

# Diabetic retinopathy is associated with oxidative stress and mitigation of gene expression of antioxidant enzymes

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**Abstract:** Type 2 diabetes is a metabolic disease associated with serious complications, including diabetic retinopathy (DR). The authors' main aim was to investigate biochemical parameters and the oxidative stress associated with the type 2 DR patients and to study gene expression of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) among patients with DR [DR(+)] compared with a control nondiabetic group. In all, 67 patients with DR included in this study were diabetic for more than 10 years. Among them, 22 patients were DR(+), and 45 patients did not have DR [DR(-)]. The subjects' age range was 14 years to 80 years old with diabetes duration range between 2 and 45 years. Body mass index (BMI) was  $31.43 \pm 5.94$  and  $32.33 \pm 6.54$ , systolic blood pressure was  $117.15 \pm 18.16$  mmHg and  $126.15 \pm 20.26$  mmHg, diastolic blood pressure was  $81.11 \pm 10.55$  mmHg and  $82.77 \pm 10.85$  mmHg, HbA<sub>1c</sub> was  $7.2 \pm 1.1$  and  $8.19 \pm 1.95$ , serum total cholesterol was  $6.61 \pm 1.11$  and  $4.11 \pm 0.31$ , serum triglycerides were  $3.52 \pm 0.89$  and  $3.42 \pm 0.79$ , serum low-density lipoprotein (LDL) was  $2.12 \pm 0.10$  and  $2.42 \pm 0.15$ , high-density lipoprotein (HDL) was  $2.66 \pm 0.30$  and  $2.55 \pm 0.21$ , SOD was  $3.12 \pm 0.87$  and  $1.53 \pm 0.14$ , GPx was  $11.14 \pm 2.21$  and  $8.2 \pm 1.84$ , CAT was  $26.43 \pm 3.34$  and  $9.60 \pm 2.14$ , for DR(-) and DR(+) patients, respectively. SOD, GPx and CAT polymerase chain reaction (PCR) products of the DR(+) patients revealed the diminished expression of CAT gene followed by GPx and SOD genes. All were significant compared with the normal controls,  $P < 0.05$ . Linear regression analysis revealed a strong significant positive correlation between the retinopathy grade and the diastolic blood pressure, diabetes duration, hemoglobin A1c (HA<sub>1c</sub>)%, and fasting blood glucose ( $P < 0.001$ ). A marginally significant positive correlation between the retinopathy grade and LDL-cholesterol was observed ( $P < 0.05$ ), and a significant negative correlation between the retinopathy grade and total cholesterol was observed ( $P < 0.05$ ). Poor glycemic control and alteration in mRNA gene expression of antioxidant enzymes are strongly associated with development of DR and the regular screening is mandatory for early detection and treatment.

**Keywords:** diabetic retinopathy, antioxidant enzymes, gene expression

## Introduction

Type 2 diabetes is a metabolic disease associated with serious micro- and macrovascular complications, including diabetic retinopathy (DR), that are aggravated by poor glycemic control, hypertension, and longer disease duration.<sup>1</sup> DR is associated with a strong genetic predisposition, highlighted by the familial clustering of DR.<sup>2,3</sup>

Diabetic retinopathy is a common and highly specific microvascular complication of diabetes mellitus and represents the leading cause of blindness among working-age individuals in developed countries.<sup>4</sup> DR occurs both in type 1 and type 2 diabetes and is strictly related to disease duration: virtually all individuals diagnosed with

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diabetes at less than 30 years of age and more than 70% of individuals diagnosed aged 30 years or older develop DR after 15 years of diabetes duration.<sup>5,6</sup> However, there are substantial differences between type 1 and type 2 diabetes in terms of clinical presentation, prevalence, and, at least in part, risk factors of DR.

Prevalence of any DR is higher in type 1 than in type 2 diabetes and also in males than in females.<sup>7</sup> However, prevalence estimates are quite variable across the world, and it is unclear whether recent improvements in diabetes care have resulted in significant reduction of this complication, and particularly, of its sight-threatening lesions.<sup>8</sup> Reanalysis of individuals under poor control in 25 years of follow-up revealed only minor improvements following implementation of improved care, even though an intensive glucose control early in the course of the disease produced significant and persistent benefits on microvascular complications including DR, both in type 1 and in type 2 diabetes.<sup>9–11</sup> In addition to the extent and duration of chronic hyperglycemia, other risk factors for DR are dyslipidemia and hypertension; however, the role of age, gender, and smoking is controversial and seems to differ between type 1 and type 2.<sup>12–16</sup>

The aim of the present study was to investigate different anthropometric and biochemical parameters among patients with DR [DR(+)] and patients without DR [DR(-)]. These included age, diabetes onset and duration, fasting and postprandial glucose, systolic and diastolic blood pressure (BP). Blood HA<sub>1c</sub> was measured as were serum triglycerides, total and high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol. The antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) were measured. A polymerase chain reaction (PCR) was also performed to quantify the expression of messenger ribonucleic acid (mRNA) for the SOD, GPx and CAT antioxidant enzymes.

## Subjects, materials, and methods

The proposal of the following prospective study was approved by the local ethics committee; written informed consent was obtained from each patient who attended the out-patient clinic of the Diabetic Patients Medical Centre in King Fahad Hospital in Al-Madinah Al-Munawarah, in Kingdom of Saudi Arabia in the academic year 2011–2012. All diabetic subjects were diagnosed using international standard criteria published by the American Diabetes Association.<sup>17,18</sup> The DR assessment was carried out according to the Diabetic

Retinopathy Grading of Tam et al in 2009<sup>19</sup> and Wilkinson et al in 2003.<sup>20</sup>

## Chemicals and kits

Chemicals and reagents were of the highest analytical grade and purchased from the Sigma-Aldrich (St Louis, MO, USA). Diagnostic kits for HA<sub>1c</sub> were purchased from NS BIOTEC (London, UK). Diagnostic kits for serum triglycerides, and total and HDL-cholesterol, were purchased from Randox Laboratories Ltd., (London, UK). Diagnostic kits for serum CAT and SOD were purchased from Cell Biolabs Inc (San Diego, CA, USA). The serum GPx activity assay kit was purchased from BioVision Inc., (Milpitas, CA, USA). The RNA extraction kit was supplied from Promega UK Ltd (Southampton, UK). The primers for SOD, GPx and CAT genes were synthesized and purchased from TIB Molbiol GmbH (Berlin, Germany).

## Patients and biochemical parameters

The 67 patients with non-insulin-dependent diabetic retinopathy used in this study were diabetic for more than 10 years. All patients filled out a structured questionnaire to collect the following information: age; diabetes onset and duration; fasting and postprandial glucose using the glucose oxidase method; systolic and diastolic blood pressure (BP) using the Richter® mercurial sphygmomanometer after 5 minutes of rest. Fresh blood samples were used for the determination of HA<sub>1c</sub>. Blood samples were withdrawn from the vein into a BD Vacutainer tube containing anticoagulant and sodium fluoride, followed by centrifugation at 6000 rpm for 10 minutes. The resulting supernatant was used for the determination of serum triglycerides, total and HDL cholesterol using standard analytical methods. However, the LDL cholesterol was calculated by the Friedwald formula.<sup>21</sup>

The serum GPx activity was determined by a spectrophotometric method based on the oxidation of GSH by GPx in the presence of hydrogen peroxide to produce glutathione disulfide (GSSG). Then, nicotinamide adenine dinucleotide phosphate (NADPH) and GR were used for production of glutathione (GSH) and NADP. Enzyme activity was determined by measuring the disappearance on NADPH at 340 nm and was expressed as mU/mL.<sup>22</sup> Serum CAT enzyme activity was determined by spectrophotometric method that based on the fact that, catalase catalyzes the breakdown of H<sub>2</sub>O<sub>2</sub>. The remaining H<sub>2</sub>O<sub>2</sub> in the reaction mixture facilitates the coupling of this reaction monitored at 520 nm. The decrease

in absorbance is proportional to the activity of CAT.<sup>23</sup> CAT activity was expressed as U/mL. The serum SOD activity was determined at 37°C by the xanthine/xanthine oxidase system that generates the superoxide anions. The presence of SOD resulted in the decrease of superoxide anions yielding a lower colorimetric signal at 490 nm. SOD was expressed as U/mL.<sup>24</sup>

## Reverse-transcriptase (RT)-PCR of SOD, CAT and GPx

RNA was extracted from the blood; 1 µg of the extracted mRNA was denatured at 72°C for 10 minutes and reversibly transcribed to cDNA by incubating with a reaction mixture containing 50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM DTT, 0.5 mM of each dNTP, 62.5 mU DNase I, 50 ng random hexamers, and 100U M-MuLV reverse transcriptase (RT). The reaction mixture was then incubated at 42°C for 60 minutes, heated to 95°C for 5 minutes, and then quickly chilled on ice. The PCR was performed to quantify the expression of mRNA for the antioxidant enzymes (SOD, GPx and CAT). The cDNA (2 µL) was amplified in a 50 µL of PCR reaction mixture containing 10 × PCR reaction buffer, 25 mM MgCl<sub>2</sub>, 4 mM dNTPs, and 15 µM each primer and 7 Unit Taq DNA polymerase. The primers' design was optimized using the (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and listed in Table 1. The PCR thermal cycler (Swift Maxi thermal cycler, ESCO Technologies Inc, St Louis, MO, USA) was programmed as follows: denaturation step at 95°C for 2 minutes followed by 30 cycles of 95°C for 1 minute; 55°C for 1 minute and 72°C for 30 seconds. A final extension cycle at 72°C for 3 minutes was performed. The PCR products were analyzed on a 2% agarose gel and visualized under a ultra violet transilluminator.

**Table 1** Primer sequences and the expected size of the PCR product for the antioxidant enzymes; SOD, GPx, and CAT<sup>45</sup>

Gene	Primer	Sequence 5'-3'	Product size
SOD	F	5'-GCAGAAGGCAAGCGGTGAAC-3'	387 bp
	R	5'-TAGCAGGACAGCAGATGAGT-3'	
GPx	F	5'-CTCTCCGCGGTGGCACAGT-3'	29 bp
	R	5'-CCACCACCGGTTCGGACATAC-3'	
CAT	F	5'-GCGAATGGAGAGGCAGTGTAC-3'	670 bp
	R	5'-GAGTGACGTTGTCTTCATTAG CACTG-3'	

**Abbreviations:** bp, base pair; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.

## Assessment of DR

The presence of DR was assessed by an expert ophthalmologist using dilated funduscopy. Patients were classified into the following categories: absent DR; mild, moderate or severe non-proliferative diabetic retinopathy; proliferative diabetic retinopathy (PDR); or maculopathy according to the Global Diabetic Retinopathy Project Group.<sup>19,20</sup> DR grade was assigned based on the worst eye (Table 2).

## Statistical analysis

Results are reported as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found to be statistically significant (*P* < 0.05), further comparisons among groups were made according to the post hoc Tukey's procedure multiple comparison test, and linear regression analysis. All statistical analyses were performed using GraphPad InStat 3 (GraphPad Software, Inc, La Jolla, CA, USA) software. Graphs were sketched using GraphPad Prism version 4 software.

## Results

In this study, we examined 67 diabetic patients for the existence of DR. Twenty-two (32.84%) patients were DR(+), and 45 (67.16%) patients were DR(-). Our data

**Table 2** Diabetic retinopathy patient distributions in the present study population according to the Global Diabetic Retinopathy Project grading<sup>19,20</sup>

Score	International term	Description	DR patients n = 67
Grade 0	No PDR	Normal retina (Background retinopathy)	45 (46.16%)
Grade 1	Mild non-proliferative	Hgs and MA only, very minor IRMAs	8 (11.94%)
Grade 2	Moderate non-proliferative	Extensive MA, intra-retinal Hgs, and hard exudates	6 (8.96%)
Grade 3	Severe non-proliferative	Venous abnormalities, large blot Hgs, cotton wool spots, venous beading, venous loop, venous reduplication, IRMAs	5 (7.46%)
Grade 4	Proliferative retinopathy	New vessel formation either at the disc neovascularization or elsewhere	3 (4.48%)

**Abbreviations:** DR, diabetic retinopathy; PDR, proliferative diabetic retinopathy; Hgs, hemorrhages; MA, micro-aneurysm; IRMAs, intra-retinal micro-vascular abnormalities.

revealed the following positive diabetic retinopathy patient distribution: grade I, eight patients; grade II, six patients; grade III, five patients; and grade IV in three patients, representing 36.4%, 27.3%, 22.7% and 13.6% of the 22 DR(+) patients, respectively (Table 2). Patients with DR (n = 22) were 14 to 80-years-old and had a diabetes duration of 2 to 45 years. The body mass index (BMI) of DR(-) and DR(+) patients was  $31.43 \pm 5.94$  and  $32.33 \pm 6.54$ , respectively. The systolic blood pressure changed significantly, from  $109 \pm 18.46$  mmHg among normal controls to  $117.15 \pm 18.16$  mmHg and  $126.15 \pm 20.26$  mmHg for DR(-) and DR(+) patients, respectively. The diastolic blood pressure (pressure) changed significantly from  $76.77 \pm 10.55$  mmHg among normal controls to  $81.11 \pm 10.55$  mmHg and  $82.77 \pm 10.85$  mmHg for DR(-) and DR(+) patients, respectively.

The fasting blood glucose changed significantly from  $74.21 \pm 11.61$  mg% among normal controls to  $142.81 \pm 31.41$  mg% and  $143.21 \pm 37.33$  mg%, for DR(-) and DR(+) patients, respectively. The postprandial blood glucose changed significantly from  $105 \pm 23.21$  mg% among normal controls to  $170.22 \pm 43.33$  mg% and  $174.22 \pm 73.33$  mg%, for DR(-) and DR(+) patients, respectively. The  $HA_{1c}$  changed significantly from  $3.29 \pm 0.95$  mg% among normal controls to  $7.2 \pm 1.1$  mg% and  $8.19 \pm 1.95$  mg%, for DR(-) and DR(+) patients, respectively (Table 3). The serum total cholesterol changed significantly from  $2.61 \pm 0.54$  mmol/L among normal controls to  $6.61 \pm 1.11$  mmol/L and  $4.11 \pm 0.31$  mmol/L for DR(+) and DR(-) patients, respectively. The serum trig-

lycerides changed significantly from  $2.52 \pm 0.23$  mmol/L among normal controls to  $3.52 \pm 0.89$  mmol/L and  $3.42 \pm 0.79$  mmol/L for DR(+) and DR(-) patients, respectively. The serum LDL-cholesterol changed significantly from  $1.52 \pm 0.12$  mmol/L among normal controls to  $2.12 \pm 0.10$  mmol/L and  $2.42 \pm 0.15$  mmol/L for DR(-) and DR(+) patients. The serum HDL cholesterol changed significantly from  $2.85 \pm 0.13$  mmol/L among normal controls to  $2.66 \pm 0.30$  mmol/L and  $2.55 \pm 0.21$  mmol/L, for DR(-) and DR(+) patients, respectively (Table 4).

The serum SOD changed significantly from  $5.44 \pm 1.12$  U/mL among normal controls to  $3.12 \pm 0.87$  U/mL and  $1.53 \pm 0.14$  U/mL, for DR(-) and DR(+) patients. The serum GPx changed significantly from  $14.31 \pm 3.14$  mU/mL among normal controls to  $11.14 \pm 2.21$  mU/mL and  $8.2 \pm 1.84$  mU/mL, for DR(-) and DR(+) patients, respectively. The serum CAT was significantly changed from  $40.13 \pm 5.94$  U/mL in case of normal control to  $26.43 \pm 3.34$  U/mL and  $9.60 \pm 2.14$  U/mL, for DR(-) and DR(+) patients, respectively (Table 5). The agarose gel electrophoresis pattern of the SOD, GPx, and CAT PCR products of the DR(+) patients revealed the diminished expression of the CAT gene, followed by GPx and SOD genes (Figure 1).

The linear regression analysis among different biochemical parameters in DR patients revealed a strong significant positive correlation between the retinopathy grade (RG) and the diastolic blood pressure ( $r^2 = +0.49$ ;  $P < 0.001$ ), diabetes duration ( $r^2 = 0.3593$ ;  $P < 0.001$ ),  $HA_{1c}$ % ( $r^2 = +0.1472$ ,  $P < 0.0001$ ) and fasting blood glucose ( $r^2 = +0.1626$ ,  $P < 0.0001$ ). A slightly significant positive correlation

**Table 3** Basic clinical biochemical parameters of diabetic retinopathy patients

	NC n = 21 (Mean $\pm$ SD)	DR(-) n = 45 (Mean $\pm$ SD)	DR(+) n = 22 (Mean $\pm$ SD)
Age (years)	$38.33 \pm 10.22$	$40.56 \pm 11.51^*$	$42.33 \pm 12.44^*$
Duration of diabetes (years)	-	$11.29 \pm 3.43^*$	$10.29 \pm 3.28^*$
Body mass index (kg/m <sup>2</sup> )	$29.33 \pm 5.94$	$31.43 \pm 5.94^*$	$32.33 \pm 6.54^*$
Systolic blood pressure (mmHg)	$109.34 \pm 18.46$	$117.15 \pm 18.16^*$	$126.15 \pm 20.26^*$
Diastolic blood pressure (mmHg)	$76.77 \pm 10.55$	$81.11 \pm 10.55^*$	$82.77 \pm 10.85^*$
Glycated hemoglobin ( $HA_{1c}$ )	$3.29 \pm 0.95$	$7.20 \pm 1.1^*$	$8.19 \pm 1.95^*$

**Notes:** Values are expressed as mean  $\pm$  SD. \*Significant change difference from normal control group at  $P < 0.05$ .

**Abbreviations:** NC, normal control group; DR(-), diabetics without retinopathy; DR(+), diabetics with retinopathy; SD, standard deviation.

**Table 4** Lipid profile of diabetic patients

	NC n = 21	DR(-) n = 45	DR(+) n = 22
Total cholesterol (mmol/L)			
Range	0.30–4.7	0.28–6.3	0.48–9.4
Mean $\pm$ SD	$2.61 \pm 0.54$	$4.11 \pm 0.31$	$6.61 \pm 1.11^*$
Triglycerides (mmol/L)			
Range	0.34–3.8	0.45–7.8	0.34–9.8
Mean $\pm$ SD	$2.52 \pm 0.23$	$3.42 \pm 0.79^*$	$3.52 \pm 0.89^*$
LDL-cholesterol (mmol/L)			
Range	0.31–3.5	0.41–5.8	0.51–6.5
Mean $\pm$ SD	$1.52 \pm 0.12$	$2.12 \pm 0.10^*$	$2.42 \pm 0.15^*$
HDL-cholesterol (mmol/L)			
Range	0.44–3.8	0.14–3.6	0.24–4.8
Mean $\pm$ SD	$2.85 \pm 0.13$	$2.66 \pm 0.30^*$	$2.55 \pm 0.21^*$

**Notes:** Values are expressed as mean  $\pm$  SD. \*Significant change difference from normal control group at  $P < 0.05$ .

**Abbreviations:** HDL, high-density lipoprotein; LDL, low-density lipoprotein; NC, normal control group; DR(-), diabetics without retinopathy; DR(+), diabetics with retinopathy; SD, standard deviation.



**Table 5** Serum antioxidant enzymes levels of patients with and without diabetic retinopathy

Parameter	NC	DR(-)	DR(+)
SOD (U/mL)	5.44 ± 1.12	3.12 ± 0.87*	1.53 ± 0.14*
GPx (mU/mL)	14.31 ± 3.14	11.14 ± 2.21*	8.20 ± 1.84*
CAT (U/mL)	40.13 ± 5.94	26.43 ± 3.34*	9.60 ± 2.14*

**Notes:** Values are expressed as mean ± SD. \*Significant change difference from normal control group at  $P < 0.05$ .

**Abbreviations:** NC, Normal control group; DR(-), diabetics without retinopathy; DR(+), diabetics with retinopathy; SD, standard deviation. SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.

between the retinopathy grade and LDL-cholesterol was observed ( $r^2 = +0.019$ ,  $P < 0.05$ ). In contrary, slightly significant negative correlation between the retinopathy grade and total cholesterol was observed ( $r^2 = +0.01732$ ,  $P < 0.05$ ); however, other parameters showed nonsignificant correlations (Figure 2).

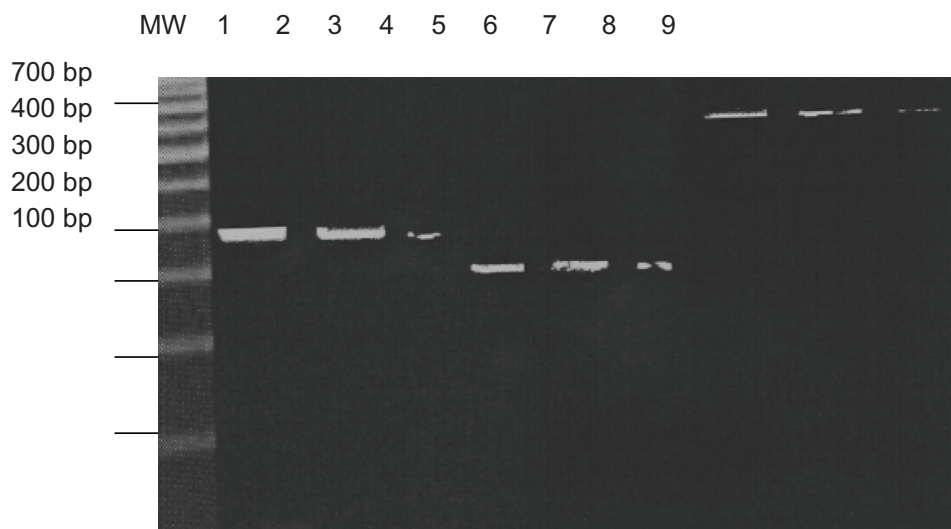
## Discussion

The prevalence of DR in our study was 32.84%. The prevalence of DR in this study was similar to that in the UK prospective diabetes study (37%),<sup>25</sup> the Melbourne visual impairment project (35.7%)<sup>26</sup>, and our previous study (36.1%);<sup>27</sup> it was also very close to that found in a study in the Pima Native Americans in Arizona (31.8%).<sup>28</sup> The low prevalence of DR in our study and the Arizona study might be attributed to a diabetes screening program in which regular diabetes screening was offered on a biennial basis to children aged  $\geq 5$  years. The prevalence of DR in our study was also higher than that in

another study conducted in Hong Kong (28.4%) in which the subjects were recruited from primary health care clinics.<sup>29</sup>

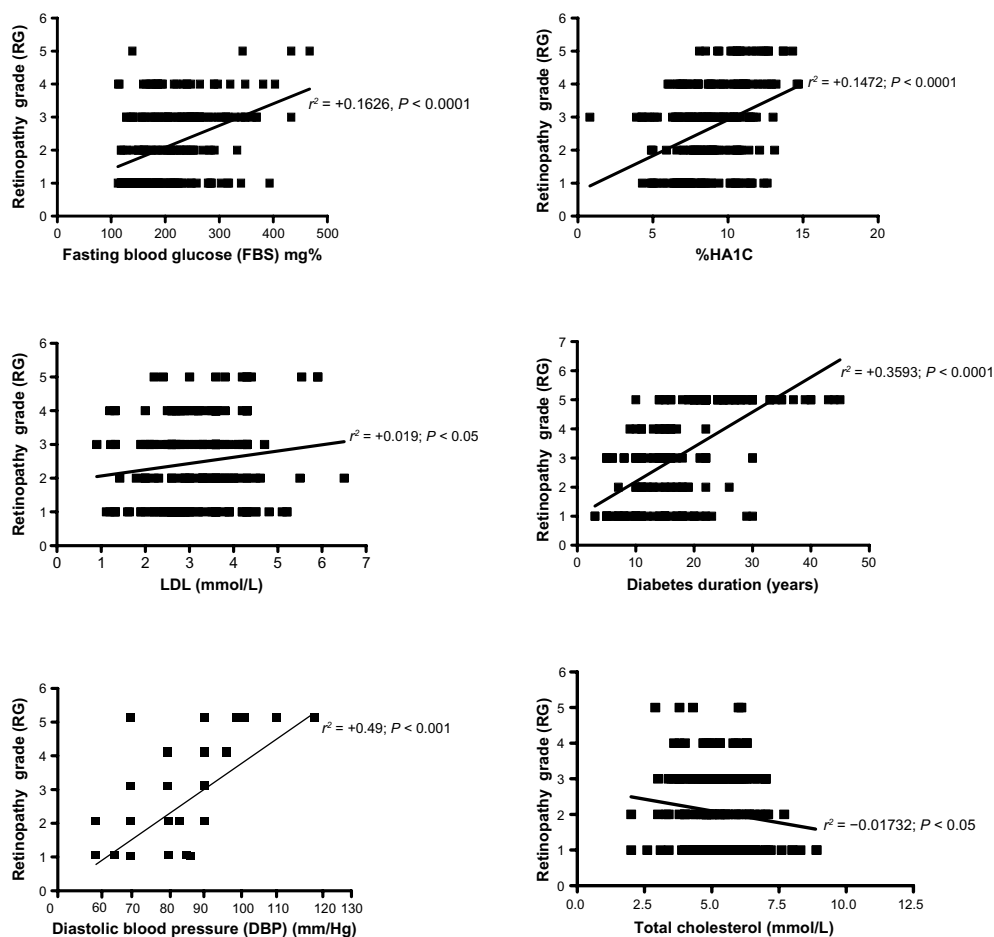
There are multiple risk factors that can affect the development and progression of DR. In this study, and as has been shown in other studies, the level of glycemic control as measured by  $H_{A_{1c}}$  is a marker for both development and progression of DR. The  $H_{A_{1c}}$  level was elevated in the DR(+) patients more than in the DR(-) patients. We also found that the serum fasting blood glucose level was associated with progression in the DR(+) patients at a higher significance level than in the DR(-) patients. This was in accordance with findings obtained from another study.<sup>30</sup> Also of interest, it has been reported that the serum postprandial plasma glucose levels determined at 2 hours after the intake of an isocaloric mixed breakfast correlated well with the progression of DR.<sup>31</sup>

The relationship between DR and hypertension was strongly suggested by the results of the United Kingdom Prospective Diabetes Study (UKPDS) trial.<sup>32</sup> However, the appropriate blood pressure control in diabetes (ABCD) trial revealed no relationship between blood pressure and incidence of DR.<sup>33</sup> The authors of the ABCD study suggested that the lack of effect might have resulted from the shorter time period of the study, lower average blood pressure control, and poorer glycemic control in the ABCD trial than in the UKPDS. Our results showed that a history of hypertension and higher systolic blood pressure were significantly associated with progression of DR. These data were in accordance with a previous study conducted in China<sup>19</sup> and consistent

**Figure 1** 2% agarose gel electrophoresis of PCR product of serum SOD, GPx and CAT.

**Notes:** Lanes 1–3 for SOD, Lanes 4–6 for GPx and Lanes 7–9 for CAT. MW = M.V. DNA 100 bp ladder. The size of SOD gene = 387 bp. The size of GPx gene = 290 bp. The size of CAT gene = 670 bp. Lanes 1, 4 and 7 are normal control. Lanes 2, 5 and 8 are DR(-) patients. Lanes 3, 6 and 9 are DR(+) patients.

**Abbreviations:** DR(-), diabetics without retinopathy; DR(+), diabetics with retinopathy; PCR, polymerase chain reaction; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase.



**Figure 2** The linear regression analysis revealed a strong significant positive correlation between the retinopathy grade (RG) and the diastolic blood pressure ( $r^2 = +0.49$ ;  $P < 0.001$ ), diabetes duration ( $r^2 = 0.3593$ ;  $P < 0.001$ ), Hemoglobin A1c ( $\text{HA}_{1c}$ ) % ( $r^2 = +0.1472$ ,  $P < 0.0001$ ) and fasting blood glucose ( $r^2 = +0.1626$ ,  $P < 0.0001$ ). A slightly significant positive correlation between the retinopathy grade and low-density lipoprotein-cholesterol was observed ( $r^2 = +0.019$ ,  $P < 0.05$ ). In contrast, slightly significant negative correlation between the retinopathy grade and total cholesterol was observed ( $r^2 = +0.01732$ ,  $P < 0.05$ ).

with the Wisconsin epidemiologic study XXIII of diabetic retinopathy evaluations after 14 years of follow-up.<sup>34,35</sup>

The possible mechanisms by which hypertension affects DR include impaired auto-regulation, hyperperfusion, and up-regulation of the vascular endothelial growth factor expression in retinal endothelial cells.<sup>36</sup> Improved monitoring and control of hypertension among diabetic patients in the United Arab Emirates, which has been shown to slow the progression of retinopathy and especially among those with poorly controlled diabetes, is strongly recommended.<sup>37</sup>

In our study, we observed that patients who developed DR had a significantly higher BMI. While most previous studies showed no association between BMI and DR, the Nottingham study showed an inverse relationship between BMI and DR in type 2 diabetic patients.<sup>38</sup> Chan et al<sup>39</sup> demonstrated that in Chinese type 2 diabetic patients lean subjects had predominant insulin deficiency and obese subjects had

features of metabolic syndrome. The lean patients had the lowest C-peptide and the highest  $\text{HA}_{1c}$  and hence, higher prevalence of microvascular complications. 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.<sup>13,40</sup>

The use of atorvastatin has been found to be associated with reduction in the severity of hard exudates and subfoveal lipid migration in clinically significant macular edema.<sup>41</sup> The present study showed significant association between hyperlipidemia and DR. This was in opposition to a previous study that showed no significant association between hyperlipidemia and DR.<sup>19</sup>

Our results showing a significant positive association between  $\text{HA}_{1c}$  levels and different grades of diabetic retinopathy are consistent with other reports.  $\text{HA}_{1c}$  levels in diabetic Saudi patients were more than 8%, and there

was a positive correlation between diabetic retinopathy and hyperlipidemia. It is strongly recommended that glycemic and lipidemic control be widely promoted and that H<sub>A1c</sub> and lipid profile investigations be carried out routinely.<sup>42</sup> The results should be compared with those of the Wisconsin Epidemiologic Study of diabetic retinopathy: XXII, which examined the 25-year cumulative progression and regression of diabetic retinopathy (DR) and its relation to various risk factors.<sup>9</sup> The Wisconsin study revealed that the higher glycated hemoglobin is associated with increased risk of incidence of PDR.

Also, the present study revealed the significant decline in serum SOD, GPx, and CAT antioxidant enzymes among the DR(+) patients. This may be attributed to the poor glycemic control of those DR patients. The levels of oxidized lipids, DNA and proteins are higher in diabetics, suggesting a diminished capacity to reduce toxic reactive oxygen.<sup>43,44</sup> These results were concomitant with the decrease of expression of the genes of the mentioned enzymes among the DR(+) patients.

## Conclusion

DR is prevalent in Saudi patients with type 2 diabetes and associated with poor glycemic control associated with low levels of mRNA expression of antioxidant enzymes. Regular screening is mandatory for early detection and management. More frequent retinopathy screening should be offered to those patients with DR and poor glycemic control in order to prevent the development and progression of DR.

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## Disclosure

The authors report no conflicts of interest in this work.

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