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ORIGINAL ARTICLE

Predisposition of *SOD1*, *GPX1*, *CAT* genetic variants and their haplotypes in cataractogenesis of type 2 diabetes mellitus in Pakistan

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Abstract

Aims Cataract formation is accelerated by hyperglycemia due to the excessive production of oxidative stress. This study aimed to examine the underlaying role of glutathione peroxidase 1 (*GPX1*) rs1800668, catalase (*CAT*) rs1001179 and superoxide dismutase 1 (*SOD1*) 50 bp Indel promotor region variants in the pathogenesis of cataract in patients with diabetes.

Methods A population-based case-control study of *n*=680 individuals was conducted which comprised of four respective groups: type 2 diabetes mellitus, diabetic cataract, senile cataract patients and controls. Screening of genotypes was performed by allele-specific (AS) and conventional polymerase chain reaction (PCR). Statistical testing was carried out using SPSS© 20.0, MedCal© and SNPStats© software's. Bioinformatics analysis of linkage disequilibrium was done by HaploView© software 7.0.

Results *GPX1* (rs1800668) showed significant association with higher susceptibility of opacification in type 2 diabetes mellitus (χ^2=23.0, Adjusted OR=1.63, 95% CI: 1.05–2.49, p<0.001). A protective role was anticipated by *CAT* variant (rs1001179) for the development of resistance against the pathogenicity of cataract with diabetes (χ^2 = 107, Adjusted OR=0.17, 95% CI: 0.10–0.29, p<0.001). Linkage disequilibrium (LD) plot of *GPX1* and *CAT* variants revealed that CTC-CTT haplotypes demonstrated the presence of linkage (D′=1.0) and co-inheritance (LOD=13.84) in patients of diabetic cataract.

Conclusions *GPX1* (rs1800668) variant may serve as an antioxidant biomarker for the assessment of risk for cataract in type 2 diabetes mellitus. GPX1 enzyme owed an antioxidant activity which can reduce the oxidative stress and hence could develop resistance in cataractogenesis. The findings could be beneficial as a potential target to the future pharmacogenomic studies of cataract prevention and eradication in diabetes mellitus.

Keywords Promotor · Antioxidant enzymes · Cataract · Senile · Type 2 Diabetes mellitus · Oxidative stress

Introduction

Cataract develops with the process of aging and considered as a secondary complication of diabetes mellitus and a leading cause of blindness. [1]. Global prevalence of cataract is 51% in accordance to World Health Organization (WHO), also confirmed by Global Burden of Eye Diseases among all types of blindness [2, 3]. Type 2 diabetes mellitus (T2DM) is one of the chronic metabolic syndromes that eventually damages vital organs such as human eye, kidneys and others [4]. Diabetic cataract is considered as the most prevalent secondary cause of T2DM and accounts for 66% of blindness in diagnosed patients [5]. The excessive accumulation of glucose and its impaired metabolism leads to activate several alternative pathways that reacts with macromolecules and change their normal structure and conformation in human eye such as glycation, oxidative stress, sorbitol accumulation and the subsequent formation of advanced glycation end products (AGES) [6]. Due to the activation of these pathways, cataractogenesis becomes accelerated up to three folds in T2DM condition [7]. Excess glucose enters into the insulin independent tissues for its metabolism which induces the production of free radicals and led to the formation of oxidative stress [8]. Mechanism of cataract pathogenesis is suggested to be modulated by the development of higher oxidative stress in patients with diabetes. Presence of high oxidative stress impaired the efficacy of body's antioxidant

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scavenging system [9]. Primary antioxidant enzyme system is comprised of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPX1) and catalase (CAT) that collectively works together for neutralizing the toxicity of reactive oxygen species in oxidative stress. Genetic variations in these crucial enzymes might disturb their normal activity which could enhance the risk of disease severity and affects the vital organs [10].

SOD1 is a primary antioxidant enzyme that diffuses free superoxide radicals. Its gene is localized on 21q22.11 chromosomal position and comprised of 5 exons [11]. While, the antioxidant enzymes CAT and GPX1 functions in a same way by metabolizing the noxious molecules into nontoxic form. High content of CAT enzyme is found in cytoplasm and GPX1 enzyme in peroxisome [12]. *CAT* gene contain 13 exons and positioned on 11p13 chromosome [13], whereas *GPX1* gene includes only 2 exons and reported to be localized on 3p21.31 position [14].

Present study targets the promotor region variants recognized in three enzymes which are involved in first line of defense that might affects their regulatory transcriptional mechanisms. In *SOD1* gene, 50bp deletion was observed in promotor sequence at 1684 bp upstream of ATG start codon. Binding site of proximal specificity protein-1 (Sp1) becomes effected by the occurrence of this 50 bp deletion in *SOD1* gene [15]. Moreover, a substitution of C to T at -262 position of *CAT* promotor region (rs1001179) located near initiator transcription site may affects the enzyme activity [16]. While, the C/T polymorphism of *GPX1* (rs1800668) promotor sequence located in 5′-untranslated region (UTR) may cause hindrance in GPX1 normal functioning [17]. Role of these genetic variations in pathogenicity of oxidative stress induced cataractogenesis in diabetes mellitus is still obscure. Therefore, current study aims to explore the three primary antioxidant enzyme genetic variants of promotor region in association with the pathogenicity of cataract in T2DM patients of Pakistan.

Materials and methods

Ethical approval and study design

The study was conducted in between the duration of January 2018 to November 2019. In accordance with the guidelines of Helsinki Declaration, the Institutional Ethical Committee of Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi permitted this research project dated on April 19, 2016 (Ref No. KIBGE/ICE/080/2016). It was a case-control study comprised of overall n = 680 study participants divided into four study groups according to the stringent study criteria: type 2 diabetes mellitus (T2DM), diabetic cataract (DC), senile cataract (SC) and controls (CL). Each group contains equal number of n =170 subjects. Study participants were selected from the outpatient department (OPD) of following two tertiary care hospitals: Baqai Institute of Diabetology and Endocrinology (BIDE) Hospital Nazimabad, Karachi, Pakistan and Prevention of Blindness (POB) Eye Hospital, Gulistan-e-Johar Karachi, Pakistan in distinctive periods. All the subjects have given their informed consent for volunteer participation in this study.

Selection criteria and sample collection

The selection of participants in four study groups was made by following the inclusion and exclusion criteria. History of patient with diabetes was recruited through online database of the relevant hospitals. Confirmation of diabetes was done by standard glycemic parameters: fasting blood glucose, random blood glucose and glycated hemoglobin (HbA1c) levels. After that, presence or absence of cataract formation was assessed by standard measures: slit lamp bio-microscopy, complete retinal examination and visual acuity in a free eye examination. Age of all the subjects was ranged between 45 to 65 years as aging was a crucial phenomenon associated with the pathogenesis of cataract considered in this study. Type 2 diabetes mellitus (T2DM) patients had more than five years of onset history, and showed no sign of cataract in eye examination. Diabetic cataract (DC) patients also had greater than five years of duration, and showed presence of cataract formation in either eye lens confirmed by standard screening. Senile cataract (SC) patients showed the presence of cataract in their lens via examination of eye with no diagnosis and history of type 2 diabetes mellitus confirmed by glycemic screening. Control (CL) subjects demonstrated neither diagnosis of type 2 diabetes mellitus nor carry any history of cataract confirmed by standard measures. Age and gender matched controls were recruited to justify the hypothesis of this study which was focused on the role of genetic variations in the progression of cataract with aging and diabetes. It is suggested that in hyperglycemic condition, the progression of cataract is accelerated which might be associated due to the presence of genetic markers that enhances the glycation of lens protein and develops opacity earlier than senility. Nevertheless, patients with any endocrine disorder, congenital cataract, traumatic cataract, eye infection or ocular diseases, pregnancy, mental retardation, diabetic microvascular and macrovascular complications, type 1 diabetes, gestational diabetes, comorbidities, use of steroid medications were excluded from the study. Approximately 5ml blood sample drawn from the cephalic vein of each participant using 5cc syringe and transferred in EDTA and fluoride vacutainers which were cautiously stored at – 80 °C until examinations. EDTA vacutainers were used for the genetic testing and glycosylated hemoglobin

(HbA1c) analysis. While, fluoride vacutainers were used for glucose estimations. The information of clinical parameters was recruited from the laboratory of BIDE hospital. While, demographic data were collected from the study participants on their follow-up visits. Furthermore, all the genetic analysis was done in the laboratory of Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi.

Genotyping of *SOD1* 50 bp Indel variant

DNA extraction was carried out by subsequent standard protocol of salting out [18]. The quantifications of DNA yield and purity was assessed by Nanodrop (IMPLEN NanoPhotometer®, Germany), whereas quality check analysis was made by gel electrophoresis. Genetic variant of *SOD1* was genotyped by conventional polymerase chain reaction (PCR) using a set of primer forward [F] AATTCCTTACCCCTG TTCTA and primer reverse [R] GGCAGATTTCAGTTC ATTGT [19]. *SOD1* 50 bp deletion was situated on the 1684 bp upstream location of ATG in the regulatory transcriptional region of promotor. Amplification and genotyping was performed using the optimized concentrations of PCR reagents: Total 20 µl of 1X reaction mixture consist of 1 µl of 0.5 mM forward and 0.5 mM of reverse primers, 2 µl of 50 ng genomic DNA, 8 µl of 2X Thermo Scientific Green PCR Master Mix ™ and nuclease free water of 8 µl was used. The PCR reactions took place in 30 cycles in Thermal cycler (T100 Bio-Rad®, California, USA) at optimized program condition comprised of following: 94 °C incubation carried out for 5 min on initiation, then 94 °C incubation was given for 30 sec on denaturation, leading to 58 °C incubation for 45 sec which undergoes annealing, 72 °C for 45 sec and at the ends 72 °C incubation was last for 7 min to complete the extension in one reaction of amplification. The genotyping of *SOD1* variant was performed by detecting the PCR products of 297 bp which indicated the insertion allele and 247 bp which showed deletion allele resolved on 2% agarose gel using ThermoFisher Scientific SYBR Safe DNA gel stain ™ in Gel Documentation System of FastGene® FAS V, Germany (Supplementary Figure 1).

Genotyping of *GPX1* (rs1800668) variant

Screening of *GPX1* gene (rs1800668) variant was carried out using well-established technique of allele-specific polymerase chain reaction (AS-PCR). Primers used for amplification of this variant were comprised of forward [F1] CGCCTG CTGGCCTCCCCTTAC or [F2] CGCCTGCTGGCCTCC CCTTAT and reverse set [R] GCAGGGAGCCCAGGCTCA CAG [20]. Genotyping of C to T substitution at 5′ untranslated region of *GPX1* gene promotor was done using PCR concentrations of 1X reaction: 1 µl of 1 mM forward (F1 or F2) and reverse primer, 2 µl of 50 ng genomic DNA, 8 µl of 2X Thermo Scientific Green PCR Master Mix ™ and nuclease free water of 8 µl was utilized for total volume of 20 µl for each reaction. PCR program conditions comprised of 30 cycles of 94 °C incubation for 5 min at initiation step, leads to 94 °C incubation for 30 sec at denaturing step, then after 65 °C incubation for 45 sec at annealing step, extension was performed at 72 °C temperature for 45 sec and then for 7 min for completing the process of amplification. PCR product of 170 bp for C or T allele of *GPX1* (rs1800668) variant with F1 or F2 primer was used for the determination of genotypes after visualization on 2 % agarose (Supplementary Figure 2).

Genotyping of *CAT* (rs1001179) variant

Genetic variant (rs1001179) of *CAT* gene was analyzed by AS-PCR using the sequence of forward [F1] GCCCTG GGTTCGGCTATC or [F2] GCCCTGGGTTCGGCTATT and reverse primers [R] GGTTTGCTGTGCAGAACACT [16]. This genetic variant contains the substitution of C to T at − 262 position of *CAT* promotor region. Amplification was performed using following concentrations of 1X PCR reaction: 1 µl of 0.5 mM of forward (F1 or F2) and reverse primers, 1 µl of 50 ng genomic DNA, 8 µl of 2X Thermo Scientific Green PCR Master Mix ™ and 8 µl of nuclease free for 20 µl reaction volume. The 35 cycles were carried out at optimized program conditions: temperature of 94 °C for 5 min was given initially followed by 94 °C for 30 sec for denaturing, 61 °C for 40 sec for appropriate annealing of primer sequences, 72 °C for 35 sec and 72 °C for 7 min for the complete extension of strands. The 2% agarose gel prepared with ThermoFisher Scientific SYBR Safe DNA gel stain ™ was utilized for resolving the 340 bp PCR product representing C or T allele of *CAT* (rs1001179) variant with either F1 or F2 primers in duplicate reactions for the purposed of genotyping (Supplementary Figure 3).

Statistical analysis

Statistical package for social sciences (SPSS) version 20.0 was used for the evaluation of the observed data and trends. One-way ANOVA statistics was particularly used for comparing the values of clinical parameters among four study groups. Allelic and genotypic frequency distribution analysis and Pearson's Chi square testing was done according to the statistics of Hardy–Weinberg equilibrium (HWE). Odds Ratio (OR) revealed the strength of association of *SOD1*, *GPX1* and *CAT* genetic variants to the susceptibility of disease by MedCal© statistical online software (version 12.1, Mariakerke, Belgium). Hypothetical genetic models were calculated to check the best fit model using two-by-two contingency as a simplest strategy of estimating an odds ratio (OR). Dominant and recessive models elaborated the

association with homozygosity and heterozygosity. These models were evaluated for the demonstration of explicit role associated with genotypes of three targeted variants to the susceptibility of disease. Distribution of eight possible haplotypes of three genetic variants and their association with cataractogenesis were estimated by SNPStats© online software [21]. Linkage disequilibrium analysis among the *CAT* (rs1001179) variant haplotypes and *GPX1* (rs1800668) variant haplotypes was done by HaploView© software version 7.0 [22] for assessing their pattern in the co-inheritance of cataractogenesis in T2DM patients.

Results

All subjects were age and gender matched among four groups of this case-control study. Anthropometric and baseline parameters included: age, body mass index (BMI), systolic and diastolic blood pressure (BP) were compared using one-way ANOVA statistics. It was observed that BMI ($p<0.01$) of all subjects included in four study groups was found to be significantly different. However, the systolic BP ($p<0.001$) was demonstrated to be significantly higher in cataract subjects as compared with others. While, the clinical parameters such as blood glucose estimations: FBG, RBG and HbA1c were shown to be significantly higher ($p<0.001$) in T2DM and diabetic cataract groups as compared to controls and senile cataract patients. Hence, the representation suggested the existence of significant association with patients of diabetes and cataract (Supplementary Table 1).

Association of genetic variants

Association of *SOD1* 50 bp Indel variant was defined with T2DM in Table 1. In genotypic distribution, the higher prevalence of I/I genotype was indicated in control group (0.74) as compared to T2DM patients (0.68) where I/D and D/D genotypes were detected in higher ratio among T2DM patients. Allelic distributions suggested that mutant D allele was observed in higher frequency in the patients of T2DM (0.18). The association of mutant D allele with pronounced incidence of T2DM was evident but showed no statistical significance (Adjusted OR=1.32, 95% CI: 0.70–2.4, p = 0.30). Genetic models suggested an insignificant effect of recessive alleles and its genotypic combinations in the development of T2DM risk (Adjusted OR=2.33, 95% CI: 0.70–7.68, $p = 0.15$). Furthermore, the association of *SOD*1 50 bp Indel variant was also assessed with susceptibility of cataract in diabetic group (Table 1). The senile group

Table 1 Association of *SOD1* 50 bp Indel variant with T2DM and diabetic cataract groups

Genotypes ($n = 340$)	T2DM ($n = 170$)	Controls ($n = 170$)	Chi square p value	Genetic models adjusted OR (95% C.I.) p value	
				Recessive model [(I/I-I/D) (D/D)]	Dominant model [(I/I) (I/D-D/D)]
I/I	117 (0.68)	126 (0.74)	$\chi^2 = 2.44$	2.33 (0.70–7.68)	1.31 (0.81–2.08)
I/D	44 (0.25)	40 (0.23)	$p = 0.29$	$p = 0.15$	$p = 0.28$
D/D	9 (0.05)	4 (0.02)			
Alleles ($n = 680$)	$n = 340$	$n = 340$			
I	139 (0.82)	146 (0.86)		Adjusted OR = 1.32 (0.70–2.4) $p = 0.30$	
D	31 (0.18)	24 (0.14)			
Genotypes ($n = 340$)	**Diabetic cataract ($n = 170$)**	**Senile cataract ($n = 170$)**	**Chi square p value**	**Genetic models adjusted OR (95% C.I.) p value**	
				Recessive model [(I/I-I/D) (D/D)]	**Dominant model [(I/I) (I/D-D/D)]**
I/I	106 (0.62)	116 (0.68)	$\chi^2 = 5.80$	3.08 (0.82–11.63)	0.75 (0.49–1.20)
I/D	61 (0.36)	45 (0.26)	$p = 0.05$	$p = 0.07$	$p = 0.24$
D/D	3 (0.02)	9 (0.05)			
Alleles ($n = 680$)	$n = 340$	$n = 340$			
I	136.5 (0.81)	138.5 (0.80)		Adjusted OR = 1.02 (0.62–1.86) $p = 0.78$	
D	33.5 (0.18)	31.5 (0.19)			

*$p<0.05$, ** $p<0.01$, ***$p<0.001$

I allele: wild type, D allele: mutant, OR: adjusted by confounding variables

was served as a comparison group. The higher frequency of I/D genotype of *SOD1* variant showed in diabetic cataract group (0.36) as compared to senile cataract group (0.26). A significant week association was suggested by chi square statistics (χ^2= 5.80, p=0.05) but no evidence suggested the possible association of this variant with the progression of cataract in diabetes (Adjusted OR=1.02, 95% CI: 0.62–1.86, $p = 0.78$). Recessive model indicated that the mutant alleles of variant genotypes plays an insignificant role in inducing cataract risk in patients with diabetes (Adjusted OR=3.08, 95% CI: 0.82–11.63, $p = 0.07$).

The association of *GPX1* variant (rs1800668) with susceptibility of T2DM subjects was represented in Table 2. The T/T genotype of *GPX1* variant showed that the predominant distribution in T2DM patients (0.13) in comparison to control individuals (0.05). Whereas, C/C genotype of *GPX1* variant exhibit significantly higher ratio in controls (0.24) as compared to T2DM patients (0.10). The Chi square analysis and odds ratio suggested that T allele of *GPX1* variant might involve in the manifestation of risk for T2DM (χ^2 = 14.1, Adjusted OR=1.56, 95% CI: 0.9–2.3, $p< 0.05$). The pathogenesis of cataract in diabetes modulated by the probability of higher risk ratio of the recessive alleles in disease group (Adjusted OR=2.64, 95% CI: 1.19–5.96, p<0.01). *GPX1* variant was also analyzed for association with diabetic cataract group (Table 2). The senile cataract group was used for comparison. T/T genotype distribution of *GPX1* variant was more pronounced in diabetic cataract group. Whereas, C/C and C/T genotypes distribution of *GPX1* variant was significantly found to be elevated in senile cataract group. Significant association of *GPX1* variant (rs1800668) was evident with risk probability of diabetic cataract group (χ^2 = 23.0, p<0.001). However, the mutant T allele of this variant may confer a higher likelihood toward the cataract formation in patients with diabetes (Adjusted OR=1.63, 95% CI: 1.05–2.49, p<0.05). Hence, our study suggested that recessive alleles of *GPX1* variant plays a resistant role toward the progression of cataract in diabetes (Adjusted OR = 0.45, 95% CI: 0.20–0.97, p<0.05).

In the end, association of *CAT* variant (rs1001179) was determined with T2DM group (Table 3). Genotypic distribution suggested that C/C frequency of *CAT* variant (rs1001179) was significantly higher in T2DM group (0.52) where C/T frequency was found to be higher in controls (0.47). An insignificant association for *CAT* variant (rs1001179) was found with the pathogenicity of T2DM pathogenicity (Adjusted OR = 1.14, 95% CI: 0.71–1.7, p = 0.60). The dominant model suggested that the

Table 2 Association of *GPX1* (rs1800668) variant with T2DM and diabetic cataract groups

Genotypes (n = 340)	T2DM (n = 170)	Controls (n = 170)	Chi square p value	Genetic models adjusted OR (95% C.I.) p value	
				Recessive model [(C/C-C/T)(T/T)]	Dominant model [(C/C)(C/T-T/T)]
C/C	18 (0.10)	41 (0.24)	χ^2 = 14.1	2.64 (1.19–5.96)	2.63 (1.42–4.75)
C/T	130 (0.76)	120 (0.70)	p < 0.001***	p < 0.01**	p < 0.001***
T/T	22 (0.13)	9 (0.05)			
Alleles (n = 680)	N = 340	N = 340			
C	83 (0.49)	101 (0.60)		Adjusted OR. 1.56 (0.9–2.3) p < 0.05*	
T	87 (0.51)	69 (0.40)			
Genotypes (n = 340)	**Diabetic cataract (n = 170)**	**Senile cataract (n = 170)**	**Chi square p value**	**Genetic models adjusted OR (95% C.I.) p value**	
				Recessive model [(C/C-C/T)(T/T)]	Dominant model [(C/C)(C/T-T/T)]
C/C	15 (0.09)	26 (0.15)	χ^2 = 23.0	0.45 (0.20–0.97)	0.55 (0.27–1.06)
C/T	114 (0.67)	134 (0.79)	p < 0.001***	p < 0.05*	p = 0.068
T/T	41 (0.24)	10 (0.06)			
Alleles (n = 680)	n = 340	n = 340			
C	72 (0.42)	93 (0.55)		Adjusted OR = 1.63 (1.05–2.49) p< 0.05*	
T	98 (0.57)	77 (0.45)			

*p<0.05, ** p<0.01, ***p<0.001

C allele: wild type, T allele; mutant, OR; adjusted by confounding variables

Table 3 Association of *CAT* (rs1001179) variant with T2DM and diabetic cataract groups

Genotypes ($n = 340$)	T2DM ($n = 170$)	Controls ($n = 170$)	Chi square p value	Genetic models Adjusted OR (95% C.I.) p value	
				Recessive model [(C/C-C/T)(T/T)]	Dominant model [(C/C)(C/T-T/T)]
C/C	89 (0.52)	76 (0.44)	$\chi^2 = 4.0$	0.75 (0.37–1.47)	1.36 (0.89–2.08)
C/T	60 (0.35)	80 (0.47)	$p = 0.13$	$p = 0.38$	$p = 0.16$
T/T	21 (0.12)	16 (0.09)			
Alleles ($n = 680$)	$n = 340$	$n = 340$			
C	116 (0.68)	119 (0.70)		Adjusted OR = 1.14 (0.71–1.7) $p = 0.60$	
T	56 (0.32)	51 (0.30)			
Genotypes ($n = 340$)	Diabetic cataract ($n = 170$)	Senile cataract ($n = 170$)	Chi square p value	Genetic models adjusted OR (95% C.I.) p value	
				Recessive model [(C/C-C/T)(T/T)]	Dominant model [(C/C)(C/T-T/T)]
C/C	28 (0.16)	121 (0.71)	$\chi^2 = 107$	0.17 (0.08–0.37)	0.09 (0.05–0.13)
C/T	102 (0.60)	40 (0.23)	$p < 0.001$***	$p < 0.001$***	$p < 0.001$***
T/T	40 (0.23)	9 (0.05)			
Alleles ($n = 680$)	$n = 340$	$n = 340$			
C	79 (0.46)	141 (0.83)		Adjusted OR = 0.17 (0.10–0.29) $p <$ 0.001***	
T	91 (0.54)	29 (0.17)			

*$p<0.05$, ** $p<0.01$, ***$p<0.001$

C allele: wild type, T allele; mutant, OR; adjusted by confounding variables

susceptibility of T2DM might enhanced by the response of dominant genotypes nevertheless fails to be supported by statistical significance (Adjusted OR = 1.36, 95% CI: 0.89–2.08, $p = 0.16$). Association analysis of *CAT* variant (rs1001179) was also tested with diabetic cataract group (Table 3). The senile cataract group was served as its comparison group. A significantly higher frequency of C/T genotype (DC = 0.60 and SC = 0.23) and T/T genotype (CC = 0.23 and CT = 0.05) of *CAT* variant (rs1001179) was found in diabetic cataract group. Nevertheless, among the patients of senile cataract group, the C/C genotype was frequently detected in higher probability (SC = 0.71 and DC = 0.16) than diabetic cataract group. A significant association of *CAT* variant (rs1001179) was defined for the risk of diabetic cataract by the statistical analysis of Chi square ($\chi^2 = 107$, $p < 0.001$). Though, it was found that mutant C allele of *CAT* variant (rs1001179) induces a protective impact against the progression of cataractogenesis in patients with diabetes (Adjusted OR = 0.17, 95% CI: 0.10–0.29, $p < 0.001$). Furthermore, both recessive and dominant genotypic models indicated defensive response against the risk of cataractogenesis in patients with diabetes ($p < 0.001$).

Distribution of Haplotypes

Role of haplotypes and their association with risk probability of diabetic cataract and T2DM was indicated in Table 4. The *CAT* (rs1001179), *GPX1* (rs1800668) and *SOD1* (50 bp Indel) genetic variants created eight haplotypic combinations. Depiction of risk was indicated by the haplotype CTI, TTI and CTD in the pathogenesis of T2DM (OR < 1.0), but no statistical significance was observed in comparison to controls. While, the haplotype CTD showed a probability of insignificant risk with diabetic cataract in comparison to senile cataract group (Adjusted OR = 3.60, 95% CI: 95% CI: 0.15–0.39, $p = 0.26$). A significant protective role of haplotypes TCI, TCD and TTI was observed against the pathogenicity of diabetic cataract ($p < 0.01$).

Linkage disequilibrium of *GPX1* and *CAT* haplotypes

Linkage disequilibrium (LD) plot between haplotypic markers of *GPX1* (rs1800668) and *CAT* (rs1001179) variants was tested by HaploView© software version 7.0. The LD plot was established between T2DM and control groups (Fig. 1). The analysis indicated that markers 5 and

Table 4 Distribution of haplotypic frequencies with risk of T2DM and diabetic cataract

CAT	*GPX1*	*SOD1*	T2DM	Control	OR	*p* value	DC	SC	OR	*p* value
C	C	I	0.355	0.301	1.00	0.23	0.129	0.360	1.00	0.12
C	T	I	0.261	0.204	1.68	0.14	0.282	0.304	0.43	0.09
T	T	I	0.139	0.107	2.33	0.06	0.060	0.154	0.06	0.00*
T	C	I	0.130	0.147	0.65	0.53	0.091	0.236	0.29	0.01
C	C	D	0.066	0.069	0.87	0.80	0.014	0.074	0.94	0.92
C	T	D	0.444	0.043	2.11	0.25	0.088	0.033	3.60	0.26
T	T	D	0	0.059	0	0	0.047	0	0	0
T	C	D	0.009	0.030	0.47	0.70	0.021	0.102	0.10	0.01

Global haplotype association *$p<0.001$

OR= adjusted by confounding variables

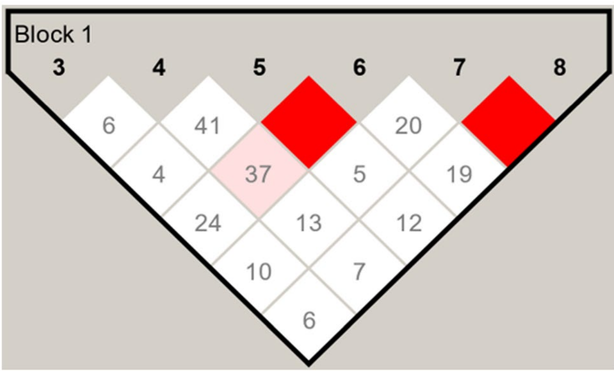


Fig. 1 Haploblocks of *GPX1* (rs1800668) and *CAT* (rs1001179) polymorphisms in LD plot constructed between T2DM (disease) and healthy individuals (control) group. Haplotypic markers 5:6 and 7:8 (red) were found in complete linkage disequilibrium and followed the pattern of co-inheritance to the susceptibility of T2DM

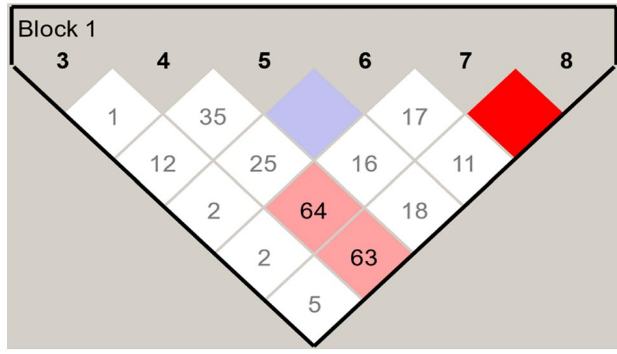


Fig. 2 Haploblocks of *GPX1* (rs1800668) and *CAT* (rs1001179) polymorphisms in LD plot developed between diabetic cataract (disease) and senile cataract (controls) groups. Haplotypic markers 7:8 (red) was found in complete linkage disequilibrium and co-inheritance in the pathogenesis of diabetic cataract

6 collectively depicted the evidence of linkage (coefficient of linkage disequilibrium (D′) =1.0) with optimum probability of co-inheritance among them in T2DM patients [likelihood of odds ratio (LOD) score=2.65]. In addition, haplotypic marker 7 and 8 also explained the occurrence of linkage D′ = 1.0 along with co-inheritance in the condition of T2DM (LOD score = 2.23). The pattern of cataractogenesis in patients with diabetes was also analyzed by LD plot (Fig. 2). The senile cataract group was used for comparative analysis. This LD plot between two cataract groups revealed one red haploblock which represented the combination of haplotypic marker 7 and 8. It states a potential linkage with each other in the pathogenicity of diabetic cataract (D′ = 1.0) and also infer the pattern of co-inheritance (LOD score = 13.84). Furthermore, the two pink haploblocks depicted that the pair of 4 and 7 markers (LOD score = 5.58) and 4 and 8 markers (LOD score = 24.45) might follows the co-inheritance pattern in the development of diabetic cataract risk with no probability of linkage (D′ < 1.0).

Discussion

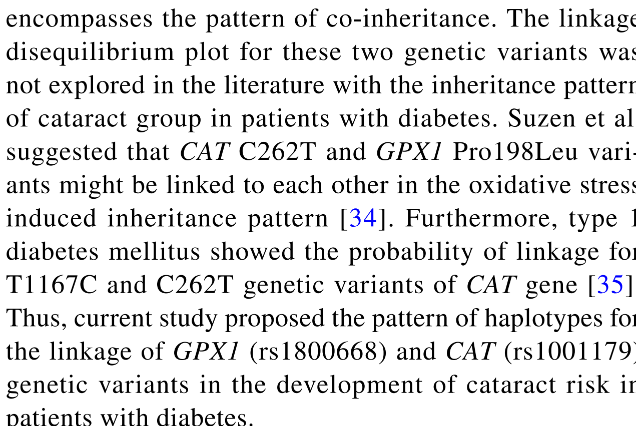
Present study suggested the association of *GPX1* (rs1800668) and *CAT* (rs1001179) and *SOD1* 50 bp Indel genetic variants with the formation of cataract risk in type 2 diabetes. The finding of this study supports the hypothesis that the genetic variations in primary antioxidant enzyme genes may modifies the risk of cataract in T2DM patients with compromised antioxidant capacity or higher oxidative stress.

Significant association of *GPX1* variant (rs1800668) suggested the higher probability of cataractogenesis in the patients of T2DM ($p < 0.05$). In this polymorphism, greater frequency of mutant T allele in disease groups infers a higher ratio in the development of risk. A previous study suggested that the risk of oxidative stress showed no evidence for the existence of significant relationship with *GPX1* rs1800668 genetic variant [23]. Another study conducted on understanding the risk of prostate cancer in association with *GPX1* rs1800668 polymorphism showed

no promising significant results [24]. T allele found to confers 2.6 times higher risk for the induction of opacification in diabetic lens ($p < 0.01$). Contrasting results suggested that C/T genotype occur significantly higher and mainly contribute in the development of risk for rheumatoid arthritis [20]. Therefore, down-regulatory mechanism for the GPX1 enzymatic activity might be triggered by the predisposing effect of mutant T allele of this genetic variant [25]. The *SOD1* 50 bp Indel genetic variant indicated no occurrence of any significant association of D allele for diabetic cataract risk (Adjusted OR = 1.02, 95% CI:0.62–1.86, $p = 0.78$). I allele may infer a protective effect against the development of the disease. Previously, it was reported that heterozygosity might prevail 33% of *SOD1* downregulation [26]. Another report suggested the lower enzymatic activity of SOD1 enzyme in the presence of *SOD1* 50 bp deletion [27]. This study provides the contradictory outcomes to previous studies with insignificant association of *SOD1* 50 bp Indel variant with risk probability of diabetic cataract. In *CAT* (rs1001179) variant, higher T/T and C/T genotype distribution in the group of diabetic cataract suggested an insignificant role of T allele in early induction of opacification process in T2DM (Adjusted OR = 0.17, 95% CI:0.10–0.29, $p < 0.001$). The *CAT* (rs1001179) variant is one of the well explored variants in multiple studies conducted to reveal its possible association in the development of risk for ischemic heart disease, diabetic nephropathy, retinopathy, type 1 diabetes, cardiovascular diseases and gestational diabetes [28, 29]. The protective role of T allele was evident by previous findings of a study conducted to evaluate the impact of this polymorphism in the oxidative stress related disorder [30].

The haplotypic frequency analysis revealed the distribution of haplotypes for targeted genetic variants in a specific set of groups [31]. The elevated CTD haplotypic frequency suggested the higher ratio of risk in the progression of T2DM and diabetic cataract. Therefore, the haplotype CTD might play a significant role in the modulation of cataractogenesis mechanism in T2DM. Thus, the outcomes might imply that the haplotypes TTI and TCI inferred a protective influence against the formation of risk for the condition of diabetic cataract. A previous study reported the *SOD1, GPX1* and *CAT* haplotypes association showed no significant observations for the probability of age-related macular degeneration risk [32]. Haplotypic analysis was conducted to reveal the cumulative effect in gene to gene interaction in order to identify the risk of a disease [33].

Functional association of CAT and GPX1 enzymes was assessed by the linkage disequilibrium plot of *CAT* (rs1001179) and *GPX1* (rs1800668) genetic variants. In present study, haplotypic markers CTC-CTT were linked to each other and the pathogenesis of diabetic cataract



encompasses the pattern of co-inheritance. The linkage disequilibrium plot for these two genetic variants was not explored in the literature with the inheritance pattern of cataract group in patients with diabetes. Suzen et al. suggested that *CAT* C262T and *GPX1* Pro198Leu variants might be linked to each other in the oxidative stress induced inheritance pattern [34]. Furthermore, type 1 diabetes mellitus showed the probability of linkage for T1167C and C262T genetic variants of *CAT* gene [35]. Thus, current study proposed the pattern of haplotypes for the linkage of *GPX1* (rs1800668) and *CAT* (rs1001179) genetic variants in the development of cataract risk in patients with diabetes.

Following are the possible limitations of this study; limited sample size for population-based study, unavailability of data for associated diseases, use of medications except steroids and paired selection of controls with three groups of cases could serve as a probable bias in this study associated with the pathogenesis of cataract in both senile and T2DM patients.

Among three polymorphisms, *GPX1* (rs1800668) variant was found to play a significant role in accelerating the mechanism of cataractogenesis in patients with diabetes. Present study indicated *GPX1* (rs1800668) variant as a potential antioxidant therapeutic biomarker for the assessment of cataract risk in diabetic condition. Since, GPX1 enzyme performs an important function in the modulation of oxidative stress. The polymorphism in promotor region may create hinderance by causing variabilities in expression and effect its function against oxidative stress which ultimately led to induced the development of opacities in lens and accelerates aging. In future, pharmacogenomic studies are needed for considering the role of *GPX1* (rs1800668) variant as a biomarker that would be beneficial to resist the progression of cataract in patients with type 2 diabetes using personalized medicines.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00592-021-01832-5.

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Author contributions SK: sample collection, optimization, experimentation, data curation, statistics, and original draft writing. SNN: performance, interpretation and revision of the genetic experimental work and manuscript. ZH: optimization, experiment, reviewing and editing of manuscript. AF: facilitation in sampling, revision and proof reading of final draft.

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Availability of data and material Additional supporting data of genotypes and clinical parameters is available upon request.

Code availability Study codes were given to the samples according to their categorization in groups.

Declarations

Conflict of interest Authors declared that they have no conflict of interest.

Ethical approval This case-control research was ethically approved by the Institutional Ethical Committee (ICE) of The Karachi institute of biotechnology and genetic engineering (KIBGE) [Ref No. KIBGE/ICE/080/2016].

Informed consent All the participants were recruited with written informed consent for the insurance of their volunteer participation in this study.

Consent to participate Written informed consent was obtained from all cases and controls.

Consent for publish Approved for publication by research institute (KIBGE, University of Karachi, Pakistan).

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