

# Association Of Gpx-198C/T Gene Polymorphism (rs1050450-198C/T) in Sudanese with Diabetic Retinopathy

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## Abstract

**Background:** Diabetic retinopathy is the common micro-vascular complication of diabetes mellitus. It is the main cause of blindness among young adults worldwide. Poor glycemic control in addition to longer diabetic duration is the main risk factors for diabetic retinopathy. Many genes have been postulated as candidates for diabetic retinopathy. Little is known about anti-oxidative enzyme gene polymorphism and its association with diabetic retinopathy, mainly for catalase enzyme and manganese superoxide dismutase and glutathione genes. The study aims to assess the role of glutathione GPX-198C/T (rs1050450) gene polymorphism in diabetic retinopathy Sudanese patients and its relation with GPX level. In addition to determine the association of FBS, HbA1c and lipid in the pathogenesis of diabetic retinopathy.

**Methodology:** The number of subjects involved were 130 which were classified into (n 60) clinically diagnosed as diabetic retinopathy and (n 70) diabetes mellitus without retinopathy as control group, age ranged from 22 to 80 years old, from Makkah Eye Complex. DNA was extracted and PCR product for GPX, gene segment were digested by Aha1 enzymes, moreover gene polymorphisms were determined. Serum GPX, activity and FBS, TG, CHOL and HbA1c level were analyzed using Cobas Int 400 using absorption photometer and immunoassay methods respectively.

**Results:** The results revealed that, retinopathy is common in female than male by approximately 2 fold =1.9:1. Type II is more common in our population that type 1. The majority of the patients had type II diabetes (128, 98.5%) and only 2(1.5%) patients were type I diabetes mellitus. The activity of GPX, was significantly higher in DNR when compared with DR (p=0.003). Mean HbA1c and FBG concentration were significantly higher among DR than DNR p=0.001 and p=0.001 respectively. In contrast, mean serum CHOL and TG level revealed insignificant differences when compared DR with DNR. The genotyping for GPX-198C/T showed that, the frequency of CC was observed in 33(47%) in control higher than cases 13(22%), these Associations for CCs, GPX-198C/T SNP rs1050450, decreased risk after correction for multiple testing (OR=0.310, 95% CI=0.176-0.486, p=0.001), While TT genotype was detected in 25(42%) cases and only 14(20%) in controls, these Associations for SNPs, TTs, GPX-198C/T SNP rs1050450, increased risk after correction for multiple testing (OR=2.4, 95% CI=1.10-5.90, p=0.004). The frequency of the allele C protective allele was found to be 48% among cases group while allele T-risky allele was higher among cases group 72%, OR=0.357 (0.216-0.433), p=0.001.

**Conclusion:** The study concludes that there is a significant association between GPX-198C/T (rs1050450) gene polymorphism and the occurrence of diabetic retinopathy in Sudanese population. There is a significant decrease in GPX levels and glycemic control in patients with the mutant allele T.

**Keywords:** Glutathione peroxidase GPX-198C/T; Gene polymorphism (rs1050450); Diabetic retinopathy, PCR-RFLP

## Introduction

No a doubt full diabetic retinopathy is the most common causes of blindness around the world. Which are a multifactorial eye disease and a major cause of the loss of lens transparency in the aging population group 20-60 years moreover has a burden on the economy and community as it's associated with loss of reproductively. Additionally, WHO added diabetic retinopathy to the priority list of eye disease as it can be partly prevented and treated. It is a well-known fact that diabetes mellitus is a risk factor for cardiovascular disease [1-3]. Poor glycemic control and longer disease duration are leading cause to the development of angiopathic complications. DR occurs both in type 1 and type 2 diabetes and is strictly related to disease duration [4,5]. The prevalence of diabetes mellitus in the Sudan is 3.4% and reaches up to 10% in some communities. While the prevalence of diabetic retinopathy was reported to be low as 28.1% in 1989 but this prevalence

is increased to 43% in 1995. This increase is attributed to poor glycemic control, long disease duration, old age and hyperlipidemia. It had been reported that Sudanese patients are more prone to develop micro and macro vascular complications. As the main cause for developing vascular complications is hyperglycemia, but this report may indicate the involvement of genetic factors in development of vascular

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complications as retinopathy [6,7]. ROS reactive oxygen species are most common processes to develop vascular complications.

## Production

ROS are generated under physiological and pathological condition, including pathogenesis of diabetes vascular complications. Recently, many studies reported that some diabetic patients develop macrovascular complications while others didn't develop any complications in spite of sharing the same level of glycemic control, mode of treatment and matching for age. This findings lead to postulation of genetic susceptibility to developing diabetes vascular complications [8,9]. ROS developed in micro vascular cells in retina mostly and the superficial fiber cells, which are highly reactive. The proper regulation of cell functions depend on a certain level of ROS, such as intracellular signal, transcription activation, cell proliferation, inflammation, and apoptosis, but higher amounts of ROS are harmful to macromolecules [10-12]. The main multifunction's of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), protection the organisms from oxidative damage [6,8,11]. SOD decomposes superoxide into hydrogen peroxide. CAT catalyzes the decomposition of hydrogen peroxide into water and oxygen, thereby preventing cell damage from high levels of ROS. GPXs are selenoproteins that reduce organic peroxides and hydrogen peroxide through the coupled oxidation of glutathione [10,11].

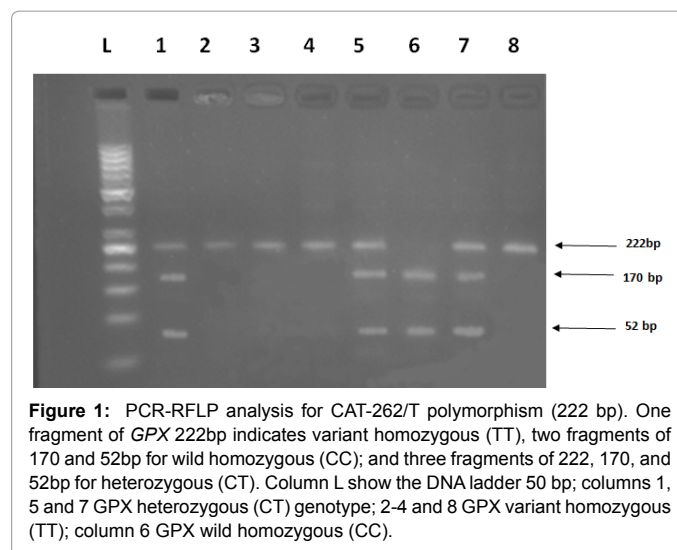
Genetic variations in the antioxidant genes coding for the SOD, CAT, and GPX enzymes alter ROS resulted from irregularity of their enzymatic activity and alter ROS detoxification [9,11]. ROS with genetic material, polymorphisms in genes coding for antioxidant enzymes have a significant function for inter-individual differences in maintaining the human genome's integrity. Genetic polymorphisms in SOD, CAT, and GPX have been included in proneness to cancer and other diseases [8,11]. These potentially significant genetic variants related to oxidative stress have already been studied extensively, including single nucleotide polymorphisms (SNP) GPX-198C/T in the promoter region of the GPX gene (SNP, rs1050450), GPX-198C/T polymorphism gene has significant value as an antioxidant enzymes. Most of these polymorphism result in changes in the levels or the activities of these enzymes, which can lead to reduced protection against oxidative stress. The effect of these variations on the lens has not yet been clarified, so we choose these candidate SNPs to study in our work [9,11,13-18].

The aim of the present study was to evaluate the possible association of JPX-198C/T gene polymorphisms with diabetic retinopathy, in addition to determine the association of FBS and HbA1c and lipid in the pathogenesis of diabetic retinopathy in the Sudanese population.

## Materials and Methods

**Methodology:** In case-control hospital based study (n 130) subject were enrolled, then classified into (n 60) clinically diagnosed as diabetic retinopathy and (n 70) diabetes mellitus without retinopathy as control group, age ranged from 22 to 80 years old, from Makkah Eye Complex. DNA was extracted and PCR product for GPX-198C/T gene segment digested by Abal enzyme was used for determination of genotype by (PCR-RFLP).

GPX-198C/T genotypes were determined by using a multiplex PCR-RFLP method (Figure 1). To form undigested fragments of 222 bp by amplifying An SNPs C→T in exon 10 (codon 399) using primers, illustrated in. PCR procedure were started by 35 cycles for the total amplification reaction completed in 1:08 h. 94°C for 5 min, as an initial denaturation temperature 94°C for 30 s, as DNA denaturation



temperature 62°C for 30 s, as primers annealing temperature 72°C for 30 s, as Taq polymerase extension temperature 72°C 10 min, as a final extension step. The 222 bp PCR products were digested with Abal (MBI Fermentas, Burlington, CA) at 37°C for 5 h and analyzed with 2% agarose gels. Abal digestion resulted in one fragment of 222 bp for wild-type (TT); two fragments of 170 and 52 bp for variant homozygous (CC); and three fragments of 222, 170, and 52 bp for heterozygous (CT) (Tables 1-3). Ethical consideration Permission of this study was obtained from the local authorities in the area of the study. This study was approved by the College Board Committee. The objectives of the study were explained and written consent was obtained from each participant in this study.

**Statistical data analysis:** Data were analyzed using SPSS software, version 20.0 for Windows (SPSS). P of <0.05 was considered statistically significant. Chi-square ( $\chi^2$ ) test was used to compare the differences between patients and control groups.

## Evaluation of DR

The experience ophthalmologist was assessed the presence of DR by using dilated funduscopy. And there for patients were divided into the following group: without singe of DR; mild moderate or proliferative diabetic retinopathy (PDR): severe non-proliferative diabetic retinopathy; or maculopathy according to the Global Diabetic Retinopathy Project Group. DR grade was assigned based on the worst eye. Diabetic retinopathy patient distributions in the present study population according to the Global Diabetic Retinopathy Project grading.

## Results

The baseline clinical and demographic features of the study patients with DR and DNR are shown in. In this study, 130 diabetic patients were enrolled, sixty patients with diabetic retinopathy (DR), (46.2%) 60 and on the other hand seventy diabetics without diabetic retinopathy (DNR), (53.8)70 as (controls). The overall male to female was 1:1.9 folds. The majority of the patients was 128(98.5%) type II diabetes and only 2(1.5%) patients were type I diabetes mellitus. The eldest patient in this study aged 80 years while the youngest was 22 years. The mean of the cases group versus controls was [59 ± 11.0 vs. 59 ± 10.5 years; p=0.317]. The mean duration of diabetes in years was significant among cases than controls (16.5 ± 7.5 vs. 16.5 ± 7.5; P=0.005)

Gene	Mutation	Forward	Primer	PCR	Restriction enzyme	Allele	PCR-RFLP
			Length	Product			Products
GPX	rs1050450	F5:TCCAGACCATTGACATCGAG:3	40bp	222bp	Aba1	C/C	52+170bp
	198C>T	R5:ACTGGGATCAACAGGACCAG:3					52+170+222bp
	Pro200Leu						222bp

Table 1: SNP Specification and Primer Sequences and PCR-RFLP Products.

Gene	Mutation	Prevalence of risk allele	Chromosome
GPX	rs1050450-198C>T	58%	3p21.3
	Pro200Leu		

Table 2: SNPs Location and Prevalence of Risk Allele among African Population.

Name of Primer	Primer Sequence
(GPX) (sense)	5': TCCAGACCATTGACATCGAG:3",
GPX (antisense)	5:ACTGGGATCAACAGGACCAG:3.

Table 3: Glutathione Peroxidase1 (GPX) Primer.

Variables	Patients	Controls	p-Value
	N=60	N=70	
Patients Gender (%)			
Male	26(43%)	30(43%)	
Female	34(57%)	40(57%)	
Patients Age, years	59 ± 11.0	59.0 ± 10.5	0.075
Period of Diabetes, years	16.5 ± 7.5	16.5 ± 7.5	0.005
Type of Diabetes (%)			
Type I	2(4)	0(0)	
Type II	58(96)	70(100)	

Significant difference considered as p-value ≤ 0.05

Table 4: Socio-Demographic Comparison between Cases and Control.

Variables	Patients	Controls	p-Value
	N=60	N=70	
GPX mU/MI	8.2 ± 1.84	11.14 ± 2.21	0.001

Significant difference considered as p-value ≤ 0.05

Table 5: Biochemical Comparison between Cases and Controls.

(Table 4). The serum GPX activity was significant different from [11.14 ± 2.21 mU/mL among DNR to 8.2 ± 1.84 mU/mL p=001], for DR patients, respectively (Table 5). HbA1c ranged between 6-10 mg/dl and found significantly high among case than control groups [8.20 ± 1.94 vs. 7.2 ± 1.1 mg/dl P=0.001]. Fasting blood glucose ranged between 72 and 282 mg/dL and found significantly high among case than control groups [190.4 ± 45.1 vs. 160.1 ± 45.8; mg/dL p=0.001], mean serum cholesterol and triacylglycerol levels were not statistically different between both groups, however, cholesterol and triacylglycerol range in this study were 104-246 mg/dL and 25-258 mg/dL, respectively (Table 6). The genotyping for *GPX-198C/T* showed that, the frequency of CC was observed in 33(47%) in control group express CC genotype higher than cases, 13(22%) this reflect the protective effect of CC genotype and this is further confirmed by calculating the odd ratio (95% confidence interval)=0.310 (0.176-0.486), p=0.001. While CT genotype was detected in 22(36%) patients in cases compared with 23(33%) among control group, OR=0.40(0.10-0.90), p=0.003. TT genotype was detected in 25(42%) patients in cases and only 14(20%) in controls, this reflect the risk of TT genotype and confirmed by OR=2.4(1.10-5.90), p=0.004. The frequency of the allele C-allele was found to be 48% among cases group while allele T-allele was higher among cases group 72%, OR=0.357 (0.216-0.433), P=0.001 (Table 7).

## Discussion

Diabetic retinopathy is the result of metabolic disorder in diabetes

Variables	Patients	Controls	p-Value
	N=60	N=70	
HBA1c, mg%	8.20 ± 1.94	7.2 ± 1.1	0.001
Fasting Blood Glucose, mg/dL	190.4 ± 45.1	160.1 ± 45.8	0.001
Cholesterol Level, mg/dL	195.7 ± 55.2	184.9 ± 39.1	0.463
Triacylglycerol, mg/dL	156.3 ± 88.6	141.3 ± 51.0	0.335

Significant difference considered as p-value ≤ 0.05

Table 6: Biochemical Comparison between Cases and Controls.

Genotype	Patients (%)	Controls (%)	OR (95% CI)	p-Value
GPX-198C/T	N=60	N=70		
CC	13 (22)	33 (47)	0.310 (0.176-0.486)	0.001
CT	22 (36)	23(33)	0.40 (0.10-0.90)	0.003
TT	25 (42)	14 (20)	2.4 (1.10-5.90)	0.004
C allele frequency	0.48	0.89		
T allele frequency	0.72	0.51	0.357 (0.216-433)	0.001

Significant difference considered as p-value ≤ 0.05

Table 7: Distribution of Genotype Frequencies of *GPX-198C/T* Polymorphisms among Cases and Controls.

and most common cause of blindness in people aged 30-60 years. After 15 years almost all patients with type 1 and two thirds of those with type 2 diabetes have a risk of retinopathy. In the retina there is increased oxygen uptake and glucose oxidation relative to any other tissue; consequently, this phenomenon renders retina more vulnerable to oxidative stress, accordingly the present study was carried out to evaluate GPX gene polymorphisms and level in patients with diabetic retinopathy (DR), and to correlate between gene polymorphism and study variables. The frequency showed that, the prevalence of DR in the present study was 60(46.1%), which is similar to previous findings that, in the India 60(42.7%), and was higher than that in the prospective diabetes studies done in Egypt 28(39.84%), KSA 22(32.84%), UK 25(37%) and Melbourne 26(35.7%). A similarity was observed with Pima Native Americans in Arizona 58(41.8%). The high prevalence of DR in our study and the Arizona study might be attributed to the poor glycemic control that increases the risk for diabetic retinopathy. Moreover, a limited period of poor glycemic control can have a prolonged effect on the incidence of diabetic retinopathy ("metabolic memory") as demonstrated by the Epidemiology of Diabetes Interventions and Complications (EDIC) cohort follow-up. The prevalence of DR in our study was also higher than that in another study conducted in Hong Kong 30(28.4%) in which the subjects were recruited from primary health care clinics [18-20].

The genotyping for *GPX-198C/T* showed that, the frequency of genotype CC was significantly lower in cases compared with control, these Associations for SNPs different by type. Among CCs, *GPX-198C/T* SNP rs1050450, decreased risk after correction for multiple testing (OR=0.310, 95% CI=0.176-0.486, P=0.001), while frequency of the TT genotype were significantly higher in cases than controls. Theses TTs, *GPX-198C/T* SNP rs1050450, increased risk after correction for multiple testing (OR=2.4, 95% CI=1.10-5.90 P=0.004). The frequency of the allele C protective allele was found to be 48% among cases group while allele T-risky allele was higher among cases group

72%, - (OR=0.35795% CI=0.216-0.433 P=0.001). However the *GPX-198C/T* polymorphism has only recently been considered as a putative candidate gene in the susceptibility of diabetes and its complications (KSA). A large number of studies on *GSTM1-0* and/or *GSTT1-0* null genotypes reported an increased risk for development and progression of rheumatoid arthritis and asthma. However, one study reported an association of *GSTM1* gene with protection from development of type 1 diabetes in a group of 14 to 20-year-old children we conclude that *GPX1* is significant to evaluate the combinatorial association of gene variants in DR in T2DM. Another study from India, reported a significant association of *GSTM1* null with T2DM with no significant association with *GSTT1* null genotype. We hypothesized that genetic variability of *GPX* enzymes regulating oxidative stress could be involved in development of microangiopathic complications in people with diabetes. From these results demonstrated that the development and progression of DR affect by the multiple risk factors. In this study, and showed in other studies. Moreover other study from India was reported that *Gpx1* 'C' allele indicated a 1.362 times higher risk of T2DM. Additionally other study from china was reported that there was no statistically significant association between the *GPX1-198C/T* gene polymorphisms and the risk of DR [18,20,21-30]. Serum anti-oxidant activity (GPx) was significantly lower among DR (P=0.001), compared with DNR individuals. This may be reverred to the poor glycemic control of DR patients. Poor glycemic control may be the key factor enhancing AGE formation, which may be associated with lower GPx activity in DR [18,22]. Interestingly, one study in Hungarian patients had reported that an increased frequency of diabetes with catalase deficiency compared with both healthy relatives and the background population [24]. Free radicals formation in diabetes mellitus and increase over time may play a role in the development of diabetic retinopathy, which is an important complication of the disease [26]. Oxidative stress can influence the expression of multiple genes, including signalling molecules; over expression of these genes may cause mitochondrial dysfunction and peroxidization of the lipid and protein structure, which induce a variety of cellular dysfunctions leading to retinopathy [23]. Elevated oxidized lipids, DNA and protein in diabetics, represent a diminished capacity to decrease toxic reactive oxygen [26]. A relation was found between Reducing Glutathione Concentration and Diabetic Complications (USA and UK) [18,25]. Reduction in Glutathione Levels Occurs in Patients with Primary Open-Angle Glaucoma (USA) [18,27]. And the level of glycemic control as measured by HbA1c, fasting blood glucose is a marker for both development and progression of DR. There was a significant increased main level of HbA1c, fasting blood glucose concentration in the DR patients when compared with DNR, (p=0.001, p=0.001), contradicted with patient. This was in accordance with findings obtained from another study [18,29]. Also of interest, it has been reported that the serum fasting plasma glucose levels correlated well with the progression of DR [18,30]. Finally, poor glycemic control increases the risk for diabetic retinopathy. Moreover, a limited period of poor glycemic control can have a prolonged effect on the incidence of diabetic retinopathy ("metabolic memory") as demonstrated by the Epidemiology of Diabetes Interventions and Complications (EDIC) cohort follow-up [23]. The association between serum lipid levels and DR has been investigated in many studies, several studies have shown that the serum cholesterol level was associated with increased risk and severity of retinal hard exudates [18,26]. Treatment of hard exudates by atorvastatin has a significant associated with reduction in the severity and decrease the risk of the lipid in clinically significant macular edema [18,23]. The present study observed that, there was significant association between hyperlipidemia and DR [18]. The current study provide evidence that the lipid profile concentration in the DR patients

when compared with DNR was insignificant (p=0.463, p=0.335, and p=0.327) this agree with result obtained by Zhang et al. [29]. Recently recommend that glycemic and lipid emic control be widely promoted and used as routinely for diagnoses [18,27]. This agrees with result obtained from Wisconsin Epidemiologic Study. The results should be compared with those of the Wisconsin Epidemiologic Study of diabetic retinopathy and its relation to various risk factors [18,23] The Wisconsin study reported that the glycated hemoglobin is associated with increased risk of incidence of PDR. Also, the result of present study show that there was significant different in main concentration of serum GPX antioxidant enzymes of DR in compared with DNR with (p=0.001). There is a decrease in GPX activity in diabetic patients with retinopathy in comparison to without retinopathy control with significant difference between diabetic patients with retinopathy than without retinopathy.

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#### References

1. Ciulla TA, Amador AG, Zinman B (2003) Diabetic retinopathy and diabetic macular edema: path physiology, screening, and novel therapies. *Diabetes Care* 26: 2653-2664.
2. Hallman DM, Huber JC, Gonzalez VH, Klein BE, Klein R, et al. (2005) Familial aggregation of severity of diabetic retinopathy in Mexican Americans from Starr County Texas. *Diabetes Care* 28: 1163-1168.
3. Uhlmann K, Kovacs P, Boettcher Y, Hammes HP, Paschke R (2006) Genetics of diabetic retinopathy. *Exp Clin Endocrinol Diabetes* 114: 275-294.
4. Congdon NG, Friedman DS, Lietman T (2003) Important causes of visual impairment in the world today. *JAMA* 290: 2057-2060.
5. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL (1984) The Wisconsin Epidemiologic Study of Diabetic Retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. *Arch Ophthalmol* 102: 527-532.
6. Elbagir MN, Eltom MA, Elmahadi EM, Kadam IM, Berne C: A high prevalence of diabetes mellitus and impaired glucose tolerance in the Danagla community in northern Sudan. *Diabet Med* 15: 164-169.
7. Elbagir MN, Eltom MA, Elmahadi EM, Kadam IM, Berne C (1996) A population-based study of the prevalence of diabetes and impaired glucose tolerance in adults in northern Sudan. *Diabetes Care* 19: 1126-1128.
8. Narendran V, John RK, Raghuram A, Ravindran RD, Nirmalan PK, et al. Diabetic retinopathy among self-reported diabetics in Southern India: A population based assessment Article. *Br J Ophthalmol* 86: 1014-1018.
9. Dandona L, Dandona R, Naduvilath T, McCarty C, Rao G (1999) Population based assessment of diabetic retinopathy in an urban population in Southern India. *Br J Ophthalmol* 83: 937-940.
10. Viswanath K, McGavin DDM (2003) Diabetic Retinopathy: Clinical Findings and Management *Community Eye Health* 16: 21-24.
11. Shinde A, Ganu J, Naik P, Sawant A (2012) Oxidative stress and antioxidative status in patients with alcoholic liver disease. *Biomedical Research* 23: 105-108
12. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI (2013) Markers of Oxidative Stress during Diabetes Mellitus. *Journal of Biomarkers* volume 2013, pp: 1-8
13. Zhang Y, Zhang L, Sun D, Li Z, Wang L, et al. (2011) Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. *Molecular Vision* 17: 2325-2332
14. Zhang X, Saaddine JB, Chou CF, Cotch MF, Cheng YJ, et al. (2010) Prevalence of diabetic retinopathy in the United States, 2005-2008. *JAMA* 304: 649-656.
15. Hammes HP, Kerner W, Hofer S, Kordonouri O, Raile K, et al. (2011) Diabetic retinopathy in type 1 diabetes- a contemporary analysis of 8784 patients. *Diabetologia* 54: 1977-1984.

16. American Diabetes Association Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, 2004; 27: 5-10.
17. American Diabetes Association Standards of medical care in diabetes, 2008. *Diabetes Care*, 2008; 31: 12-S54.
18. El-Bab MF, Zaki NS, Mojaddidi MA, AL-Barry M, El-Beshbishy HA (2013) Oxidative Stress and Diabetic Retinopathy. *Int J Gen Med* 19: 799-806.
19. Tam VH, Lam EP, Chu BC, Tse KK, Fung LM (2009) Incidence and progression of diabetic retinopathy in Hong Kong Chinese with type 2 diabetes mellitus. *J Diabetes Complications* 23: 185-193
20. Pourvali K, Abbasi M, Mottaghi A (2016) Role of Superoxide Dismutase 2 Gene Ala16Val Polymorphism and Total Antioxidant Capacity in Diabetes and its Complications. *Avicenna J Med Biotech* 8: 48-56
21. Choudhuri S, Dutta D, Chowdhury IH, Mitra B, Sen A, et al. (2013). Association of hyperglycemia mediated increased advanced Glycation and erythrocyte antioxidant enzyme activity in different stages of diabetic retinopathy. *Diabetes research clinical practice* 100: 376-384.
22. Hovnik T, Dolžan V, Bratina NU, Podkrajšek KT, Battelino T (2009) Genetic Polymorphisms in Genes Encoding Antioxidant Enzymes Are Associated With Diabetic Retinopathy. *Diabetes Care* 32: 2258-2262.
23. Santos KG, Canani LH, Gross JL, Roisenberg I (2006) The Catalase-262C/T Promoter Polymorphism and Diabetic Complications in Caucasians with Type 2 Diabetes. *Committee on Publication Ethics* 22: 355-359.
24. Vats P, Sagar N, Singh TP, Banerjee M (2015) Association of Superoxide dismutases (SOD1 and SOD2), Glutathione peroxidase1 (GPx1) and CAT21 C/T gene polymorphisms with Type 2 diabetes mellitus. *Free Radical Research* 49.
25. Gürler B, Vural H, Yilmaz N, Oguz H, Satici A, et al. (2000) The role of oxidative stress in diabetic retinopathy. *Eye* 14: 730-735.
26. ICPPE 2017 4th International Conference on Petroleum and Petrochemical Engineering 21 January 2017-23 January 2017. Bangkok, Thailand.
27. ICFAE 2017 3rd International Conference on Food and Agricultural Engineering (ICFAE 2017). 10 May 2017 -12 May 2017. Budapest, Hungary.
28. Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, et al. (2006) Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age dependent skeletal muscle atrophy. *Free Radic Biol Med* 40: 1993-2004.
29. Zhang Y, Zhang L, Sun DL, Li ZS, Wang L, et al. (2011) Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. *Mol Vis* 17: 2325-2332.
30. Klein R, Knudtson MD, Lee KE, Gangnon R, Klein BE (2009) The Wisconsin Epidemiologic Study of Diabetic Retinopathy XXIII: the twenty-five-year incidence of macular edema in persons with type 1 diabetes. *Ophthalmology* 116: 497-503.