Increased Oxidative DNA Damage in Lean Normoglycemic Offspring of Type 2 Diabetic Patients

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Key words
- oxidative DNA damage
- offspring
- Type 2 diabetes

Abstract

Objective: Several studies have shown increased oxidative stress in patients with pre-diabetes and newly diagnosed Type 2 diabetes mellitus (T2DM). It has been proposed that oxidative stress initiates insulin resistance in genetically predisposed individuals. The aim of this study was to evaluate the markers of oxidative stress in the offspring of patients with T2DM.

Material and Methods: We examined 60 lean normoglycemic offspring of Type 2 diabetics, and 52 age, sex and body mass index matched subjects without family history of T2DM as controls. Anthropometric, biochemical and carotid intima media thickness (IMT) measurements and oral glucose tolerance test (OGTT) were performed. Erythrocyte superoxide dismutase and glutathione peroxidase activities, serum nitric oxide, plasma total sulfhydryl (tSH) groups, plasma total antioxidant status, plasma malondialdehyde and serum 8-hydroxydeoxy-guanosine (8-OHdG) levels were compared between 2 groups.

Results: 2 groups were similar for the measurements of anthropometric, blood pressure, lipids, fasting glucose, HOMA-IR and carotid IMT. Glucose levels during OGTT were significantly higher in the offspring of Type 2 diabetics than controls ($p=0.035$). The offspring of Type 2 diabetics showed a significant increase in serum 8-OHdG level ($p=0.005$) and plasma tSH groups ($p=0.032$) when compared to the controls. Significant differences were not obtained in other oxidative stress marker levels between 2 groups.

Conclusion: Main finding of our study was the presence of increased oxidative DNA damage in lean normoglycemic offspring of Type 2 diabetic patients. There is a need for further clinical studies in order to explain whether oxidative stress is present in genetically predisposed subjects and induces the insulin resistance.

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Introduction

Reactive oxygen species (ROS) are oxygen derivatives playing roles in several biochemical processes under physiological conditions. The term oxidative stress refers to a condition in which cells are subjected to excessive amount of ROS as a result of a general increase in ROS generation, a depression of the antioxidant systems, or both [1,2]. The ROS can not be inactivated by antioxidant systems react with cellular macromolecules and enhance the process of lipid peroxidation and DNA damage and induce protein modifications. The breakdown of lipid peroxides in biological systems produces a number of aldehydes. Malondialdehyde (MDA) is the most abundant and dominant aldehyde resulting from lipid peroxidation. 8-hydroxydeoxy-guanosine (8-OHdG) is an oxidatively damaged promutagenic base and has been proposed as a reliable biomarker of oxidative DNA damage. Furthermore, ROS can react with tyrosine to produce tyrosyl radicals, which can oxidize nitric oxide (NO) to generate nitrotyrosine. A series of antioxidant enzymes, primarily superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) and numerous endogenous and dietary antioxidant compounds are members of antioxidant systems. SOD catalyzes the dismutation of superoxide radical to the less reactive hydrogen peroxide. GPX and catalase convert hydrogen peroxide to water and molecular oxygen. The capacity of a subject to resist oxidative stress is indicated by the total antioxidant status (TAS). Many authors have examined the relationship between oxidative stress and several diseases such as cancer [3], neurodegenerative disorders [4], cardiovascular diseases [5], obesity [6], polycystic ovary syndrome [7,8], diabetes mellitus Type 1 [9,10] and Type 2 [11,12].

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder of heterogeneous etiology as a consequence of an interaction between environmental and genetic factors [13]. 2 main abnormalities in the development of T2DM are insulin resistance and defective insulin secretion in pancreatic β-cells in response to glucose. Although the contribution of oxidative stress to development and progression of diabetic complications [14–21] is well known, its role in the pathogenesis of T2DM is unclear. There is a relationship between oxidative stress and insulin resistance in metabolic syndrome-related manifestations such as atherosclerosis, hypertension, T2DM [1,2]. Markers of oxidative stress are elevated in clinical T2DM, but there are only limited data relating the degree of oxidative stress to insulin resistance in pre-diabetic states [22]. Several studies indicate that increased oxidative stress and endothelial dysfunction are present in early phases of diabetes, impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) [22–27].

Genetic predisposition is important in the pathogenesis of T2DM [28]. The offspring of Type 2 diabetics have a higher risk for developing insulin resistance and T2DM. Therefore, healthy first-degree relatives of the patients with T2DM are logical candidates for studies of factors involved in the pathogenesis of T2DM [29]. Systemic oxidative stress can lead insulin resistance in cultured cells and animals [22,29,30]. But, it remains unclear whether oxidative stress may play a role in the development of insulin resistance in humans. It has been proposed that metabolic impairments in these genetically predisposed subjects could trigger insulin resistance through increased oxidative stress. To our knowledge there is not any paper investigating the markers of oxidative stress and anti-oxidant status in lean normoglycemic offspring of Type 2 diabetics. Therefore, we evaluated MDA as a marker of lipid peroxidation, antioxidants such as SOD, GPX, total sulfhydryl (tSH) groups and TAS, and 8-OHdG as a marker of oxidative DNA damage in lean, normoglycemic subject with family history of T2DM.

Material and Methods

We included 60 lean (BMI between 18.5 and 25 kg/m^2), non-diabetic offspring of Type 2 diabetics, and 52 normoglycemic control subjects without family history (FH) of T2DM, matched in terms of gender, age, and body mass index (BMI). The participants were recruited from hospital staff, medical students and offspring of patients with T2DM admitted to the Outpatient Clinic of Endocrinology and Metabolism at Ege University Hospital. Family history of diabetes was assessed by questionnaire. Relatives of Type 2 diabetics were included in the study if both of the parents, or one parent and one first-or-second-degree relative had T2DM. All subjects were nonsmokers, aged between 18 and 35 years, free from acute or chronic infections, known ischemic heart disease, peripheral vascular disease, hypertension, dyslipidemia and any other serious medical problems. The study protocol was approved by the Ethical Committee of Ege University Hospital. Written informed consent was taken from all subjects before participating in the study.

First, all subjects were evaluated by physical examination, anthropometric measurements. Their body weights were measured on a two-point bioelectrical impedance apparatus (Tanita TBF 300, TANITA Corp. Tokyo, Japan) validated for adults; and their heights were measured by a wall-mounted stadiometer. BMI was calculated as body weight (kg) divided by height (m) squared. Waist circumference was measured according to a standard procedure described earlier [31]. Blood pressure was recorded as the last of 2 measurements with the subjects seated using a sphygmomanometer. The blood samples were taken from the antecubital vein for appropriate laboratory tests after a 12-h fast. The subjects who had metabolic impairment were excluded.

Before OGTT, 250 g of carbonhydrate-containing diet was advised for all subjects for 3 days. OGTT was performed between 08:00 and 10:00 h after an overnight fast. Both insulin and glucose levels were measured at baseline, 1 and 2 h after 75 g glucose ingestion. Plasma glucose was measured immediately by the glucose oxidase method using a glucose analyzer (photometer 5010). Serum insulin level was measured with enzyme-labeled chemiluminescent immunometric assay (Immulite 2000, UK). The persons with normal glucose metabolism according to ADA criteria [32] were evaluated for oxidative stress and anti-oxidant status.

After an overnight fast, venous blood samples were drawn from an antecubital vein into heparinized and no-anticoagulant tubes and centrifuged at 3000 rpm for 20 min. Serum, plasma, and erythrocyte lysate (EL) were stored at −80 °C until the analyses were performed. EL was prepared according to the method published earlier [33]. Plasma was removed from the blood samples drawn into heparin containing tubes, and packed erythrocytes were washed 2 times with 9 g/L NaCl solution and hemolyzed with ice-cold distilled water (1/5, v/v). Erythrocyte SOD and GPx activities in hemolyzates were determined by using commercial kits (Randox, UK) and an automated Roche P800 analyzer. Serum NO level was determined by measuring stable NO end-products, nitrite and nitrate levels by using Miranda's spectrophotometric method [34]. Nitrate was reduced to nitrite with vanadium (III); and then nitrite level was measured by using Griess reagents. This reflects total nitrate and nitrite amount in the sample. Serial dilutions of Na nitrate (Merck, Germany) were used as standard. Levels of plasma tSH groups were measured by using the method described by Sedlak and Lindsay [35]. Serial dilutions of Glutathione (Sigma, USA) were used for standard curve. Plasma TAS (mmol glutation eq/L) was measured by spectrophotometric method of Erel [36]. Plasma MDA levels indicated lipid peroxidation were measured as described by Armstrong and al-Awadi [37], who modified Yagi method [38]. Calibration curve was prepared with 1, 1, 3, 3-tetramethoxypropane (Sigma, USA). Serum 8-OHdG level, a biomarker of oxidative DNA damage, was determined by using commercial kits (Oxis, USA).

For all participants, the measurements of carotid intima-media thickness (IMT), which is a sensitive marker to detect early arteriosclerosis, were performed on the mid portion of the common carotid artery using an equipment of Sonoline Elegra system (Siemens, Erlangen, Germany) with a 7.5 MHz linear-array transducer by radiologist experienced in US examinations.

Statistical analysis

All of the analyses were conducted by using SPSS 18.0. Each variable was checked for normality of distribution by the Shapiro-Wilk normality test. When normality test passed, unpaired t test was used to compare the offspring of Type 2 diabetics and controls for each of the variables. The Mann-Whitney test was used for non-normally distributed variables. Normally distributed data were expressed as mean ± S.D. Non-normally distributed variables were presented as median ± IR. The repeated measure ANOVA was used to analyze the relations between family history and glucose and insulin levels during OGTT. Pearson's correla-

Table 1 Baseline characteristics of FH+ and FH- subjects.

	FH+	FH−	p-value
n	60	52	–
sex (female/male)	44/16	37/15	0.797
age (years)	24.1±3.8	23.7±2.6	0.450
BMI (kg/m²)	21.9±1.9	21.7±1.8	0.712
waist circumference (cm)	75.0±7.8	76.2±8.8	0.477
systolic BP (mmHg)	106.4±11.9	110.9±11.8	0.052
diastolic BP (mmHg)	70.3±8.2	72.4±8.3	0.170
total cholesterol (mg/dl)	163.6±23.4	161.4±22.8	0.616
triglycerides (mg/dl)	72.8±26.9	69.0±24.3	0.448
HDL-cholesterol (mg/dl)	59.9±13.4	59.9±12.8	0.999
LDL-cholesterol (mg/dl)	89.0±20.8	87.7±22.8	0.768
fasting glucose (mg/dl)	79.0±9.1	76.3±8.5	0.107
fasting insulin (mIU/ml)	6.2±3.2	5.7±3.2	0.366
HOMA-IR	1.28±0.74	1.09±0.66	0.158
Hs-CRP (mg/dl)¶	0.094±0.113	0.063±0.081	0.058
fibrinogen (mg/dl)	339.2±80.3	327.8±70.8	0.459
right carotid IMT (mm)	0.726±0.076	0.742±0.074	0.619
left carotid IMT (mm)	0.735±0.089	0.732±0.079	0.499

FH: Family history; BP: Blood pressure; normally distributed data were expressed as means±SD, non-normally distributed data were presented as median±IR
¶ non-normally distributed variable

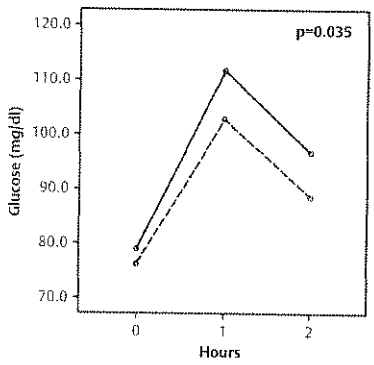

Fig. 1 Glucose levels during OGTT in the offspring of diabetic patients (—) and control subjects (---).

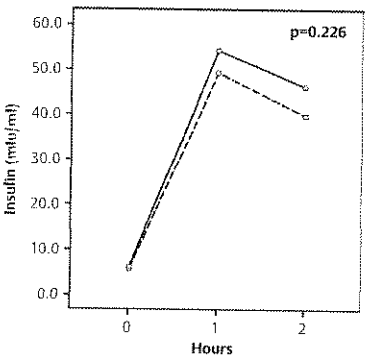

Fig. 2 Insulin levels during OGTT were not statistically significant in the offspring of diabetic patients (—) than control subjects (---).

tion coefficients were performed for correlations. The level of significance was accepted at $p<0.05$.

Results

Baseline characteristics of the groups in this study were shown in ⊙ **Table 1**. 2 groups were similar in terms of age, gender, anthropometric measurements, blood pressure, lipids, fasting glucose and insulin, HOMA-IR and fibrinogen levels ($p>0.05$). FH+ group had higher values for blood glucose than FH- group from 1 up to 2 h after oral glucose load ($p=0.035$) (⊙ **Fig. 1**). Although insulin levels during OGTT were greater in FH+ group than FH- group, the difference was not statistically significant ($p>0.05$) (⊙ **Fig. 2**). FH+ group had elevated level of Hs-CRP than FH- group, but difference was not statistically significant ($p=0.058$). Carotid IMT measurements were also similar in offspring of Type 2 diabetics and controls ($p>0.05$).

Table 2 Parameters of antioxidant status, oxidative stress and oxidative DNA damage in 2 groups.

	FH+	FH−	p-value
SOD (U/g hb)	498.4±273.4	1418.5±187.9	0.103
GPX (U/g hb)	34.9±11.6	33.4±7.9	0.412
NO (microMol/L)¶	43.2±18.4	40.1±16.8	0.513
TSH (mMol/L)	1.16±0.43	0.97±0.47	0.032
TAS (mmol glutation eq/L)	0.461±0.067	0.459±0.074	0.937
MDA (nmol/mL)¶	3.8±3.3	3.8±3.9	0.568
8-OHdG (ng/ml)¶	0.245±0.715	0.126±0.238	0.005

FH: Family history; normally distributed data were expressed as means±SE, non-normally distributed data were presented as median±IR
¶ non-normally distributed variable

Table 3 Relationship between erythrocyte GPx activity, TAS, MDA levels and glucose and insulin levels during OGTT.

	GPx activity	TAS	MDA
glucose; fasting	–	–	–
1 h	r=−213, p=0.028	r=236, p=0.012	–
2 h	–	–	–
insulin; fasting	–	r=366, p=0.001	–
1 h	–	r=209, p=0.027	r=261, p=0.006
2 h	–	r=227, p=0.016	–

2 groups were analyzed in terms of oxidative stress, anti-oxidant status and oxidative DNA damage (⊙ **Table 2**). Erythrocyte SOD and GPx activities, serum NO, plasma TAS; and plasma MDA levels were found to be similar in both groups. In comparison with the subjects without FH, the offspring of Type 2 diabetics showed a significant increase in the levels of serum 8-OHdG ($p=0.005$) and plasma tSH groups ($p=0.032$).

In study population, correlation analysis was conducted between oxidative stress, anti-oxidant status, oxidative DNA damage, and glucose, insulin levels. Erythrocyte GPx activity was negatively correlated with glucose level at 1 h of OGTT ($r=-213$, $p=0.028$), TAS was positively correlated with glucose level at 1 h of OGTT ($r=236$, $p=0.012$), insulin levels at fasting ($r=366$, $p=0.001$), 1 h ($r=209$, $p=0.027$) and 2 h ($r=227$, $p=0.016$) of OGTT, MDA was correlated positively with insulin level at 1 h of OGTT ($r=261$, $p=0.006$) (⊙ **Table 3**).

Discussion

The main finding of our study was the presence of the evidence for oxidative DNA damage in lean, normoglycemic offspring of Type 2 diabetics. A few study have shown increased oxidative DNA damage in patients with impaired glucose regulation (IGR) and newly diagnosed T2DM [24,27]. Song and colleagues [24]

have revealed a significant correlation between DNA damage and hyperglycemia, insulin resistance, and beta-cell dysfunction. In this study, plasma MDA and antioxidant status (TAS, erythrocyte GSH content and SOD activity) have also been evaluated. Authors have proposed that hyperglycemia in IGR state caused the predominance of oxidative stress over antioxidant defense systems, leading to oxidative DNA damage, which possibly contributes to Beta-cell dysfunction, insulin resistance, and more apparent hyperglycemia. In order to examine the effect of glucose excursion on the activation of oxidative stress, Zheng and colleagues [27] have compared the data of continuous glucose monitoring system (CGMS) with levels of oxidative stress markers, 8-iso-PGF2α, 8-OH-dG, and protein carbonyl content in the subjects with normal glucose regulation (NGR) and IGR. Finally, they have reported that glucose excursions in subjects with IGR and T2DM trigger the activation of oxidative stress.

In vitro, prolonged culture of beta cells with elevated glucose decreases insulin release, insulin content and insulin secretion [39]. Since beta-cells have low antioxidant capacity, they are extremely sensitive towards oxidative stress associated with excess glucose metabolism [40]. Moreover, chronic exposure to glucose and free fatty acid can result in increased production of ROS, and activation of stress-sensitive pathways [41]. Our findings are partially consistent with these results supporting the relationship between oxidative stress and glucose levels. Although we have found that both the levels of glucose during OGTT and serum 8-OHdG were meaningfully higher in offspring of Type 2 diabetics than the subjects without FH, we could not show a correlation between glucose and 8-OHdG levels.

Al-Aubaidy has emphasized that serum 8-OHdG is an early oxidative stress marker in the patients with pre-diabetes and T2DM [42]. Besides, no significant change in MDA and erythrocyte reduced glutathione levels has been observed, it has been found that 8-OH-dG level was significantly higher in the pre-diabetics when compared to control group. In our study, 8-OHdG level was higher in the offspring of Type 2 diabetics than control group, while MDA level was not different. Therefore, we also think that serum 8-OHdG measurement may be more sensitive when compared to other oxidative stress markers, especially in early states of the diseases.

Skeletal muscle and adipose tissues are the most important tissues involved in the pathogenesis of insulin resistance. Recently, several researchers have advocated that oxidative stress is one of the pathophysiological mechanisms of insulin resistance. A few hypothesis related to the role of oxidative stress in insulin resistance have been reported. When caloric intake exceeds the energy consumption; superoxide generation is increased on mitochondrial electron transport chain. It has been proposed that insulin resistance may be considered as a compensatory mechanism that protects the cells against further insulin stimulated glucose and fatty acid uptake, and thus oxidative damage. Several animal and human studies have shown that antioxidants improve insulin sensitivity, supporting this hypothesis [30]. Furthermore, ROS-induced mitochondrial dysfunction can lead to disruptions of lipid metabolism, increasing the intracellular lipid content and contribute to lipid-dependent insulin resistance in myocytes [11]. In-vitro exposure to oxidant stress leads to insulin resistance of distal insulin signaling and diminished insulin-stimulated glucose transport activity [43,44]. Beta-cells and endothelial cells may be more affected by overfeeding, because they are not insulin dependent for glucose uptake. So, they can not down-regulate the influx of nutrients by means of insulin resistance, and must allow intracellular concentrations to increase further. Beta cells have also low antioxidant capacity, and this makes them extremely sensitive to oxidative stress [40].

The Framingham Offspring Study has revealed that systemic oxidative stress is associated with insulin resistance in non-diabetic individuals and among subgroups at elevated risk of diabetes even after accounting for BMI [22]. Recently, Samocha-Bonet and colleagues have proposed that metabolic inflexibility in subjects genetically predisposed to T2DM could trigger insulin resistance through increased oxidative stress. Mitochondria are significant source of superoxide radicals; and mitochondrial dysfunction plays a role in the development of diabetes and insulin resistance [45]. It has been shown that rates of muscle mitochondrial substrate oxidation were decreased in lean, insulin resistant offspring when compared to insulin sensitive control subjects by using magnetic resonance spectroscopy [46].

Insulin resistance is present even in non-diabetic lean first degree relatives of Type 2 diabetic subjects [47]. In our study, HOMA-IR, insulin levels during OGTT were higher in FH+ subjects than control group, but the difference was not statistically significant. The reason for the fact that we could not determine a difference between 2 groups in terms of insulin resistance may be related to the method used for measuring the insulin resistance. The gold standard for measuring insulin resistance is hyperinsulinemic clamp technique. Since this method is required considerable experience and extensive equipments. Homeostasis model assessment of insulin resistance (HOMA-IR) which is easier method for estimating insulin resistance is used in present study. But this index has low sensitivity and specificity for detecting insulin resistance in non-diabetic individuals [48]. Plasma tSH groups as well as 8-OH-dG levels were also increased in the offspring of Type 2 diabetics when compared to the subjects without FH. This result suggests an increase in oxidative DNA damage and a compensatory increase in tSH level in subjects with FH of T2D. However, differences were not observed between 2 groups for other oxidative stress markers and antioxidant status. Correlation analysis conducted between oxidative stress, antioxidant status, and glucose and insulin levels also revealed different results. Erythrocyte GPx activity was correlated negatively with glucose level at 1 h of OGTT; TAS was correlated positively with glucose level at 1 h of OGTT, insulin level at 0, 1 and 2 h of OGTT; and MDA was positively correlated with insulin level at 1 h of OGTT.

In this study, increased oxidative DNA damage was found in lean normoglycemic offspring of Type 2 diabetics. However, we could not show a definite relationship between oxidative stress and glucose metabolism or insulin resistance. There is a need further clinical studies in order to explain whether oxidative stress is present in genetically predisposed subjects and induces the insulin resistance.

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Conflict of Interest: None.

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