ORIGINAL RESEARCH

Omur Tabak MD¹
Remise Gelisgen PhD²
Hayriye Erman MD²
Fusun Erdenen MD¹
Cüneyt Muderrisoğlu MD¹
Hale Aral PhD³
Hafize Uzun PhD²

¹ Istanbul Education and Research Hospital,
Internal Medicine Clinic, İstanbul, Turkey

² Department of Biochemistry, Istanbul
University, Cerrahpasa Medical Faculty, İstanbul,
Turkey

³ Istanbul Education and Research Hospital,
Biochemical Laboratory, Turkey

Oxidative lipid, protein, and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus

Abstract

Purpose: The purpose of this study was to determine the effects of diabetic complications on oxidation of proteins, lipids, and DNA and to investigate the relationship between oxidative damage markers and clinical parameters.

Methods: The study group consisted of 69 type 2 diabetic patients (20 patients without complication, 49 patients with complication) who attended internal medicine outpatient clinics of Istanbul Education and Research Hospital and 19 healthy control subjects. In serum samples of both diabetic patients and healthy subjects, 8-hydroxy-2'-deoxyguanosine (8-OHdG), as a marker of oxidative DNA damage, N^ε-(hexanoyl)lysine (HEL) and 15-F2t-iso-prostaglandin (15-F2t-IsoP), as products of lipoxidative damage, advanced oxidation protein products (AOPP), as markers of protein damage, and paraoxonase1 (PON1) as antioxidant were studied.

Results: 15-F2t-IsoP ($p < 0.005$) and AOPP ($p < 0.001$) levels were significantly higher in diabetic group than control group while there were no significant differences in levels of 8-OHdG and HEL between the two groups. AOPP ($p < 0.001$) and 8-OHdG ($p < 0.001$) were significantly higher in diabetic group with complications compared to diabetic group without complications.

Conclusions: Increased formation of free radicals and oxidative stress, under conditions of hyperglycaemia, is one of the probable causes for evolution of complications in diabetes mellitus. Our study supports the hypothesis that oxidant/antioxidant balance is disturbed in diabetic patients.

Manuscript submitted 8th February, 2011

Manuscript accepted 19th April, 2011

Clin Invest Med 2011; 34 (3): E163-E171.

Correspondence to:
Prof. Dr. Hafize Uzun (PhD)
Department of Biochemistry,
Istanbul University,
Cerrahpasa Medical Faculty,
İstanbul, Turkey
E-mail: huzun59@hotmail.com

Type 2 diabetes mellitus (DM) and its complications are frequently seen today, and with an increasing incidence. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death [1].

N^ε-(hexanoyl)lysine (HEL), an early byproduct of lipid peroxidation, is formed from a lipid hydroperoxide and a lysine residue. HEL is considered to be one of the stable oxidative stress markers for lipid peroxide and protein modification [2]. An important role for oxidative stress in the increased HEL seen in diabetes is suggested by experimental and clinical studies [3,4].

Recently, a new marker of protein oxidation, advanced oxidation protein products (AOPP), has begun to attract the attention of various investigators [5-8]. AOPP are proteins, predominantly albumin and its aggregates, which have been damaged by oxidative stress [9].

Reactive oxygen species (ROS) can cause strand breaks in DNA and base modifications, including oxidation of guanine residues to 8-hydroxy-2'-deoxyguanosine (8-OHdG) - an oxidized nucleoside of DNA that is the most frequently detected and studied DNA lesion [10]. In recent years, several clinical studies have analyzed levels of 8-OHdG in human organs, leukocyte DNA and urine in relation to oxidative stress and diabetes mellitus [11-13].

8-iso-prostaglandin F_{2α}, or 8-epi-prostaglandin F_{2α}, was renamed 15-F_{2t}-IsoP [14]. Among the isoprostanes, the 15-F_{2t}-IsoP has been shown to be both stable and biologically active, and it is produced in abundance during oxidative stress [15]. F₂-isoprostanes may transduce the effects of the oxidant stress associated with complex metabolic disorders into specialized forms of cellular activation. In particular, the low-grade inflammatory state, characterizing metabolic disorders such as obesity, hypercholesterolemia, type 2 diabetes mellitus, and homozygous homocystinuria, may be the primary trigger of thromboxane-dependent platelet activation; mediated, at least in part, through enhanced lipid peroxidation [16].

Paraoxonase (PON1) is associated with high-density lipoprotein (HDL). PON1 prevents lipid oxidation: not only of LDL, but also of HDL itself [17]. This protection is probably related to the PON1-hydrolysing activity of some activated phospholipids [18] and/or lipid peroxide [19] products. Human PON1 (aryldialkylphosphatase, EC 3.1.8.1) is a 43 kDa esterase associated with apolipoprotein A1 (apoA-I) and clusterin (apolipoprotein J) in HDL. PON1 was shown to possess a peroxidase-like activity, which can contribute to its protective

effect against lipoprotein oxidation, as well as a homocysteine-thiolactonase activity, which may be linked with its anti-atherogenic properties [19, 20].

In this study, we examine the role that oxidative stress plays in DM and its complications by measuring products of oxidative damage in the plasma: 8-OHdG, as a marker of oxidative DNA damage, HEL and 15-F_{2t}-IsoP as products of lipooxidative damage and AOPP as markers of protein damage were studied.

Materials and Methods

The study included 69 type 2 diabetic patients; 20 of them without any of the vascular complications often associated with type 2 diabetes mellitus (DM), 37 patients had microvascular complications either retinopathy, nephropathy, or neuropathy, four patients had macrovascular complications such as coronary artery disease, eight patients had mixed micro- and microvascular complications, as well as 20 healthy age-matched volunteers. All patients were clinically stable without any acute or chronic infection. Patients attending outpatient internal medicine clinics of Istanbul Education and Research who presented with febrile illness, diabetic ketoacidosis, or renal failure and those who were suffering from chronic diseases were excluded from the study. The patients did not receive any medications, including statins. Diabetic patients were given a diabetic diet. While dietary guidelines from the American Diabetes Association (ADA) were used to frame nutrition recommendations, additional content was adapted from the Turkey Diabetes Mellitus Association (TDMA) guidelines. A total of seven subjects were treated with insulin therapy (after failure of oral hypoglycemic agents), 57 subjects with oral hypoglycemic agents (sulfonylureas or sulfonylureas plus biguanides) and five with the combination of insulin and oral hypoglycemic agents. All subjects were non-smokers. Members of the control group were selected from the hospital staff without personal or family history of either diabetes or dyslipidemia and with normal thyroid, hepatic and renal functions. Neither DM patients nor healthy controls have received vitamin or mineral supplements. Two-hour glucose load was used to rule out impaired glucose regulation in healthy controls. Diabetes mellitus and impaired glucose tolerance (IGT) were diagnosed according to the 1998 WHO criteria [21]. Control groups were not taking any drugs known to affect carbohydrate or lipid metabolism. Among the diabetic patients, 20 were free from any evidence of vascular complications while 49 diabetics had various kinds of vascular complications. Demographic (gender, age) and anthropometric data (weight, height, waist circumference), medical history (duration of disease, genetic background) and type of treat-

ment were recorded. The study was approved by the Ethical Committee on human research of Istanbul Education and Research Hospital. Written informed consent was obtained from all subjects.

Blood was drawn after 12-14 hours of fasting in the morning. Serum and plasma were obtained after at least 30 minutes of clotting by centrifugation at 2500xg for 15 minutes. Serum samples were removed and used directly for measurements of routine parameters. Other serum and plasma samples were stored at -80°C until use. All icteric or haemolytic blood samples were discarded. All reagents were analytical grade and purchased from Sigma Chemical Co (St. Louis, USA) and Merck (Darmstadt, Germany).

Routine analyses

Routine parameters were determined on the Olympus AU 800 analyzer by enzymatic methods using commercial kits (Roche Diagnostics, GmbH, Mannheim, Germany). Plasma insulin was measured by radioimmunoassay using a commercial kit (DSL-1600, USA). Homeostatic model assessment (HOMA) is a method for assessing beta cell function and insulin resistance (HOMA-IR) from basal (fasting) glucose and insulin. HOMA-IR was calculated by the formula $[\text{fasting glucose (mmol/L)} \times \text{fasting insulin } (\mu\text{U/ml})] / 22.5$.

Assay of serum HEL levels

Serum HEL levels were determined by ELISA using a commercial kit (Northwest Life Science Specialties, Vancouver, USA). HEL studies were performed using a competitive immunoassay kit according to the manufacturer's directions. The coefficients of intra- and interassay variations for HEL assay were 6.9% (n = 10) and 7.5% (n = 10), respectively.

Assay of serum 15-F2t-IsoP levels

Serum 15-F2t-IsoP levels were determined by ELISA using a commercial kit (Northwest Life Science Specialties, Vancouver, USA). Serum 15-F2t-IsoP was measured using a competitive immunoassay kit according to the manufacturer's directions. The coefficients of intra- and interassay variations for 15-F2t-IsoP assay were 6.7% (n = 10) and 7.3% (n = 10), respectively.

Assay of 8-OHdG levels

The plasma 8-OHdG levels were determined using an enzyme linked immunosorbent assay detection kit (Northwest Life Science Specialties, Vancouver, USA). Plasma 8-OHdG was

measured using a competitive immunoassay kit according to the manufacturer's directions. The coefficients of intra- and interassay variations for 8-OHdG assay were 6.4% (n = 10) and 7.0% (n = 10), respectively.

Assay of advanced protein oxidation products (AOPP) levels

Spectrophotometric determination of AOPP levels was performed by a kit according to the manufacturer's directions (Immundiagnostik, Bensheim, Germany). The coefficients of intra- and interassay variations for AOPP assay were 4.3% (n = 10) and 6.2% (n = 10), respectively.

Assay of PON1 activity

Plasma PON1 activity was assayed using synthetic paraoxon (diethyl-*p*-nitrophenyl phosphate) as substrate. PON1 activity was determined by measuring the initial rate of substrate hydrolysis to *p*-nitrophenol, the absorbance of which was monitored at 412 nm in the assay mixture containing 2.0 mM paraoxon, 2.0 mM CaCl₂ and 20 μ l of plasma in 100 mM Tris-HCl buffer (pH 8.0). The blank sample containing incubation mixture without plasma was run simultaneously to correct for spontaneous substrate breakdown. Enzymatic activity was calculated from the molar extinction coefficient 18,290 M⁻¹cm⁻¹ and is expressed as units per milliliter. One unit of PON1 activity is defined as 1 nmol of 4-nitrophenol formed per minute under the above assay conditions [22]. The intra-assay and inter-assay coefficients of variation were 6.8% (n = 25) and 3.3% (n = 25), respectively.

Statistical analyses

Statistical analyses were carried out by using SPSS 11 package. Data are presented as the mean $\pm$ standard deviation (SD). Mann-Whitney U and Kruskal Wallis were used in comparison of groups. Correlations between changes in variables were tested using Pearson's correlation. P < 0.05 was considered statistically significant.

Results

Demographic and biochemical values of diabetic patients and control group are shown in Table 1. There was no difference in age between the groups. Blood lipid levels, including LDL-cholesterol, HDL-cholesterol and triglycerides, were not significantly different between the groups. BMI, glucose, HbA1c, microalbuminuria, HOMA-IR, fibrinogen, homocysteine and N-terminal-pro-brain-type natriuretic peptide (NT-proBNP) levels were significantly higher in patients with DM in com-

TABLE 1. Demographic and biochemical values for diabetic and control subjects.

	Control	DM	P
Age (years)	52.60±6.64	57.41±8.93	NS
Sex (F/M)	9/10	45/24	-
BMI (kg/m ²)	27.35±3.36	30.25±4.84	0.014
Glucose (mmol/L)	5.24±0.64	10.89±69.26	0.001
HbA1c (%)	5.45±0.86	7.81±1.82	0.001
Urea (mg/dl) (mmol/L)	9.90±3.16	11.84±4.68	NS
Creatinine (μmol/L)	79.56±17.68	77.79±15.91	NS
Microalbuminuria (mg/day)	5.82±1.65	45.78±84.98	0.039
Uric acid (μmol/L)	300.97±73.16	262.31±93.38	NS
Total Cholesterol (mmol/L)	4.85±0.86	5.26±1.26	NS
HDL (mmol/L)	1.28±0.21	1.24±0.33	NS
LDL (mmol/L)	3.00±0.61	3.17±1.03	NS
Triglycerides (mmol/L)	1.73±1.03	2.04±1.01	NS
Total Protein (g/L)	74.7±3.6	75.6±3.2	NS
Albumin (g/L)	41.5±2.0	42.2±5.3	NS
Total Bilirubin (μmol/L)	12.65±6.33	13.34±15.05	NS
Direct Bilirubin (μmol/L)	5.30±4.28	5.47±6.67	NS
Ferritin (pmol/L)	278.94±232.03	223.89±210.05	NS
Fasting Insulin (pmol/L)	65.91±28.13	71.19±43.55	NS
C-peptide (nmol/L)	0.91±0.44	0.88±0.39	NS
HOMA-IR	2.20±0.88	4.68±2.83	0.001
Fibrinogen (μmol/L)	8.44±3.03	9.64±2.20	0.05
Homocysteine (μmol/L)	5.88±2.14	8.59±1.00	0.001
hsCRP (nmol/L)	3.33±4.10	6.38±9.52	NS
NT-ProBNP (ng/L)	58.23±68.74	117.91±130.11	0.05

BMI: body mass index; HOMA-IR: Homeostatic model assessment–insulin resistant; hsCRP: high sensitive C-reactive protein; NT-proBNP: N-terminal-pro-brain-type natriuretic peptide; NT-ProBNP; NS: not significant.

TABLE 2. Oxidative stress parameters for conidiabetic and control subjects.

	Control	DM	P
HEL (nmol/L)	45.33±19.69	46.98±20.69	0.753
AOPP (μmol/L)	41.28±6.54	67.46±15.34	0.001
8-OHdG (ng/ml)	1.80±0.54	2.24±0.97	0.055
15-F2t-IsoP (pg/ml)	0.39±0.36	1.17±1.12	0.003
PON 1 (U/ml)	260.55±76.00	113.42±62.38	0.001

HEL: N^ε-(hexanoyl)lysine; AOPP: advanced oxidation protein products; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; 15-F2t-IsoP: 15-F2t-iso-prostaglandin; PON1: paraoxonase1

FIGURE 1. Pearson's correlation coefficients between 15-F2t-IsoP and glucose in all patients.

parison with the control group. There were no differences in other parameters between the groups.

Oxidative stress parameters of control and patient groups were shown in Table 2. AOPP and 15-F2t-IsoP levels were significantly higher in diabetic group in comparison with the control group ($p=0.001$ and $p=0.003$, respectively). On the other hand, PON 1 was significantly lower in the diabetic group in comparison with the control group ($p=0.001$). There were no significant differences in HEL and 8-OHdG levels between the groups.

Demographic characteristics and clinical features of patients with and without complications are shown in Table 3. Duration of diabetes was significantly longer in diabetics with complications ($p=0.01$). Similarly, fasting plasma glucose and HbA1c were significantly higher in diabetics with complications ($p=0.001$ and $p=0.01$, respectively).

Oxidative stress parameters of patients with and without complications are shown in Table 4. AOPP and 8-OHdG were significantly higher in the group with complications ($p=0.001$ and $p=0.001$, respectively); however, PON 1 was significantly lower in the group with complications (0.01). There was no significant difference in HEL and 15-F2t-IsoP levels between the groups.

As shown in Fig. 1, 15-F2t-IsoP levels were significantly positively correlated with fasting glucose ($P<0.003$, $r = 0.36$) in all patients, but no correlation was found between oxidative markers and other clinical parameters. Pearson's correlation coefficients between 8-OHdG and HEL and AOPP in all patients are shown in Fig. 2 ($P<0.047$, $r = 0.24$ and $P<0.038$, $r = 0.25$, respectively). Similarly, Pearson's correlation coefficients between 15-F2t-IsoP and both fasting glucose and HbA1c in diabetic patients with complications are shown in Fig. 3 ($P<0.001$, $r = 0.49$ and $P<0.019$, $r = 0.34$, respectively).

TABLE 3. Demographic characteristics and clinical features of patients with and without complications.

	Without complications (20-%29)	With complications (49-%71)	P
Age (years)	57.20±9.13	57.49±8.95	0.84
Sex (F/M)	14/6	31/18	
BMI (kg/m ²)	28.80±3.61	30.84±5.18	0.19
Duration of diabetes (year)	4.70±3.64	9.35±7.18	0.01
Glucose (mmol/L)	9.04±2.81	11.65±3.98	0.001
HbA1c (%)	6.92±1.72	8.17±1.75	0.01
Urea (mmol/L)	11.42±2.14	12.01±5.39	0.88
Creatinine (μmol/L)	71.76±6.86	79.56 ±15.25	0.13
Microalbuminuria (mg/day)	15.60±7.21	58.09±98.37	0.08
Uric acid (μmol/L)	230.80±86.85	275.42±93.99	0.18
Total Cholesterol (mmol/L)	5.02±1.49	5.36±1.16	0.76
HDL (mmol/L)	1.23±0.20	1.25±0.37	0.99
LDL (mmol/L)	3.22±1.10	3.14±1.01	0.99
Triglycerides (mmol/L)	1.77±0.62	2.16±1.12	0.19
Total Protein (g/L)	75.3±3.89	75.6±2.95	0.92
Albumin (g/L)	42.1±3.10	42.1±6.00	0.99
Total Bilirubin (μmol/L)	18.47±26.68	11.12±4.62	0.93
Direct Bilirubin (μmol/L)	5.13±2.57	5.64±7.87	0.96
Ferritin (pmol/L)	203.29±163.60	232.32±227.31	0.91
Fasting Insulin (pmol/L)	82.30±44.73	66.67±42.71	0.47
C-peptide (nmol/L)	1.05±0.43	0.82±0.36	0.11
HOMA-IR	4.68±2.74	4.69±2.89	0.92
Fibrinogen (μmol/L)	9.23±1.59	9.81±2.40	0.20
Homocysteine (μmol/L)	8.54±0.59	8.61±1.13	0.98
hsCRP (nmol/L)	3.71±2.38	7.52±11.05	0.23
NT-ProBNP (ng/L)	119.99 ±126.17	117.06±132.96	0.99

BMI: body mass index; HOMA; IR: Homeostatic model assessment–insulin resistant; hsCRP: high sensitive C-reactive protein; NT-proBNP: N-terminal-pro-brain-type natriuretic peptide NT-ProBNP. NS; not significant.

TABLE 4. Oxidative stress parameters in patients with and without complications.

	Without Complications	With Complications	P
HEL (nmol/L)	45.15±16.88	47.73±22.17	0.99
AOPP (μmol/L)	54.60±10.78	72.70±13.80	0.001
8-OHdG (ng/ml)	1.39±0.40	2.58±0.93	0.001
15-F2t-IsoP (pg/ml)	1.11±1.06	1.20±1.15	0.55
PON 1 (U/ml)	124.85±59.44	108.75±63.54	0.01

HEL: N^ε-(hexanoyl)lysine; AOPP: advanced oxidation protein products; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; 15-F2t-IsoP: 15-F2t-iso-prostaglandin; PON1: paraoxonase1

Discussion

Hyperglycemia is a major factor in the development of diabetic complications although the mechanisms by which increased glucose levels contribute to these changes have not been fully elucidated. Numerous studies have demonstrated oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, but there is controversy about which of these is the most reliable and suitable marker for clinical practice. This study highlights a direct link between oxidative stress and diabetic complications through the measurement of a number of markers of oxidative stress. There are multiple sources of oxidative stress in diabetes, including nonenzymatic, enzymatic and mitochondrial pathways. Direct evidence of oxidative stress in diabetes is based on studies that focused on the measurement

FIGURE 2. Pearson's correlation coefficients between 8-OHdG and HEL and AOPP in all patients.

FIGURE 3. Pearson's correlation coefficients between 15-F2t-IsoP and both fasting glucose and HbA1c in diabetic patients with complications.

of oxidative stress markers such as plasma and urinary F2-isoprostane [23]. In this study, levels of 15-F2t-IsoP, a biologically active product of lipid peroxidation, were significantly higher in diabetic patients than in the control group. On the other hand, there was no significant difference between diabetics with and without complications. Also, a positive correlation was determined between 15-F2t-IsoP and both fasting glucose and HbA1c in the diabetic group with complications. Similarly, there was a positive correlation between 15-F2t-IsoP and fasting glucose in all diabetic patients. 15-F2t-IsoP in the diabetic group with complications, not well-regulated with medication, would suggest an interaction between hyperglycemia and glucose variability with respect to the formation of reactive oxygen species. Increased levels of F2-isoprostanes can be found in the plasma or urine of patients affected by several diseases, including diabetes, and are used as *in vivo* indicators of lipid peroxidation [24-28]. Increased isoprostane levels in diabetic patients with chronic heart failure have been shown to be

correlated with antioxidant status and disease severity [29]. Consequently, modulation of enzymes as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in target organs such as heart and kidney, prone to diabetic complications, may be beneficial in the prevention and management of heart and kidney failure [23]. Although our results indicate a close relation between hyperglycemia and lipid peroxidation, there are no differences in HEL between all groups, which is the earliest marker of lipid peroxidation. While many previous studies report an increase of lipid peroxidation products in diabetic patients [30], several report no significant increase [31]. Extensive HEL staining was observed in db/db mice in experimental study [32, 4]. Urinary excretion of Ne-(hexanoyl) has also been shown to be significantly higher in patients with diabetes than in control subjects [3]. F2-isoprostanes increase in both type 1 and type 2 diabetes, according to a study by Gopaul *et al.* [33]. They reported that 8-iso-PGF₂α (a major F2-isoprostane) was three times higher in

type 2 diabetics than in healthy people. Vascular complications represent the major cause of morbidity and mortality in diabetic patients. Since oxidative stress plays a key role in diabetic complications, isoprostanes are biologically active products of arachidonic acid metabolism that is related to vascular diseases [34].

There is extensive experimental evidence that oxidative damage permanently occurs in lipids of cellular membranes, proteins and DNA. In nuclear and mitochondrial DNA, 8-OHdG or 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is one of the predominant forms of free radical-induced oxidative lesions and has therefore been widely used as a biomarker for oxidative stress and disease [35]. Kakimoto *et al.* [12] have also shown that the levels of 8-OHdG are increased in kidney tissues of streptozotocin-induced diabetic rats. In the present study, we showed that diabetic patients, especially patients with complications, demonstrated oxidative DNA damage. 8-OHdG levels in urine and mononuclear cells of type 2 diabetic patients with either retinopathy or nephropathy were much higher than those in patients without complications [36]. Diabetic patients have more severe oxidative DNA damage than normal subjects. Levels of oxidative DNA damage are increased in diabetic patients, suggesting that increased oxidative stress may be associated with the progression of diabetes, especially in diabetic patients with nephropathy [37]. In combination with previously published results, these results suggest that serum 8-OHdG is a potentially useful biomarker for evaluating the severity of complications in patients with DM.

AOPP is produced during oxidative stress. This procedure is carried out by myeloperoxidase in active neutrophils with the effects of hypochloric acid and chloramines. These products are identified as proteins that have crosswise conjunctions with tyrosine. Additionally, these are reliable markers to measure oxidative modification of proteins. Biochemical characterization of AOPP in plasma reveals that both the high- and low-molecular-weight AOPP peaks contain oxidized albumin in aggregate-forming or monomeric form [38]. In the present study, the occurrence of protein oxidation in plasma of diabetic patients was also confirmed by a novel marker (AOPP) that provides information on the degree of oxidative damage to proteins. Moreover, patients with diabetic complications also had significantly higher AOPP levels than diabetic patients without vascular complications. Pan *et al.* [39] also showed that serum AOPP and protein carbonyl were significantly higher in type 2 DM compared with normal subjects. Diabetic patients with retinopathy had significantly higher AOPP and protein carbonyl compared with diabetic patients without retinopathy [39]. These data are in agreement with those of other

authors [39-42]. These findings suggest that oxidative protein damage begins in early stages of diabetes and continues to increase as the disease progresses and that AOPP is also a valid biomarker for assessing oxidative stress in diabetes.

PON1 is an enzyme located on serum HDL that has been observed in *in vitro* experiments. Its function is to decrease the accumulation of lipid peroxides on LDL [43,44]. In this study, 15-F2t-IsoP and AOPP levels were shown to increased significantly in diabetes, while PON1 activity decreased. These data confirm the previous observations [45-49] of low PON activity in type 2 diabetes. DM is accompanied by the development of oxidative protein damage as evaluated by an increase in the circulating oxidized proteins [40,42]. This change could be related to an increased risk of endothelial damage. Indeed, these biochemical modifications could affect PON1 activity and, as a result, lead to an increased susceptibility of plasma proteins to protein oxidation in diabetic individuals. Therefore, the reduction of the PON1 activity, as well as its antioxidant activity, could negatively affect the atheroprotective properties of plasma proteins in DM. Paraoxonase activities are affected by PON1 genetic variability in Turkish NIDDM patients and controls. PON1 polymorphisms and low PON activity levels were suggested as an independent risk factor for CHD in diabetic patients [47]. There is evidence of a possible relationship between increased plasma protein oxidation parameters and decreased PON1 activity. Inactivation of PON1 itself may lead to a predisposition to atherosclerosis as well as increased markers of oxidative stress. It would be interesting to clarify whether the decrease in PON1 activity occurs selectively in preference to a range in other redox-sensitive enzymes with thiols in their active site, or there is a general diminution of enzyme activity.

Results of the present study showed that there were severe lipid peroxidation and protein oxidation, and DNA oxidative damage in patients exhibiting complications due to diabetes mellitus. Though the oxidative stress plays a role in the development of diabetic complications and acute effects of hyperglycemia may be counterbalanced by antioxidants, further investigation is needed in order to confirm the relationship between these phenomena.

Acknowledgments

This work was supported by The Research Fund of Istanbul Education and Research Hospital.

References

- Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003;17:24-38.
- Kato Y, Mori Y, Makino Y, Morimitsu Y, Hiroi S, Ishikawa T *et al.* Formation of *N*^ε-(hexanonyl)lysine in protein exposed to lipid hydroperoxide. A plausible marker for lipid hydroperoxide-derived protein modification. *J Biol Chem* 1999; 274:20406-14.
- Kato Y, Yoshida A, Naito M, Kawai Y, Tsuji K, Kitamura M *et al.* Identification and quantification of *N*^ε-(Hexanoyl)lysine in human urine by liquid chromatography/tandem mass spectrometry. *Free Radic Biol Med* 2004;37:1864-74.
- Tomaru M, Takano H, Inoue K, Yanagisawa R, Osakabe N, Yasuda A *et al.* Pulmonary exposure to diesel exhaust particles enhances fatty change of the liver in obese diabetic mice. *Int J Mol Med* 2007;19:17-22.
- Woods AA, Linton SM, Davies MJ. Detection of HOCl mediated protein oxidation products in the extracellular matrix of human atherosclerotic plaques. *Biochem J* 2003;370:729-35.
- Kaneda H, Taguchi J, Ogasawara K, Aizawa T, Ohno M. Increased level of advanced oxidation protein products in patients with coronary artery disease. *Atherosclerosis* 2002;162:221-25.
- Skvarilová M, Bulava A, Stejskal D, Adamovská S, Bartek J. Increased level of advanced oxidation products (AOPP) as a marker of oxidative stress in patients with acute coronary syndrome. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2005;149:83-7.
- Cakatay U. Protein oxidation parameters in type 2 diabetic patients with good and poor glycaemic control. *Diabetes Metab* 2005;31:551-57.
- Kalousová M, Zima T, Tesar V, Dusilová-Sulková S, Skrha J. Advanced glycoxidation end products in chronic diseases-clinical chemistry and genetic background. *Mutat Res* 2005;579:37-46.
- Nishikawa T, Sasahara T, Kiritoshi S, Sonoda K, Senokuchi T, Matsuo T *et al.* Evaluation of Urinary 8-Hydroxydeoxy-Guanosine as biomarker of macrovascular complications in type 2 diabetes. *Diabetes Care* 2003;26:1507-12.
- Del Guerra S, Lupi R, Marselli L, Masini M, Bugliani M, Sbrana S *et al.* Functional and molecular defects of pancreatic islets in human type 2 diabetes. *Diabetes* 2005;54:727-35.
- Kakimoto M, Inoguchi T, Sonta T, Yu HY, Imamura M, Etoh T *et al.* Accumulation of 8-Hydroxy-2'-Deoxyguanosine and mitochondrial DNA deletion in kidney of diabetic rats. *Diabetes* 2002;51:1588-95.
- Kanauchi M, Nishioka H, Hashimoto T. Oxidative DNA damage and tubulointerstitial injury in diabetic nephropathy. *Nephron* 2002;91:327-29.
- Taber DF, Morrow JD, Roberts LJ 2nd. A nomenclature system for the isoprostanes. *Prostaglandins Other Lipid Mediat* 2004;73:47-50.
- Cracowski JL, Durand T, Bessard G. Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications. *Trends Pharmacol Sci* 2002;23:360-6.
- Davi G, Falco A, Patrono C. Determinants of F2-isoprostane biosynthesis and inhibition in man. *Chem Phys Lipids* 2004;128:149-63.
- Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol* 2005;69:541-50.
- Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM *et al.* Protective effect of high density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 1995;96:2882-91.
- Gugliucci A, Luncford N, Kinugasa E, Ogata H, Schulze J, Kimura S. Acrolein inactivates paraoxonase 1: changes in free acrolein levels after hemodialysis correlate with increases in paraoxonase 1 activity in chronic renal failure patients. *Clin Chim Acta* 2007;384:105-12.
- Jaouad L, de Guise C, Berrougui H, Cloutier M, Isabelle M, Fulop T *et al.* Age-related decrease in high-density lipoproteins antioxidant activity is due to an alteration in the PON1's free sulfhydryl groups. *Atherosclerosis* 2006;185:191-200.
- Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539-53.
- Cakatay U, Kayali R, Uzun H. Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population. *Clin Exp Med* 2008;8:51-7.
- Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc Diabetol* 2005;4(1):5.
- Davi G, Ciabattini G, Consoli A, Mezzetti A, Falco A, Santarone S *et al.* In vivo formation of 8-iso prostaglandin F2 alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 1999;99:224-29.
- Roberts LJ, Morrow JD. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med* 2000;28:505-13.
- Basu S. Isoprostanes: novel bioactive products of lipid peroxidation. *Free Radic Res* 2004;38:105-22.
- Davi G, Falco A, Patrono C. Lipid peroxidation in diabetes mellitus. *Antioxid Redox Signal* 2005;7:256-68.
- Calabrese V, Mancuso C, Sapienza M, Puleo E, Calafato S, Cornelius C *et al.* Oxidative stress and cellular stress response in diabetic nephropathy. *Cell Stress Chaperones* 2007;12:299-306.
- Cristina Polidori M, Pratico D, Savino K, Rokach J, Stahl W, Mecocci P. Increased F2 isoprostane plasma levels in patients with congestive heart failure are correlated with antioxidant status and disease severity. *J Card Fail* 2004;10:334-38.

30. Memisogullari R, Taysi S, Bakan E, Capaoglu I. Antioxidant status and lipid peroxidation in type 2 diabetes mellitus. *Cell Biochem Funct* 2003;21:291-96.
31. Kajanachumpol S, Komindr S, Mahaisiriyodom A. Plasma lipid peroxide and antioxidant levels in diabetic patients. *J Med Assoc Thai* 1997;80:372-7.
32. Tomaru M, Takano H, Inoue K, Yanagisawa R, Osakabe N, Yasuda A et al. Pulmonary exposure to diesel exhaust particles enhances fatty change of the liver in obese diabetic mice. *Int J Mol Med* 2007;19:17-22.
33. Gopaul NK, Anggård EE, Mallet AI, Betteridge DJ, Wolff SP, Nourooz-Zadeh J. Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Lett* 1995;368:225-29.
34. Mezzetti A, Cipollone F, Cuccurullo F. Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm. *Cardiovascular Research* 2000;47:475-88.
35. Huang HY, Helzlsouer KJ, Appel LJ. The effects of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev* 2000;9:647-52.
36. Suzuki S, Hinokio Y, Komatu K, Ohtomo M, Onoda M, Hirai S et al. Oxidative damage to mitochondrial DNA and its relationship to diabetic complications. *Diabetes Res Clin Pract* 1999;45:161-68.
37. Pan HZ, Chang D, Feng LG, Xu FJ, Kuang HY, Lu MJ. Oxidative damage to DNA and its relationship with diabetic complications. *Biomed Environ Sci* 2007;20:160-3.
38. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 1996;49:1304-13.
39. Pan HZ, Zhang H, Chang D, Li H, Sui H. The change of oxidative stress products in diabetes mellitus and diabetic retinopathy. *Br J Ophthalmol* 2008;92:548-51.
40. Kalousova M, Skrha J, Zima T. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. *Physiol Res* 2002;51:597-604.
41. Witko-Sarsat V, Friedlander M, Nguyen Khoa T, Capeillère-Blandin C, Nguyen AT, Canteloup S et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 1998;161:2524-32.
42. Piwowar A, Knapik-Kordecka M, Warwas M. AOPP and its relations with selected markers of oxidative/antioxidative system in type 2 diabetes mellitus *Diabetes Research and Clinical Practice* 2007;77:188-92.
43. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett* 1991;286:152-54.
44. Mackness MI, Arrol S, Abbott CA, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis* 1993;104:129-35.
45. Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuashia B et al. Serum paraoxonase PON1 55 and 192 polymorphism and paraoxonase activity and concentration in non insulin dependent diabetes mellitus. *Atherosclerosis* 1998;139:341-49.
46. Mackness B, Durrington PN, Abuashia B, Boulton AJM, Mackness MI. Low paraoxonase activity in type 2 diabetes mellitus complicated by retinopathy. *Clin Science* 2000;98:355-63.
47. Agachan B, Yilmaz H, Karaali Z, Isbir T. Paraoxonase 55 and 192 polymorphism and its relationship to serum paraoxonase activity and serum lipids in Turkish patients with non-insulin dependent diabetes mellitus. *Cell Biochem Funct* 2004;22:163-68.
48. Poh R, Muniandy S. Paraoxonase 1 activity as a predictor of cardiovascular disease in type 2 diabetes. *Southeast Asian J Trop Med Public Health* 2010;41:1231-46.
49. Jornayvaz FR, Brulhart-Meynet MC, James RW. Myeloperoxidase and paraoxonase-1 in type 2 diabetic patients. *Nutr Metab Cardiovasc Dis* 2009;19:613-9.