was collected by venipuncture in an anticoagulant (EDTA) containing tube (purple top). The entire blood collection process was performed by the experienced phlebotomist. Once the blood sample was collected, it was immediately stored at -80 °C till further use.

Detection of SNPs in SOD, GPX, and CAT Genes

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).

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

S. No	SNP and gene symbol	Primer Sequence
1	rs1050450 (GPX)	GPX1 left 5'- CCCCGAGACA-GCAGCACT – 3'
		GPX1 Right 5'- ACCATTGA-CATCGAGCCTGA– 3'
2	rs7943316 (CAT)	Catalase Left 5'-CGAGCAGC-CAATCAGAAGG– 3'
		Catalase Right 5'-GCCAT-AGCGTGCGGTTTG– 3'

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 10seconds, and annealing temperature were 60°C for 60seconds run 40 cycles.

For the HRM curve, the temp 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.

Statistical Analysis:

We utilized IBM-SPSS (version 21.0; SPSS Inc., Chicago, IL) for statistical analyses. We performed a 2-tail Chi-square test to determine the correlation 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) and significance < 0.05.

RESULTS

Demographic Factors for Cataract

The mean age of the cataract patients (n=250) was 43.97± 13.36 years. The mean height of the patients was 2.63 ± 0.25 m^2. The mean weight in Kg was 80.12 ± 9.04 and the mean BMI of the patients was 30.60 ± 3.64. (Table 1)

Table 1: Demographic description of the cases

Variable	Mean	SD	Range	Min	Max
Age in years	43.97	13.36	61	18	79
Height in m^2	2.63	0.25	1.63	1.81	3.44
Weight in kg	80.12	9.04	65	45	110
BMI kg/m^2	30.60	3.64	25.16	17.03	42.19

Table 2 shows risk factors associated with age among cataract cases. Most of the cases were males (n=150, 60%). (Majority (n=112, 74.7%) of the males and females (n=57, 57%) were between the ages 31 to 60 years. (p value = 0.014). 62% (n=155) cases had positive family history of cataract and it was significantly associated with age (p value = 0.000). The most common subtype of cataract was found to be nuclear (n = 110, 44%) with 74.5% (n=82) cases were found in ages between 31 to 60 years. The cortical cataract was found among (n = 80, 32%) cases while posterior capsular subtype was found in 24% (n = 60) cases. (p value = 0.000). Majority of the cases were non-smokers (n = 198, 79.2%) and had significant association with age groups. (p value = 0.014).

Table 2: Risk factors associated with age groups among cataract cases (N=250)

Risk Factors	Age Groups (N= 250)						Total	p-value
	Less than 30 years (n=48)		31 – 60 years (n= 169)		Greater than 60 years (n=33)			
	n	%	n	%	n	%		
Gender								
Male	23	15.3	112	74.7	15	10.0	150	0.014*
Female	25	25.0	57	57.0	18	18.0	100	
Family History of Cataract								
Yes	17	11.0	121	78.1	17	11.0	155	0.000*
No	31	32.6	48	50.5	16	16.8	95	
Type of Cataract								
Nuclear	12	10.9	82	74.5	16	14.5	110	
Cortical	18	22.5	45	56.3	17	21.3	80	0.000*
Posterior	18	30.0	42	70.0	0	0.0	60	
Smoking								
Yes	3	5.8	43	82.7	6	11.5	52	0.014*
No	45	22.7	126	63.6	27	13.6	198	

* The p-value was considered significant at < 0.05; values less than <0.01 were very significant and those < 0.001 were highly significant

SNP in GPX and CAT gene polymorphism as the risk of Cataract

The distribution of both GPX and CAT genes polymorphisms in both control and cataract groups with their respective percentages are shown in Table 3. There was a significant difference between the case and control groups in the GPX genotype. We observe that 30% of cases had SNP in their genome in the control group and this ratio was increased in the cataract group (46%). The statistical analysis revealed a possible dangerous effect of the *GPX*genotype (OR=1.98, 95% CI) in the development of cataract.

Similarly, a significant difference was also observed between the case and control groups in the CAT genotype. We observe that 44% of cases had SNP in their genome in the control group and this ratio was increased in the cataract group (52%). The statistical analysis revealed a possible dangerous effect of the *CAT* genotype (OR=1.33, 95% CI) in the development of cataract as shown in Table 3.

Table 3: Distribution of Genetic polymorphisms among antioxidant GPX and CAT genes.

	Control				Cataract				Odds ratio	P-value
	Mutant		non-Mutant		Mutant		non-Mutant			
	n	%	n	%	n	%	n	%		
GPX	75	30	175	70	115	46	135	54	1.98	0.0002
CAT	112	44	138	55.2	130	52	120	48	1.33	0.1

* The p value was considered significant at < 0.05; values less than <0.01 were very significant and those < 0.001 were highly significant

Figure 1. A: shows the high resolution melting curves for GPX and CAT genes. The three lines representdifferent genotypes of the gene. Wild type AA or CC is present in the graph on the right side. The presence of heterozygous genotype (AT) of this gene shifted HRM curve about 1 °C towards lower temperature while homozygous (TT) genotype shifted only 0.5 °C (Figure 1).

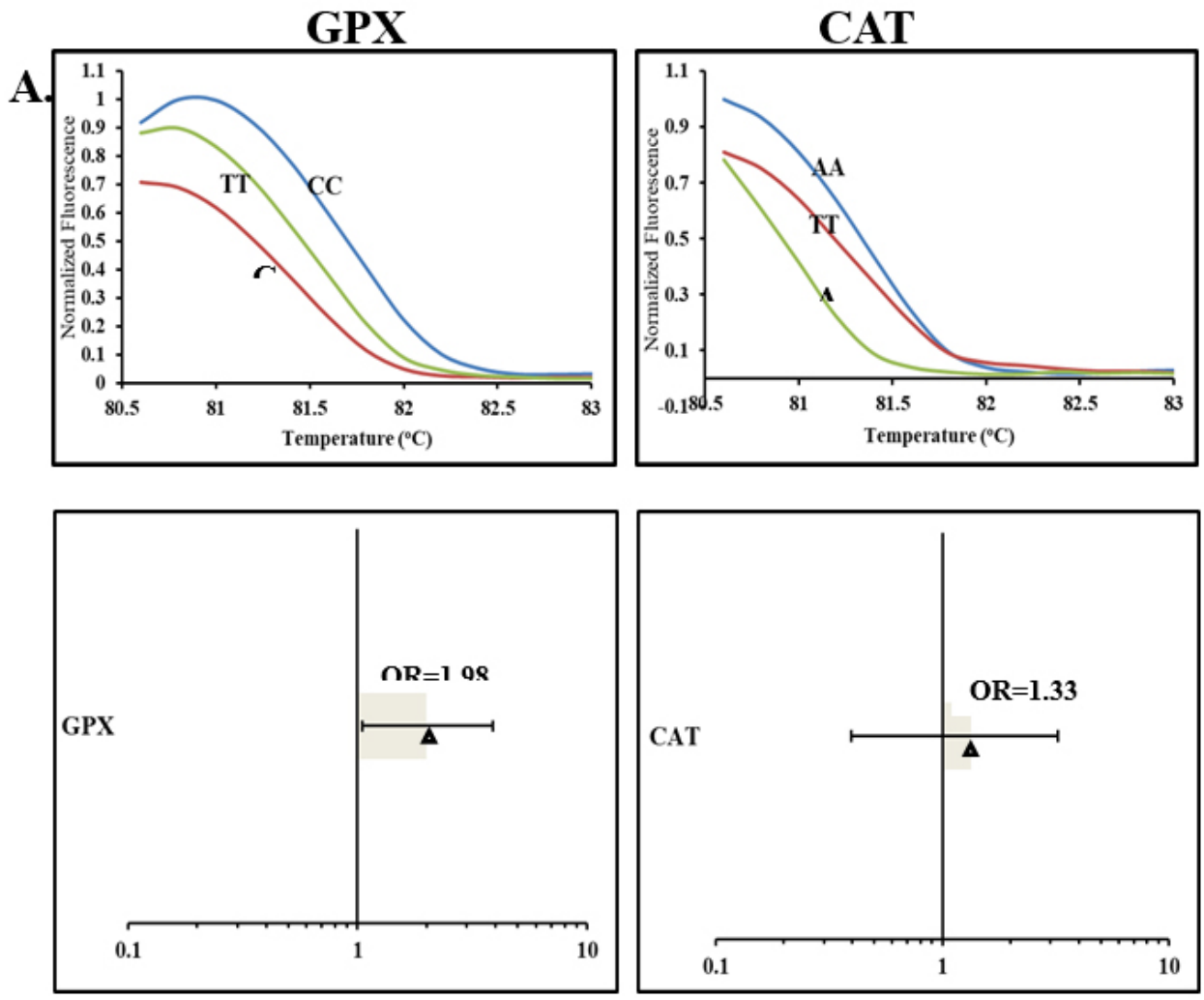

Figure 1: Genetic polymorphisms in GPX and CAT genes.

A) High **re**solution melting curves of GPXgene
B) graphical representation of odds ratios of GPX gene
C) High **re**solution melting curves of CAT gene
D) graphical representation of odds ratios of CAT gene

Distribution of genotype

The genotypic frequencies of AA, AT, and TT between the control and cataract group are illustrated in Table 4. We observed significantly increased risk for the development of cataract associated with mutant CT (OR=1.98; p= 0.007) and TT genotype polymorphism (OR = 1.98; p = 0.1) in GPX gene while there was no significant risk estimated in CAT gene associated with mutant AT (OR=1.33; p= 0.13) and TT genotype polymorphism (OR = 0.94; p = 0.46).Further, we also calculated allelic distribution among case and control. We observed a higher frequency of the C allele (82%) ofthe GPX gene in controls while the T allele was estimated at only 18%. However, the C allele frequency was down to 72% in the cataract group and the T allele 27%. This data clearly indicated that T allelebecame more prevalent (18% in control vs 27% in the cataract group; OR = 1.73, p = 0.0003) (Table 4). In the case of the CAT gene, the frequency of the A allele was calculated at 65% in controls while the T allele was estimated at only 35%. Similar to control, the frequency of the A allele was 64% in the cataract group and the T allele was 36%. There was not much difference between T allele in cases and control (35% in control vs 36% in the cataract group; OR = 1.0, p = 0.4) (Table 4).

Table 4: Distribution of GPX C/T variant (rs1050450) and CAT A/T variant (rs7943316) between control and cataract patients.

Genotypes	Control		Cataract		Odds Ratio	p-value
	n	%	n	%	(CI 95%)	
GPX gene						
C/C	175	70	135	54		
C/T	60	24	92	36.8	1.98 (1.34,2.95)	0.007*
T/T	15	6	23	9.2	1.98(0.99, 3.95)	0.1
Frequency of the C alleles	410	82	362	72	1.73 (1.28-2.34)	0.0003*
Frequency of the T alleles	90	18	138	27		
CAT gene						
A/A	130	52	120	48		
A/T	65	26	80	32	1.3 (0.88 , 2.0)	0.13
T/T	55	22	50	20	0.94 (0.62 , 1.55)	0.46
Frequency of the A allele	325	65	320	64	1.0 (0.720 - 1.516)	0.4
Frequency of the T alleles	175	35	180	36		

* The p value was considered significant at < 0.05; values less than <0.01 were very significant and those < 0.001 were highly significant

DISCUSSION

Epithelial cells of the eye lens play crucial roles in the maintenance of the entire organ. They not only control transport to the lens but also have direct contact with the aqueous humor and are most vulnerable to phototoxic damage.[15] Oxidation induced damage to lens epithelial cells includes bulk protein oxidation,[16] inactivation of key enzymes, DNA breaks [17] and lipid peroxidation.[18]

Antioxidant enzyme GPX1 is mainly acting on hydrogen and lipid peroxides in the lens membrane. The compromised function of the GPX enzyme leads to the accumulation of lipid peroxides, finally opacification in the eye and cataract formation. Similar results including deficiency of GPX1 enzyme, membrane damage and cataract formation has been reported in GPX1–knockout mice.[19] Further, several studies demonstrated that single nucleotide polymorphisms in the *GPX*gene could alter the amino acid,[20] decreased the ability to scavenge ROS[21]and may link to different kinds of diseases.[22,23] In our experiments, we found that the *GPX* polymorphisms were associated with an increased risk of cataract between the controls and patients while the CAT gene had little impact on the development of cataract. Similar to our results, it was earlier reported that GPX activity decreased in the nuclear region of lens while there is no changes in the activity of catalase with the progression of cataract.[24] However, another Chinese study reported that there was no association of both GPX1–198C/T and CAT–21A/T polymorphisms with cataract formation.[14] This may be due to the exposure and interaction of other genes participating in antioxidant recognition, repair and cell cycle regulation may have altered the effect of *GPX1* polymorphisms. These findings support the hypothesis that genetic variations in antioxidant defense may modify the risk of cataract among individuals with increased oxidative stress or decreased antioxidant capacity.

Our results is suggested an association between the polymorphisms of GPX genes with cataract in the Pakistani population. However, there is a need for other ethnicities and locality to confirm our findings, and to fully examine the possible relationship between antioxidant enzyme genes' polymorphisms with cataract. These data emphasize that the importance of antioxidant enzyme genes' polymorphisms and antioxidant enzymes as pharmacological targets to reduce ROS production

may provide a strategy to prevent or slow the progression of age-related cataract formation.

CONCLUSION

Our findings indicate potential genetic variations in antioxidant genes particularly GPX and CAT genes polymorphisms as risk factors for cataract. This is study the first studyto report the antioxidant SNPs associated with the developmentof cataract in the Pakistani population.

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Authors' Contribution:

Dr. Atif Mahmood, Main investigator, analysis & interpretation of data.

Dr. Qamer Aziz, Revising manuscript critically

Dr. Ruqaya Nangrejo, Critical Interpretation of Data

Dr. Mohammad Saleh Soomro, Concept & drafting the work

Dr. Iftikhar Ahmed Siddiqui, Final Approval of manuscript

Dr. Iffat Ara, Lab Technical support

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