Original Research Article

Statin is associated with antioxidant gene polymorphism as the risk factor of cataracts in the Pakistani population.

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Abstract:

Introduction: Cataract is one of the major causes 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.

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

Methods: This was a multi-centric case controlled study in Karachi, Pakistan between September 2019 and 2020. A single nucleotide polymorphism (SNP) at rs2070424 locus for SOD1 gene, at rs1050450 for GPX and rs7943316 locus for catalase, were 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. The risk ratio with statin was seen and found that it was 1.5 times increased in SOD1, 1.2 times in the GPX and slightly up (ratio: 1.1) in the CAT gene.

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Results: Statin is a risk factor for cataracts in those patients who have mutated antioxidant genes and the risk ratio of cataracts was found to be increased in the mutated genes of patients as compared with non-mutated ones.

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Conclusion: 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

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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). The awareness of the risk factors of cataracts could have an important benefit by reducing patients' dependency on society. Although there are several factors involved in the initiation of cataract formation, age is the principal risk factor related to the development of cataract and lens opacities (5, 6)

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In addition to demographic factors, genetic variations in antioxidant enzymes may modulate disease risk (3). To see the association of antioxidant genes with cataracts, we checked possible polymorphism in three major antioxidant genes superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Further, anti-lipidemic drugs are also reported as a risk factor for cataracts such as statin (7). These drugs not only reduce lipid production but also up-regulate the anti-oxidant activity (8). However, some researchers claim that statin does not show any effect against oxidative stress (9) or might even work as a pro-oxidant (10). The possible mechanism of this controversial effect of statin on these enzymes is not very clear. We hypothesized that statins show lesser antioxidant properties in those patients who have nucleotide polymorphism in their antioxidant enzyme genes.

We studied the association of statin in cataract patients with defective antioxidant enzyme genes in Pakistani population.

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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 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 of 500 patients (cataract 250, control 250) 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'

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Anyways, no worries, that info may be omitted if the authors don't have an intention to include it.

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 95ºC for 10 minutes, denaturation temperature 95ºC for 10 seconds and annealing temperature was 60ºC for 60 seconds run 40 cycles.

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 < .05.

Results

Demographic risk factors of cataracts (Table 1)

80.8% of cataract cases were above the age of 50 years while only 20% 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 = .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= .807).

Another risk factor observed in this study was a family history of cataracts. 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=<.01). Smoking, an important risk factor, was also found to be significantly associated with cataracts (p= .02). 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	**.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	**.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	**.015***
No	31	32.6	35	36.8	29	30.5	95	

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Ethnicity								
Sindhi	10	58.8	3	17.6	4	23.5	17	
Punjabi	3	37.5	1	12.5	4	50.0	8	
Pathan	34	41.5	30	36.6	18	22.0	82	**.283**
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	**.020***
No	93	47.0	55	27.8	50	25.3	198	

* The P-value was considered significant at < .05; values less than <.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 the higher risk factor for 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. Fig1 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 (Fig1 B).

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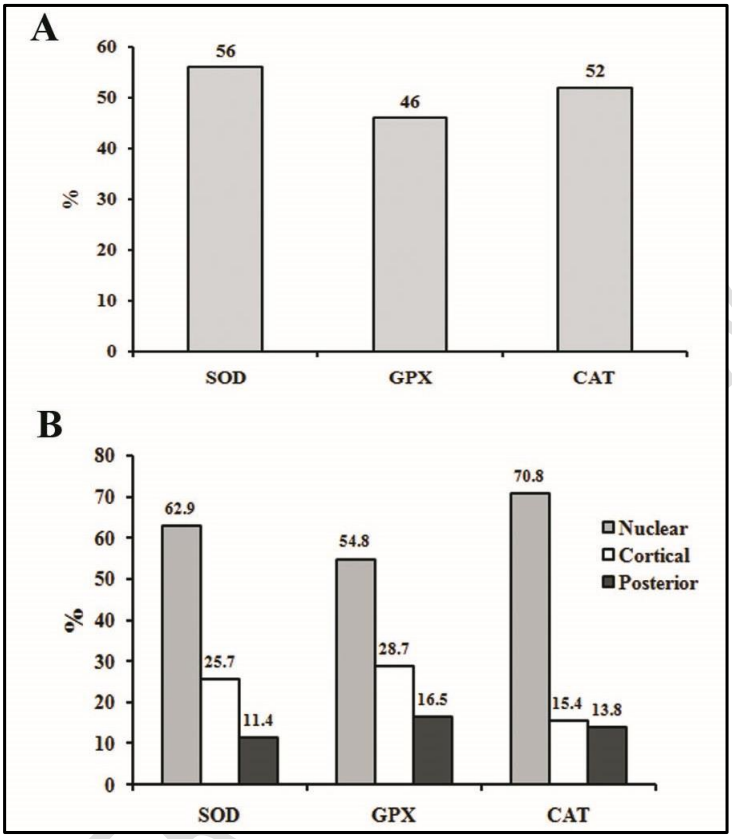

Figure.1. Percentages of gene mutations in subtypes of cataract patients

Statin increased the risk of cataracts in mutated antioxidant genes.

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 of patients as compared with non-mutated cases. The risk ratio of cataracts was 1.5 times increased in SOD1, 1.2 times in the GPX and slightly up (ratio: 1.1) in the CAT gene as shown in Table 2.

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in the non mutant arm, statin use was associated with higher number of cataracts (65 versus 60) but when mutation occurred the incidence of cataract was noted to be higher (75 versus 50) – so can argue that statin use is associated with high incidence of cataract in the presence of SOD mutation.

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it is 1.2 times LESSER (beneficial effect)
57+58 = 115 cataract pts were on statin
66+60 = 126 cataract pts were not on statin

Another way of interpretation to favor the authors (as noted in my comment above) , shall be that in the non mutant arm, statin use was associated with a lesser number of cataracts (57 versus 66) however, in the mutant arm, the reduction was less significant (58 versus 60) - thereby showing the statin BENEFIT WAS REDUCED in GPX gene mutation

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In the non mutant arm, statin use was associated with 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)	Odds ratio
SOD1	Statin user	65	75	1.5
	Non- user	60	50	
GPX	Statin user	57	58	1.2
	Non- user	66	60	
CAT	Statin user	65	68	1.1
	Non- user	60	57	

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Discussion

The study revealed that different types of cataracts are associated with different risk factors in the Pakistani population of which age, smoking and family history of cataracts have proven to be stronger risk factors for the development of cataracts. 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.

Our demographic data showed that a family history of cataracts is the major risk factor for the development of cataracts. Most cataracts possibly occured due to heredity determinants (11), and this occurrence has been frequently observed in various studies which include family and twins (12, 13). 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 (14). Long term exposure to reactive oxygen species (ROS) has caused oxidative damage in older individuals (15). Several studies have proven that oxidative species can damage lens proteins (16) membrane lipids (17) and DNA (14).

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 cataracts 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 (18). Similar to our results, it was reported that GPX activity decreased in the nuclear region of the lens (19). It seems to corroborate with out finding that nuclear cataracts were the commonest type of senile cataracts in our study population. No changes in the activity of catalase with the progression of cataract was found in some studies (20). However, in our study we found that the incidence of cataract was higher in patients having Catalase gene mutations who took statins.

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

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Yu S, Chu Y, Li G, Ren L, Zhang Q, Wu L. Statin Use and the Risk of Cataracts: A Systematic Review and Meta-Analysis. *J Am Heart Assoc*. 2017;6(3):e004180. Published 2017 Mar 20. doi:10.1161/JAHA.116.004180

KINDY CONSIDER THIS ARTICLE – IT'S A META ANALYSIS OF MANY STUDIES THAT HAVE ANALYZED STATINS AND CATARACT in the journal of American heart association PMID: 28320745 – A total of 6 cohort studies, 6 case–control studies, and 5 randomized controlled trials, together involving more than 313 200 patients were analyzed and they inferred that there was no clear evidence

Instead, could include this article as one of your references and relate your regional observation in comparison to their study and mention that yours is more unique as you have compared the effect of statins on specific gene mutation patients compared to ones that did not have gene mutation. Further, you are also doing it at your geographic location to look for any difference in results

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1-You could modify your conclusion and specify that there is 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

2- You may mention that your study corroborates with other studies that age is a risk factor for development of cataracts

3-You may also mention that in your study, the incidence of cataracts seemed lesser among smokers than non-smokers which is another unique finding. However, we must clarify that smoking cannot be considered as a protective factor against cataract as numerous studies have shown that smoking increases cataract. So, You could just mention this observation and that this finding needs validation with a larger nationwide study.

Well done, best wishes to the authors !
Hopefully by slightly tweaking the interpretation, this study shall be of more value.

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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