Original Research Article

Statin as a risk factor for cataract in cases of antioxidant gene polymorphism in Pakistani population

Abstract:

Introduction: Cataract is main cause of reversible blindness and visual impairment. Risk factors include anti-hyperlipidemic drugs such as statin. However, the mechanism of statins as a risk factor for cataracts is not clear. The antioxidant effect of statin is reported in some studies while other studies showed negative results. This study was conducted to understand the association of cataract in statin users with antioxidant gene abnormalities.

Objective: To investigate the risk factor of statin in the formation of cataracts in the Pakistani population.

Methods: This was a multi-centric case-control study in Karachi, Pakistan between September 2019 and 2020. A single nucleotide polymorphism (SNP) at rs2070424 locus for superoxide dismutase (SOD1) gene, at rs1050450 for glutathione peroxidase (GPX) and rs7943316 locus for catalase (CAT), were examined with polymerase chain reaction (PCR) using high resolution melting curve (HRM) technique in 250 cataract patients.

Results: The risk ratio with statin was seen and found that it was 1.5 times increased in SOD1 gene mutations, the statin benefit was 1.2 times reduced in GPX gene mutation and ratio was 1.1 in the CAT gene mutations.

Conclusion: Statin is a risk factor for cataracts in those patients who have mutated antioxidant genes. The risk ratio of cataracts was found to be increased in the mutated genes of patients compared with non-mutated ones. This study proved the effect of statin as a risk factor associated with antioxidant genes in the development of cataracts in the Pakistani population.

Keywords: Cataract, statin, antioxidant gene mutations, SOD1, CAT, GP

Introduction

A cataract is the main reason for blindness and the leading cause of visual impairment. According to WHO estimates, there are a total of 37 million blind people throughout the world and cataract is present in greater than 17 million of those (1, 2). Cataract has several heredity determinants which have been frequently reported in various studies and includes family and twin studies (3). The heredity determinants have not been restricted to congenital cataracts only but have also been involved in the progression of nuclear as well as cortical opacities concerning age phenomena (4), a principal risk factor related to the development of cataract and lens opacities (5, 6). Although there are several unavoidable factors like genetics or age involved in the initiation of cataract formation, some modifiable risk factors like controlling over blood pressure and cholesterol may help to lower the incidence of cataract (7).

On the other hand, use of anti-lipidemic drug such as statin is also reported as risk factor for cataract (8). It is convincingly reported that statins not only reduce lipid production but also up-regulate the anti-oxidant enzymes (9) including superoxide dismutase (SOD) (10), glutathione peroxidase (GPX) (11) and catalase (CAT) (12). It means increased expression of these enzymes in the eye lenses may reduce the risk of cataract (13). However, some researchers claim that statin does not show any effect against oxidative stress (14) or might even work as a pro-oxidant (15). The possible mechanism of this controversial effect of statin on these enzymes is not very clear. One possibility of diverse effect of statin is pharmacogenomics which is based on the individual's genetic profile. Some genetic variations in antioxidant enzymes may lead to impaired regulation of enzyme activity and showed association with cataracts (3). In this study, we checked possible polymorphism in three major antioxidant genes SOD, GPX and CAT in cataract patients'. These polymorphisms may play an important role for inter-individual differences in maintaining the human genome's integrity. The polymorphisms of *SOD1*–251A/G, *GPX1*–198C/T and *CAT*–21A/T significantly reduced antioxidant capacity in the cataract group suggested that these genes polymorphisms are risk factor for the diseases (16).

We hypothesized that statins user show lesser antioxidant properties in those patients who have nucleotide polymorphism in their antioxidant enzyme genes. This study aims to provide the association of statin in cataract patients with defective antioxidant enzyme genes in Pakistani population that affect the expression and activity of enzyme. This study help to provide safe use of statins for those having SNP in their antioxidant genes.

Methodology:

Demographics data collection:

This case-control study was performed on cataract patients who attended outpatient departments of Fatima Hospital, Baqai Medical University and LRBT hospital. Age and sex-matched cases and controls were collected from unrelated volunteers from the same hospitals / OPD's.

The total sample size of 250 cataract patients was calculated using Rao Soft sample size calculator. Cataracts due to a secondary disease like diabetes, hypertension, trauma and those induced by 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 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 an 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:

SOD1 Left	5'- CTGAAAACTAGTCGAGACTCCAT – 3'
SOD1 Right	5' –CAAGGCTTCACGTCTACACA– 3'
GPX1 left	5'- CCCCGAGACAGCAGCACT – 3'
GPX1 Right	5'- ACCATTGACATCGAGCCTGA– 3'
Catalase Left	5'-CGAGCAGCCAATCAGAAGG– 3'
Catalase Right	5'-GCCATAGCGTGCGGTTTG– 3'

For detection of DNA sequence variations, we used a ready-to-use master mix (Thermo Scientific, cat no K1031) for High-Resolution Melt (HRM) analysis.

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^{o}C for 10 minutes, denaturation temperature 95^{o}C for 10 seconds and annealing temperature was 60^{o}C for 60 seconds run 40 cycles. For HRM curve, temp range was 65-90 ^{o}C with the increment of 0.2 ^{o}C. For detection of possible SNPs, data was included in the graph from 80-90 ^{o}C. At least three samples were run on gel for confirmation of SNP for each gene.

Statistical Analysis:

For statistical analyses, we used IBM-SPSS (version 21.0; SPSS Inc., Chicago, IL). The student's *t*-test and chi-square were performed on different variables to obtain frequencies, percentages and associations respectively. The P-value was considered significant at <0.05.

Results

Demographic risk factors of cataracts (Table 1)

80.8% of cataract cases were above the age of 50 years whileonly20% were below 50 indicating age as a risk factor. The majority of the cases had the nuclear type of cataract indicating the propensity of this subtype among our geographic location (p= 0.007). Men had a slightly higher prevalence than women (60% male versus 40% women) but the percentage of males and females in each subtype of cataract was almost equal (p= 0.807).

Family history was found to be positively correlated with cataract as spontaneous onset cataracts are usually part of ageing process and all secondary and complicated cataract were already excluded. According to our data, 62% of cases had a history of cataracts in their family and nuclear type of cataract was the most prevalent type among others (p=<0.01). Smoking, on the other hand, was also found to be negatively correlated with cataracts. Only 20% of total cases were active smokers in our study (Table 1). Similarly, we observed that ethnicity was not a significant risk factor for cataracts and their subtypes.

Table.1. Risk factors associated with the type of cataract among cases (n=250)

Risk Factors	Types of Cataract						Total	*P*-value
	Nuclear		Cortical		Posterior			
	N	%	n	%	n	%		
Age								
>50 years	98	48.5	62	30.7	42	20.8	202	**0.007***
<50 years	12	25.0	18	37.5	18	37.5	48	
Gender								
Male	68	45.3	48	32.0	34	22.7	150	**0.807**
Female	42	42.0	32	32.0	26	26.0	100	
Family History of Cataract								
Yes	79	51.0	45	29.0	31	20.0	155	**0.015***

No	31	32.6	35	36.8	29	30.5	95	
Ethnicity								
Sindhi	10	58.8	3	17.6	4	23.5	17	**0.283**
Punjabi	3	37.5	1	12.5	4	50.0	8	
Pathan	34	41.5	30	36.6	18	22.0	82	
Baloch	7	58.3	4	33.3	1	8.3	12	
Muhajir	53	41.4	42	32.8	33	25.8	128	
Other	35	100.0	0	0	0	0	3	
Smoking								
Yes	17	32.7	25	48.1	10	19.2	52	**0.020***
No	93	47.0	55	27.8	50	25.3	198	

*The P-value was considered significant at <0.05; values less than <0.01 were very significant and those <.001 were highly significant

Distribution of antioxidant genes polymorphism in cataract patients

Our demographic data showed that a family history of cataracts is positively correlated with the development of cataracts in the patients. Therefore, we tested possible SNPs in antioxidant genes (SOD1, GPX and CAT) in cataract patients to see the role of these genes in cataract formation. We found that most of the cataract patients (≈ 50%) have nucleotide mutation in their antioxidant genes. Fig 1 A. showed the percentages of mutated antioxidant genes in cataract patients. We found that the SOD1 gene had the highest prevalence (56%) while GPX had 46% and CAT 52% in cataract patients. We also distributed all three genes polymorphism within the different subtypes of cataract. Nuclear cataract was the most abundant subtype among all three gene polymorphism groups; 62.9% in SOD, 54.8% in GPX and 70.8% in CAT (Fig 1 B).

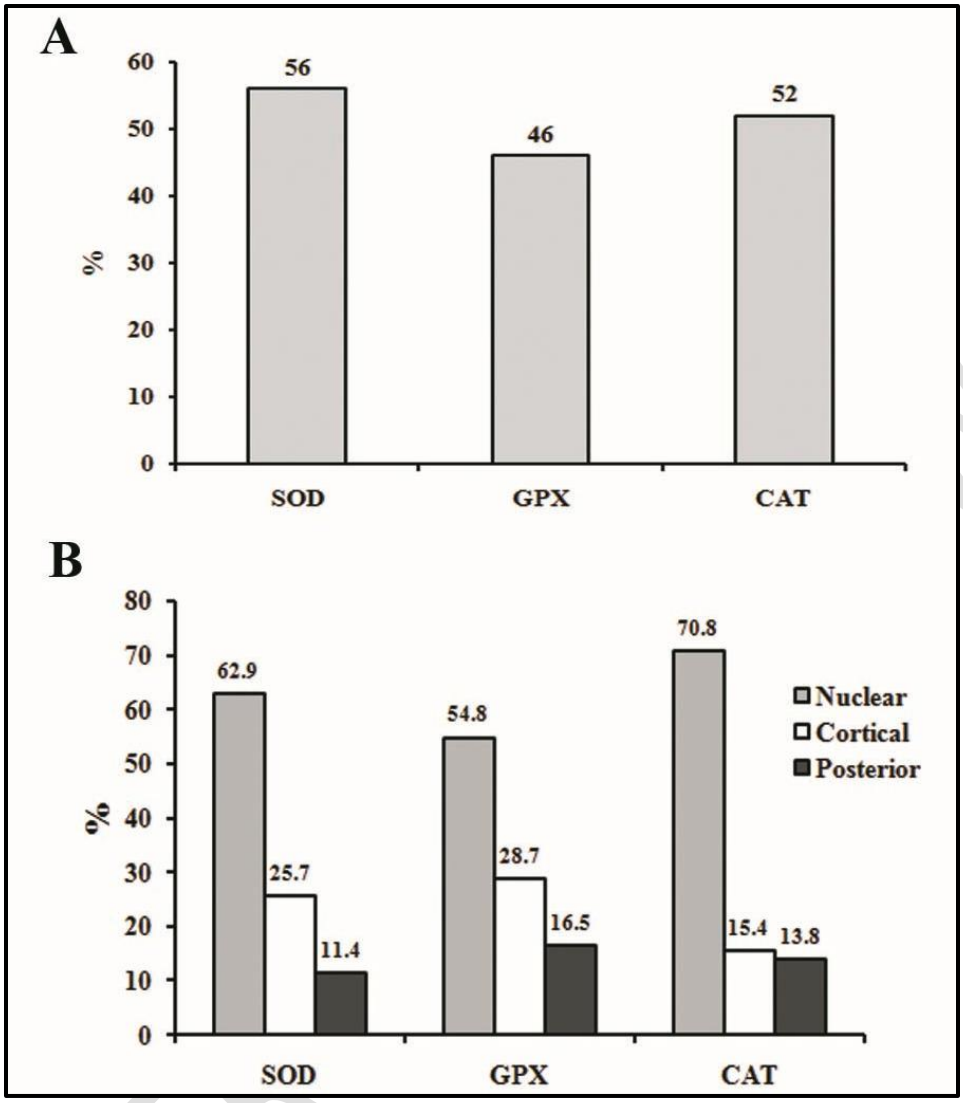

Figure.1. Percentages of gene mutations in subtypes of cataract patients

Effect of statin on the risk of cataracts in mutated antioxidant genes (Table 2)

To see the association of statin with cataracts, we categorized our data into two groups: statin-users and non-users; and calculated the cataract risk ratio between these groups among all three gene polymorphisms. Our results indicated that the risk ratio of cataracts was found to be increased in the mutated gene patients as compared with non-mutated cases.

For SOD1, in the non-mutant arm, statin use was associated with a higher number of cataracts (65 versus 60) but when the mutation occurred, the incidence of cataract was noted to be higher (75 versus 50) which shows that statin use is associated with a high incidence (1.5 times) of cataract in the presence of SOD mutation.

For GPX, in the non-mutant arm, statin use was associated with a lesser number of cataracts (57 versus 75), however, in the mutant arm, the reduction was less significant (58 versus 60) thereby showing the statin benefit was reduced in GPX gene mutation., It shows 1.26 times Less beneficial effect.

For CAT, In the non-mutant arm, statin use was associated with a higher number of cataracts (65 versus 60) while in the mutant arm statin use was associated with an even higher number (68 versus 57) thereby showing that statins may play a contributory role in cataract formation in CAT gene mutations.

Table 2: Comparison of risk ratios of cataract between statin-user and non-user in three genes polymorphisms

Antioxidant Genes		Cataract (non-mutant)	Cataract (mutant)	Total (n=250)	Odds ratio
SOD1	Statin user	65	75	140	1.5
	Non- user	60	50	110	
GPX	Statin user	57	58	115	1.26
	Non- user	75	60	135	
CAT	Statin user	65	68	133	1.1
	Non- user	60	57	117	

Discussion

The study revealed that different types of cataracts are associated with different risk factors in the Pakistani population of which age has proven to be stronger risk factors for the development of cataracts. Smoking has shown to be negatively co-related with cataract whereas family history was found to be positively correlated with cataract development. The effect of age represents the summative effect of all the complex reactions of different exposures that took place over a while and contributed towards the development of senile cataracts.

Most cataracts possibly occurred due to heredity determinants(17),and this occurrence has been frequently observed in various studies which include family and twins(18, 19). With the progression of age, some antioxidant genes were mutated due to environmental and other factors which may play a role in cataract development. Spector (1989) has shown that progressive and widespread oxidative damage led to the development of senile cataracts which is the most common type of cataract in humans. His data showed that cataracts in older individuals had reportedly shown extensive oxidation of lens protein and lipid whereas controls of similar age have shown very little oxidation in lens protein which was present only in membranous components(20). Long term exposure to reactive oxygen species (ROS) has caused oxidative damage in older individuals (21). Several studies have proven that oxidative species can damage lens proteins (22) membrane lipids (23) and DNA (20).

Our results showed that all three antioxidant genes represented higher SNP detection within the cataract patients. We found that SOD1 gene polymorphism was most prevalent in the cataract patients and nuclear cataract is the most abundant sub-type in all three mutated gene groups. Our results of SOD1 gene polymorphism are similar to a Chinese study which found that the genotype frequency of the GG and AA of SOD1–251A/G was significantly different in cataract patients but they found different results in the other two genes (16). It seems to corroborate without finding that nuclear cataracts were the commonest type of senile cataracts in our study population (24).No changes in the activity of catalase with the progression of cataract was found in some studies(25). However, in our study we found that the incidence of cataract was higher in patients having Catalase gene mutations who took statins.

These polymorphisms in antioxidant genes are associated with cataracts. Further, the long term use of statin was found to be linked with cataract development and a possible mechanism is increased oxidative stress. Recently, a systemic review concluded that the use of statin significantly increases the concentrations of GPX and SOD enzymes while it does not affect the concentration of Catalase (26). If these antioxidant genes have a polymorphism in a particular position then they are unable to overcome statin-induced ROS production. Our results report similar outcomes describing that the individuals with prolonged use of statin showed higher response as the risk of cataract in the mutant antioxidant gene.

Conclusion:

In conclusion, this study has suggested an association of statin as a risk factor for cataracts in patients suffering from antioxidant gene abnormalities in the Pakistani population. There is an increased association of possible statin-induced cataracts among the SOD1 and CAT gene mutations, but possibly reduction of statin benefit in GPX mutation thereby correlating the increased risk statins can induce in cataract formation if such antioxidant genes are defective. The study corroborates with other studies that age is a risk factor for the development of cataracts.

In this study, the incidence of cataracts seemed lesser among smokers than non-smokers which is another unique finding. However, smoking cannot be considered as a protective factor against cataract as numerous studies have shown that smoking increases cataract formation.

However, there is a need for other larger nationwide studies to validate our findings, and detailed genetic studies to fully examine the possible relationship between other genes with cataracts and also with other systemic diseases. This may provide a strategy to prevent or slow the progression of age-related cataract formation.

Ethical Approval and Consent

The study was approved by the ERC/ BASR of Baqai Medical University and written informed consent was obtained from all the participants.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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