British Journal of Medicine & Medical Research
1(4): 371-384, 2011

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Imbalance of Oxidant/Antioxidant Status and Risk Factors for Saudi Type 2 Diabetic Patients with Retinopathy

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Research Article

Received 4th July 2011
Accepted 10th August 2011
Online Ready 25th August 2011

ABSTRACT

Aims: To estimate the oxidant/antioxidant status in type 2 diabetic patients with retinopathy; and to correlate a number of independent variables (age, gender, education, body mass index, duration of diabetes, glycosylated haemoglobin, hypertension) to development of retinopathy.
Study design: Case-control study.
Place and Duration of Study: Research laboratories, Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University from April 2010 to April 2011.
Methodology: One-hundred diabetic patients with retinopathy recruited from King Fahad Specialist Hospital- Buraidah were included in the study. The control groups were: control group 1 consisted of sixty type 2 diabetic patients without retinopathy recruited from Diabetes and Endocrinology Center, KFSH, Buraidah, KSA; and control group 2 consisted of sixty healthy "non diabetic subjects" recruited from public places, i.e. Estarahes (party lounges). Plasma, serum, and erythrocyte lysate were prepared from blood of each subject. Human serum 8-OHdG, plasma MDA, and erythrocyte

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lysate Cu-Zn SOD were estimated by using commercial kits supplied by Northwest, U.S.A. Data was analyzed using SPSS software and Win Episcope software. P- values < 0.05 were considered significant.

Results: Age, education, duration of diabetes, poor glycaemic control, and hypertension were consistently associated with development of retinopathy (OR 5.891, 4.44, 10.420, 1.699, 1.820 respectively). Moreover, increased plasma MDA, increased serum 8-OHdG levels, decreased Cu-Zn SOD activity were observed in diabetic patients with retinopathy compared to subjects in control groups. In addition, negative correlations were found between plasma MDA and Cu-Zn SOD activity, HbA1c & Cu-Zn SOD activity as well in all subjects.

Conclusion: This report emphasizes the important role of oxidative stress in the development of retinopathy in type 2 diabetes suggesting that blocking of oxidative stress is a crucial step for delayed progression of diabetic retinopathy and hence the need for antioxidant supplements to postpone the severity of diabetic retinopathy.

Keywords: Type 2 diabetes; diabetic retinopathy; risk factors; oxidative stress; malondialdehyde; 8-hydroxy-2`-deoxyguanosine; superoxide dismutase;

1. INTRODUCTION

Diabetic retinopathy (DR) is the commonest microvascular complication of diabetes and remains one of the leading causes of blindness worldwide among adults aged 20–74 years old. The two most important visual complications of DR are diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) (Abu El-Asrar et al., 2009). With a prevalence rate of 24% among the adult population, diabetes is a common disorder in the Kingdom of Saudi Arabia (KSA) (Salman and Al-Rubeaan, 2009). Recently, Khan et al. (2010) reported that the prevalence of DR in Eastern Saudi Arabia was 30% with the odd ratios among diabetics residing in an urban area significantly higher than diabetics residing in rural areas.

Hyperglycemia, the characteristic feature of diabetes, has been reported to be responsible for the elevated levels of free radicals in the plasma such as hydroxyl radicals ($OH^{\cdot -}$), superoxide anions($O_2^{\cdot -}$), and hydrogen peroxide (H_2O_2) (Mahreen et al., 2010; Cetinkaya et al., 2005). These reactive oxygen species (ROS) may interfere with cell function by reacting with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue (Winkler et al., 2007). Under normal physiological conditions, there is a critical balance in the generation of ROS and antioxidant defense systems in cells (Yang et al., 2008). However, when this balance is disrupted due to excess ROS or reduced activity of antioxidant defenses or both; this provokes a situation of oxidative stress (Rattan and Nayak, 2008; Soliman, 2008).

Lipid peroxidation occurs in response to elevated levels of ROS with the liberation of reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (Buddi et al. 2002). Moreover, hydroxyl radicals may attack DNA strands causing the addition to DNA bases, which lead to the generation of a variety of oxidation products such as 8-hydroxy-2`-deoxyguanosine (8-OHdG) which is the most commonly detected by-product of DNA damage and considered as a biomarker of oxidative stress (Valavanidis et al., 2009; Schulpis et al., 2007; Woo et al., 2010).

This case-control study aimed: to estimate the oxidant/antioxidant status in type 2 diabetic patients with DR; and to correlate a number of independent variables (age, gender, education, body mass index "BMI", duration of diabetes, glycosylated haemoglobin "HbA1c", and hypertension) to development of retinopathy. Based on the outcomes of the study, novel therapeutic strategies may be suggested at least to postpone the development of this sight threatening disease by prescribing antioxidant supplements as early as diabetes being diagnosed by endocrinologists.

2. MATERIALS AND METHODS

2.1 Study Design and Population

This case - control study was conducted in the research laboratories, Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University from April 2010 to April 2011. One-hundred type 2 diabetic patients with retinopathy recruited from King Fahad Specialist Hospital (KFSH) – Buraidah- KSA were enrolled in this study. They were examined for evidence of DR by an ophthalmologist and then graded as mild nonproliferative, moderate nonproliferative, moderately severe nonproliferative, severe nonproliferative, or proliferative according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS).

The control groups were: control group 1 consisted of sixty type 2 diabetic patients without retinopathy recruited from Diabetes and Endocrinology Center, KFSH, Buraidah, KSA; and control group 2 consisted of sixty healthy "non diabetic subjects" recruited from public places, i.e., Estarahes (party lounges). Diabetes was defined as a fasting serum glucose ≥7.0 mmol/L (126 mg/dL), the use of anti-diabetic agents, or both. The inclusion criteria for subjects in control group 2 were neither had been diagnosed by a physician as having diabetes or use hypoglycaemic medication nor be hypertensive or any known disease.

2.2 Measurement of BMI

Body weight and height were recorded for each subject. Weight was measured using calibrated electronic weighing scales (Proton Digital Scale, Model PHC 309 MD) and height was measured using a Portable Height Scale (Mentone Educational, Model PE087, Australia). BMI was calculated as weight (in kilograms) divided by height (in metres) squared. The WHO classification for BMI was used to estimate the degree of obesity. Subjects were categorized as normal if BMI was less than 25 kg/m^2, overweight if BMI was between 25-29.9 kg/m^2, and obese if BMI was greater than or equal to 30 kg/m^2.

2.3 Samples Collection & Preparation

Blood samples were collected as per the Clinical and Laboratory Standards Institute (CLSI) document (Ernst et al, 2008). Samples collected in one heparanised vacutainer (4 ml) and one plain vacutainer (4 ml) for each subject.

For plasma preparation, samples were spun in a refrigerated centrifuge at 4^0C at 3000 RPM for 10 minutes. Plasma was collected and stored at -80^0C for MDA and glucose analysis. For erythrocyte lysate preparation, bottom red cells were mixed with almost 20 times of volume of cold saline and centrifuged at 4^0C at 3000 RPM for 5 minutes. Extraction of hemoglobin

from RBCs lysate was done by the ethanol-chloroform 62.5/37.5 (v/v) method. The erythrocyte lysate was collected and stored at - 80℃ for Cu-Zn SOD assay.

The plain vacutainer after centrifugation at 3000 RPM for 10 minutes at 4^0C; the serum was separated and transferred directly into AMICON ULTRA-4 CENTRIFUGAL FILTER DEVICE (cut off 10, 000 kDa) and spun at 4^0C for 20 minutes at 7500 g [as per the instruction Amicon-Ultra, Millipore, Ireland]. The ultra filtrate sample was stored in Cryovial® at - 80℃ until analysis of 8-OHdG.

2.4 Laboratory Analysis

2.4.1 Parameters of oxidative stress

Markers of oxidative stress were estimated using commercial kits supplied by Northwest, U.S.A. Human serum 8-OHdG levels were measured by utilizing a competitive enzyme-linked immunosorbent assay method on bioMerieux Reader version. Plasma MDA levels were determined on Cary Varian spectrophotometer. Erythrocyte lysate Cu-Zn SOD activity was estimated on the VERSA max tunable microplate reader. Briefly, the method was based on monitoring the autooxidation rate of hematoxylin. One unit SOD activity is defined as the amount of enzyme that will inhibit the rate of cytochrome c reduction by half under specific conditions.

2.4.2 Estimation of random blood glucose and HbA1c

Glucose level was measured on the plasma samples by GOD-PAP method using Hospitex Eos Bravo Plus clinical chemistry analyzer. HbA1c was measured by immunoturbidometric method using commercially available kit supplied by Vital Diagnostic, Italy. HbA1c ≥ 7% was considered as an indicator of poor glycaemic control.

2.5 Statistical Analysis

The data collected and analyzed using the statistical package for social sciences (SPSS) software (version 13). Data expressed as mean ± standard deviation (SD) or number (percentage) as appropriate. Comparison of variables between two groups performed with an unpaired t-test and chi-square test for continuous and categorical variables, respectively. The p values were considered significant at $P < 0.05$. The Pearson correlation was used to examine the relation between selected variables. Odd ratios “OR” and 95 percent confidence interval “CI” were calculated using Win Episcope (version 2.0) to find the association between independent risk factors (age, gender, education, BMI, duration of diabetes, HbA1c, and hypertension) and development of DR.

2.6 Ethical Consideration

Ethical approval was obtained from the Ethics and Research Committee, KFSH- Buraidah. Participation was voluntary and verbal consent was acquired from each participant. Confidentiality of all participants was maintained as no names were requested.

3. RESULTS

3.1 Characteristics of the Study Participants

One-hundred type 2 diabetic patients with retinopathy participated in this study; their gender ratio was 1.0 male: 1.3 female. The male: female ratios in control groups 1 and 2 were 1.0: 1.5 and 1.1: 1.0 respectively. Regarding age of participants, patients with DR were found to be older (61.52±6.6 years) than subjects in control groups 1 and 2 (51.08±9.6 years and 44.28±10.7 years respectively). The mean BMI of patients with DR and subjects in control groups approximate 30 Kg/m^2, which considered to be indicative of obesity. Moreover, plasma glucose level was significantly higher in patients with DR (13.37±3.8 mmol/l) than subjects in control groups 1 and 2 (11.50±4.6 mmol/l, 5.55±1.9 mmol/l, respectively). The mean duration of diabetes in patients with and without DR was 16.39 years and 8.43 years respectively. Patients with and without DR were found of poor glycaemic control, i.e., HbA1c > 7%. The demographic and clinical characteristics of participants are shown in table 1.

Table 1. Demographic and clinical characteristics of the study participants: comparing type 2 diabetic patients with retinopathy to type 2 diabetic patients without retinopathy (control group 1) and to healthy non-diabetic subjects (control group 2)

Variable	Patients with DR	Control group1	Control group2	*p*-value [a]	*p*-value [b]
No. of subjects	100	60	60		
Gender (male /female)	43/ 57	24/ 36	31/ 29		
Age (yr)	61.52±6.6	51.08±9.6	44.28±10.7	.000*	.000*
Weight (kg)	75.65±19.5	82.02±17.3	74.88±15.0	.795	.040*
Height (cm)	155.10±11.1	159.82±15.7	159.72±14.5	.027*	.029*
BMI (kg/m^2)	30.25±5.8	31.40±6.8	29.49±6.4	.452	.263
Blood Glucose (mmol/l)	13.37±3.8	11.50±4.6	5.55±1.9	.000*	.019*
HbA1c (%)	10.04±2.2	8.64±2.0	4.99±1.4	.000*	.000*
Duration of DM (years)	16.39±6.7	8.43±5.3	----	……	.000*

Abbreviations: BMI, body mass index; DR, diabetic retinopathy; DM, diabetes mellitus; HbA1c, glycosylated haemoglobin

*Data presented as mean ± SD for all variables; * p-value <0.05; a: p value compared patients with DR with subjects in control group 2 (non-diabetic subjects); b: p value compared patients with DR with subjects in control group 1 (type 2 diabetic patients without DR)*

3.2 Risk Factors for Development of Retinopathy

In table 2, odd ratios were calculated to assess the association between independent variables (gender, age, educational level, BMI, hypertension, duration of diabetes, control of glucose) and development to retinopathy compared with patients in control group 1. Data showed that increasing age as well as having low education seemed to have positive effect on development of retinopathy. Patients who aged more than 55 years were six times more have retinopathy (OR 5.891, CI 2.082 to 16.670) and also those who had the low level of education 'primary" were four times more to develop retinopathy (OR 4.44, CI 0.611 to 32.330).

Table 2. Odd ratios calculation for risk factors: patients with DR vs. patients without DR (control group 1)

Risk factor		Patients with DR Number (%)	Control group 1 Number (%)	OR	95% CI
Gender	M (R)	43 (43)	24 (40)	1	
	F	57 (57)	36 (60)	0.884	0.461-1.694
Age (yr)	< 45 (R)	08 (8)	13 (21.7)	1	
	45-55	15(15)	24(40.0)	1.016	0.341-3.026
	56-65	58(58)	16(26.7)	5.891	2.082-16.670
	> 66	19(19)	7(11.7)	4.411	1.282-15.170
BMI (kg/m2)	< 25 (R)	15(15)	09(15)	1	
	25 -29.9	40(40)	16(26.7)	1.500	0.547-4.116
	≥ 30	45(45)	35(58.3)	0.771	0.302-1.969
Smoking status	No (R)	97(97)	56(93.3)	1	
	Yes	03(03)	04(6.7)	0.433	0.094-2.003
Education	University (R)	03(03)	02(3.3)	1	
	Secondary	01(01)	05(8.3)	0.133	0.008-2.181
	Intermediate	12(12)	08(13.3)	1.00	0.135-7.392
	Primary	40(40)	06(10)	4.44	0.611-32.330
	Illiterate	44(44)	39(65)	0.752	0.119-4.738
Hypertension status	No (R)	37(37)	31(51.7)	1	
	Yes	63(63)	29(48.3)	1.820	0.951-3.483
Duration of DM (yr)	< 5 (R)	06(6)	20(33.3)	1	
	5 – 10	22(22)	17(28.3)	4.314	1.421-13.090
	11 -15	25(25)	8(13.3)	10.420	3.104-34.960
	> 15	47(47)	15(25)	10.440	3.541-30.810
HbA1c (%)	< 7(R)	14 (14.0)	13 (21.7)	1	
	≥ 7	86(86.0)	47(78.3)	1.699	0.738-3.914

Abbreviations: OR, odd ratio; CI, confidence interval; BMI, body mass index; DR, diabetic retinopathy; DM, diabetes mellitus; HbA1c, glycosylated hemoglobin

Moreover, data showed that level of glycaemic control (as measured by HbA1c status) was highly associated with retinopathy in that patients with poor glycaemic control (i.e., HbA1c ≥ 7%) were nearly twice more to have retinopathy (OR 1.699, CI 0.738 to 3.914).

In addition, retinopathy development was found to be associated with duration of diabetes (OR = 10.420, 95% CI = 3.104–34.960). Furthermore, data from this study showed that hypertensive diabetic patients were nearly double at risk to develop retinopathy compared to normotensive diabetic patients (OR 1.820, CI 0.951-3.483). Regarding gender and smoking; our data failed to establish the relationship.

3.3 Parameters of Oxidative Stress

As shown in table 3, the activity of antioxidant enzyme Cu-Zn SOD level in the erythrocyte lysate from type 2 diabetic patients with retinopathy was significantly lower compared to subjects in control groups 1 and 2 (*p*= .023 and = .000 respectively). Moreover, the oxidative stress markers' plasma MDA and serum 8-OHdG were found to be significantly increased in type 2 diabetic patients with retinopathy compared to subjects in both control groups (*p* =.000 for all).

Table 3. Plasma MDA, serum 8-OHdG, and RBC lysate Cu-Zn SOD levels in diabetic patients with retinopathy compared to control group 1 (type 2 diabetic patients without retinopathy) and to control group 2 (non-diabetic subjects)

Parameters	Patients with DR	Control group 1	Control group 2	p-value[a]	p-value[b]
MDA (µmol/l)	3.204±0.467	2.742±0.400	2.629±0.494	.000*	.000*
8-OHdG (ng/ml)	1.652±1.249	0.590±0.452	0.540±0.455	.000*	.000*
Cu-Zn SOD (U/ml)	190.779±45.640	206.942±33.628	224.418±31.463	.000*	.023*

Abbreviations: MDA, malondialdehyde; 8-OHdG, 8-hydroxy 2-deoxyguanosine; Cu-Zn SOD, Cu-Zn superoxide dismutase;
*Data presented as mean ± SD for all parameters; * p-value ≤0.05;*
a: p value compared patients with DR with subjects in control group 2 (non-diabetic subjects)
b: p value compared patients with DR with subjects in control group 1 (type 2 diabetic patients without DR)

Furthermore, we compared the markers of oxidant/antioxidant status between patients with nonproliferative retinopathy (NPDR) and patients with proliferative retinopathy (PDR). We could not find a significant difference in levels of serum 8-OHdG between the two groups.

However, patients with PDR were found to have significantly high plasma MDA and reduced activity of Cu-Zn SOD in the erythrocyte lysate compared to patients with NPDR (p =.032 and =.000 respectively). Data have been shown in table 4.

A negative correlation was estimated between activity of erythrocyte lysate Cu-Zn SOD and plasma MDA and also between the same antioxidant enzyme (Cu-Zn SOD) and HbA1c (see figures 1 and 2).

Table 4. Plasma MDA, serum 8-OHdG, and RBC lysate SOD levels in patients with proliferative retinopathy (PDR) compared to patients with nonproliferative retinopathy (NPDR)

Parameters	Patients with NPDR n= 30	Patients with PDR n= 70	p-value[a]
MDA (µmol/l)	3.150±0.417	3.391±0.583	.032*
8-OHdG (ng/ml)	1.573±1.237	1.666±1.365	.783
Cu-Zn SOD (U/ml)	204.114±29.718	148.348±60.381	.000*

*Abbreviations: MDA, malondialdehyde; 8-OHdG, 8-hydroxy 2-deoxyguanosine; Cu-Zn SOD, Cu-Zn superoxide dismutase; Data presented as mean ± SD for all parameters; *p-value ≤0.05; a: p value compared patients with NPDR to patients with PDR*

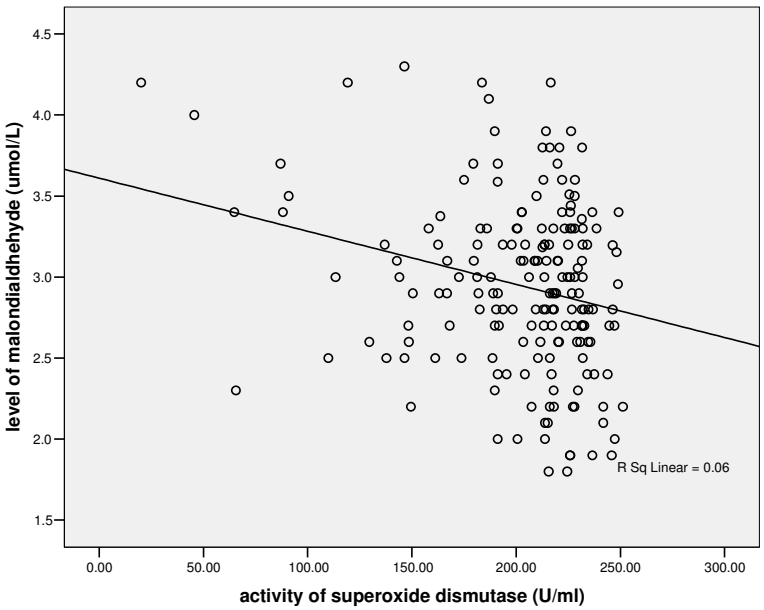

Fig. 1. Relationship between level of plasma MDA (µmol/l) and activity of Cu-Zn SOD (U/ml) in all subjects

r = -0.245, p = .001; * correlation is significant at the 0.05 level (2-tailed)*

4. DISCUSSION

DR is a common microvascular complication in patients with diabetes and may have a sudden and debilitating impact on visual acuity (VA), eventually leading to blindness. Advanced stages of DR are characterized by the growth of abnormal retinal blood vessels secondary to ischemia. These blood vessels grow in an attempt to supply oxygenated blood to the hypoxic retina (Ciulla et al., 2003).

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) showed that 1.6% of patients with type 2 diabetes (T2DM) were legally blind. However, Up to 21% of T2DM patients have DR at the time of diagnosis of diabetes (Tam et al., 2009). This could be due to a time lag between onset and clinical diagnosis (Smith et al., 2007). Within the next 30

years, the number of people worldwide at risk of developing vision loss from diabetes is predicted to double over (Antonetti et al., 2006). The current management strategy for DR requires early detection and optimal glycaemic control to slow the progression of disease. However, adherence to these recommendations is hampered by the fact that the condition is generally asymptomatic at early stages (Ciulla et al., 2003). Therefore, it is imperative to develop better means to identify, prevent, and treat retinopathy in its earliest stages rather than wait for the onset of vision-threatening lesions (Antonetti et al., 2006).

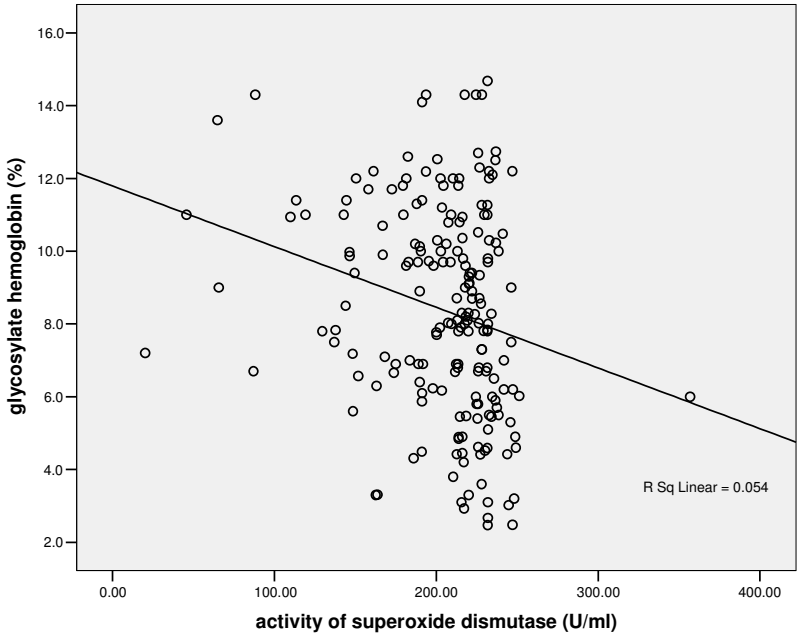

Fig. 2. Relationship between glycosylated haemoglobin (HbA1c) and activity of Cu-Zn SOD (U/ml) in all subjects

r = -0.233, p = .001; * correlation is significant at the 0.05 level (2-tailed)*

Although the pathogenesis of retinopathy in diabetes is still not fully understood, a number of risk factors have been identified. Targeting these modifiable risk factors aggressively and regular screening to allow timely intervention will reduce the progression to proliferative retinopathy and vision loss. Such primary and secondary prevention strategies are believed to be cost effective (Smith et al., 2007).

As shown in table 2, a number of independent variables had been studied as risks for progression of DR. Our data illustrated that DR was more common among elder type 2 diabetic patients compared to younger ones. Patients who aged more than 55 years seemed to be six- fold more to have retinopathy (OR 5.891, CI 2.082 to 16.670, P = .000). This is consistent with the findings of Al-Maskari F and El-Sadig M (2007) in a cross-sectional study in the United Arab Emirates, who found significant association ($p < 0.001$) between retinopathy and increasing age of patients. However, in contrast to the studies done by Javadi et al. (2009), Tam et al. (2009), and Longo-Mbenza et al. (2008), our data had not shown a consistent pattern of gender variation in DR prevalence.

Regarding the association between the level of education and DR, the odd ratios of DR were greater in patients with the low level of education "primary education" compared to those with high level education "university education" (OR 4.44, CI 0.611 to 32.330, P = .000). This contrasts to the finding from West et al. (2002) who did not find any relationship between DR and education in their analyses of a Mexican-American population.

Furthermore, our data showed that uncontrolled blood sugar was significantly associated with the presence of DR. Patients with poor glycaemic control (i.e., HbA1c ≥ 7%) were found to be nearly twice more to have retinopathy compared to those having values of glycosylated hemoglobin less than seven percent (OR 1.699, CI 0.738 to 3.914). This finding supported by Guillausseau et al. (1998) in a longitudinal cohort study who reported that relative risk for developing retinopathy was 7.2 (IC 95 %: 1.61–32.4) in T2DM patients with a mean HbA1c during follow-up above the median value of the cohort (8.3 %) compared with patients with HbA1c during follow-up below this value. Moreover, intervention studies in diabetic patients have shown that tight glucose control reduces the risk of retinopathy (van Leiden et al., 2003).

In addition, DR development was found to be strongly associated with duration of diabetes (OR = 10.420, 95% CI = 3.104–34.960, P = 0.000). Wong et al. (2006) in a prospective cohort study describing the frequency of risk factors for diabetic retinopathy in four racial/ethnic populations in six United States communities reported that longer duration of disease was significant independent predictors of any retinopathy. The role of diabetes duration in retinopathy development has been confirmed in other studies (Niazi et al., 2010; Fong et al., 2004) in which the researchers reported that the duration of diabetes is probably the strongest predictor for development and progression of retinopathy.

Data showed that hypertensive diabetic patients were nearly double at risk to develop DR compared to normotensive diabetic patients (OR = 1.820, 95% CI = 0.951-3.483, P = 0.000). The possible mechanisms by which hypertension affects DR are haemodynamic (impaired autoregulation and hyperperfusion) and secondly through vascular endothelial growth factor (VEGF), as it has been observed that hypertension independent of hyperglycaemia upregulates the VEGF expression in retinal endothelial cells and ocular fluids (Srivastava and Rema, 2005).

There is controversy regarding cigarette smoking and the risk of DR (Ojaimi et al., 2011; Esteves et al., 2009; Moss et al., 1996). However, in this study no consistent association was found between status of smoking and incidence of DR. Fewer of our diabetic retinopathy patients (< 5%) reported that they were current smokers. Moreover, this study couldn`t demonstrate any association between BMI and DR.

Regarding markers of oxidative stress, the results of the present research indicated significantly higher concentrations of plasma MDA and serum 8-OHdG in type 2 diabetic patients with retinopathy compared to the subjects in control groups. These results supported by the findings of Pan HZ et al. (2008) who reported significant elevation in the concentrations of MDA and 8-OHdG in patients with diabetic retinopathy compared to patients with diabetes without retinopathy ($p<0.05$). Based on the severity of the disease, we could not find a significant difference in the concentrations serum 8-OHdG ($p = 0.783$) between patients with NPDR and patients with PDR, although a high level of plasma MDA was observed in patients with PDR ($p = 0.032$). This finding highlights the significance of measurement of MDA in the blood which provides useful information for the prognosis of diabetes in which secondary disorders are often fatal (Al-Rawi, 2010). There are a few

biochemical mechanisms that explain the reason for such a rise of MDA. The increase in the blood free fatty acid levels in diabetic patients depending on the degree of lipolysis, which results in an increase in MDA production. Moreover, the increased MDA levels of diabetic individuals may take origin from the peroxidative damage of the membrane lipids. Since lipid peroxides play a major role in the formation of vascular tissue damage, it is suggested that an increase in MDA in diabetes can be effective in the pathogenesis of diabetic angiopathy (Samuel et al., 2010).

Furthermore, ROS can be evaluated indirectly by the measurement of some antioxidant enzyme levels such as superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (Bulut et al, 2007). The literature provides conflicting evidence of serum SOD and diabetes. Some studies have reported reduced levels of SOD activity, whereas others found no association between diabetes and SOD (Soliman, 2008; Hartnett et al., 2000; Kimura et al., 2003). This study demonstrated significantly decreased erythrocyte lysate Cu-Zn SOD in type 2 diabetic patients with retinopathy compared to patients in control group 1 (190.779±45.640 vs. 206.942±33.628 U/ml, p = .023) and to subjects in control group 2 (190.779±45.640 vs. 224.418±31.463 U/ml, p = .000). Furthermore, patients with PDR were found to have significant lower activity of Cu-Zn SOD compared to patients with NPDR (148.348±60.381 vs. 204.114±29.718 U/ml, p = .000). Although SOD provides effective intracellular defense against superoxide radical-mediated toxicity, this scavenging system might be impaired in diabetes due to diminished synthesis and/or deactivation of the enzyme by glycation (Kurtul et al., 2005). Moreover, products of membrane lipid peroxidation and other oxidants like H2O2 may react with SOD resulting in oxidative modification thereby causing loss of enzyme activity (Gupta and Chari, 2005). These explanations may be supported by our findings of negative correlations between plasma MDA and the activities of Cu-Zn SOD as shown in figure 1 and also between glycosylated haemoglobin (HbA1c) and the antioxidant enzyme Cu-Zn SOD as shown in figure 2.

5. CONCLUSION

- In the present study; age, education, duration of diabetes, poor glycaemic control, and hypertension were consistently associated with development DR. Moreover, we observed evidence of significant increased DNA oxidative damage and lipid peroxidation in type 2 diabetic patients with retinopathy compared to subjects in the control groups. These increased oxidant products were associated with a significant decrease in activity of Cu-Zn SOD in diabetic patients with retinopathy which implied reduction in the antioxidant enzymes and increased vulnerability to free radical damage.
- Our findings emphasize the important role of oxidative stress in the development of retinopathy in type 2 diabetes suggesting that blocking of oxidative stress is a crucial step for delayed progression of DR and the severity as well.
- Further longitudinal prospective studies are needed to validate the effect of antioxidant nutrients intake in the postponement of DR development. Such antioxidant supplements may be of great help to type 2 diabetic patients by providing an opportunity to live out their normal life expectancies with minimal complications such as retinopathy.

ACKNOWLEDGEMENTS

This work is supported by grant (Limited Grants Program No. 14-23, 2010) from the King Abdulaziz City for Science and Technology, Saudi Arabia. The authors are thankful to Mr. Marghoob Hasan for the technical help he provided. Furthermore, authors acknowledge Dr. Mostafa Taha and Mr. Nafea Al-Sobihy for their support.

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