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Oxidative Damage to DNA and lipids: Correlation With Protein Glycation in Patients With Type 1 Diabetes

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Diabetic hyperglycemia is associated with increased production of reactive oxygen species (ROS). ROS reacts with DNA resulting in various products, such as 8-hydroxydeoxyguanosine (8-OHdG), that excrete in urine owing to DNA repair processes. Urinary 8-OHdG has been proposed as an indicator of oxidative damage to DNA. This study aimed to evaluate relationship between oxidative damage to DNA and protein glycation in patients with Type 1 diabetes. We measured urinary 8-OHdG level in diabetic patients and healthy subjects and discussed its relationship to glycated hemoglobin (HbA_{1c}) and glycated serum protein (GSP) levels. Furthermore plasma malondialdehyde (MDA) level monitored as an important indicator of lipid peroxidation in diabetes. We studied 32 patients with Type 1 diabetes mellitus and compared the measured factors with those of 48 age-matched nondiabetic controls. GSP and MDA were measured bycolorimetric assay. Urinary 8-OHdG measurement was carried out using ELISA. In this study urinary 8-OHdG, HbA_{1c}, plasma MDA, and GSP levels were progressively higher in diabetics than in control subjects ($P<0.05$). Furthermore we found significant correlation between urinary 8-OHdG and HbA_{1c} ($P<0.05$) in diabetic group. Correlation between fasting blood sugar and GSP were significant. We also found significant correlation between fasting blood sugar and MDA. This case–control study in young diabetic patients showed increased blood glucose and related metabolic disorders result in oxidative stress and oxidative damage to DNA and lipids. Furthermore oxidative damage to DNA is associated to glycemic control level, whereas lipid peroxidation level was not significantly correlated with glycemic control level. J. Clin. Lab. Anal. 24:72–76, 2010.

Key words: oxidative damage to DNA; diabetes Type 1; 8-hydroxydeoxyguanosine; glycated hemoglobin

INTRODUCTION

Hyperglycemia is an important etiologic factor of diabetes mellitus complications (1); however, mechanisms that indicate its relationship to cellular function disorders have not been established. Several pathways (e.g., hexosamine and polyol pathways, activation of protein kinase C, advanced glycation end products (AGE) formation, etc.) have been proposed to explain the mechanism by which hyperglycemia leads to chronic complications of diabetes mellitus (2).

The common theme in most of these pathways is the induction of oxidative stress during hyperglycemia. AGE can be formed by nonenzymatic glycosylation of proteins or autoxidation of glucose (3). In nonenzymatic glycosylation process, glucose reacts nonenzymatically and covalently with amino groups of proteins in which reversible Shiff base is formed. These Shiff base intermediates undergo Amadori rearrangement to stable ketoamine derivative (Amadori products) (4). Amadori products degrade into α-dicarbonyl compounds that are

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mainly responsible for forming AGEs, glycated hemoglobin (HbA_{1c}), and glycated serum proteins (GSPs) (4).

In autoxidation process, glucose may undergo metal-catalyzed autoxidation to produce reactive carbonyl precursors of AGEs. Free radicals are formed in this process, which can cause DNA oxidation, lipid peroxidation, and other cellular lesions (5).

Probably nuclear and mitochondrial DNA is the most important target for oxidative attack by free radicals. 8-hydroxydeoxyguanosine (8-OHdG) is produced by the oxidation of deoxyguanosine, which induces mutations (6,7).

8-OHdG is popular as a sensitive, stable, and integral marker of oxidative damage in cellular DNA. Biomonitoring in human has demonstrated that 8-OHdG can be excreted in the urine. As 8-OHdG represents dynamic equilibrium between DNA oxidative damage and DNA repair rate, its measurement in urine is useful to evaluate DNA damage in whole body (8,9). In a study by Dondana et al., urinary 8-OHdG level in diabetic patients was higher than those of healthy subjects; however, they did not find any correlation between urinary 8-OHdG and blood glycated hemoglobin(HbA_{1c})(10). Tsukahara et al. reported higher excretion of 8-OH-dG in Type 1 diabetic patients compared with those of controls (11). They also found the urinary excretion of 8-OHdG was higher in patients with microalbuminuria (11). Ikue et al. measuring the urinary 8-OHdG indicated that increased oxidative stress and the risk of vascular complications may be present at early stages of Type 1 diabetes (12).

In the other hand polyunsaturated fatty acids (PUFA) are also susceptible to oxidation by free radicals and other reactive species. Oxidative damage to PUFA results in decreased membrane fluidity and physiological activity. Malondialdehyde (MDA) is a byproduct of lipid peroxidation process and its measurement is an indicator of lipid peroxidation level (13). Increased lipid peroxidation in diabetes has been suggested in earlier studies; however, it is not clear whether lipid peroxidation increases in diabetic patients without microvascular complications. Piconi et al indicate that measurement of biomarkers such as 8-OHdG, MDA, and isoprostanes is useful tool to assess the oxidation stress of organism (14).

This study aimed to evaluate oxidative damage to DNA and lipids in patients with Type 1 diabetes. The other aim of our study was to determine correlation between DNA oxidative damage and HbA_{1c}.

METHODS

Subjects

We studied 32 patients with Type 1 diabetes (18 female and 14 male; mean age $\pm$ SD, 19.03 ± 5.1 y) and 48 healthy controls (26 female and 22 male, mean age 19.8 ± 3.1 y).

We ensured that all the subjects have not been taking any medicines other than insulin. The questionnaire was intended to elicit information on the subjectõs age, smoking habits, alcohol consumption, and medical usage. All of the patients had normal serum creatinine (< 1.2 mg/dl) and normal renal function. Our patients did not have any history of smoking. The postgraduate council in Hamadan University of Medical Sciences approved the study protocol.

Sample Collection

Early morning urine samples were obtained from each subject. The samples were centrifuged and the supernatants were stored at $-20^{\circ}C$ until analysis. The fasting venous blood was drawn from diabetic patients and healthy subjects. The serum and plasma were separated by standard methods.

Urinary 8-OHdG Measurement

Urinary 8-OHdG level was measured by competitive ELISA kit (GENTAUR Corporation, Brussels, Belgium) that uses highly specific monoclonal antibody. The 8-OHdG concentration was expressed relative to urinary concentration of creatinine (ng/mg Cr).

HbA_{1c} Measurement

Blood HbA_{1c} level was measured by ion-exchange chromatography kit (Biosystem Company, Barcelona, Spain). In this method, after preparing the hemolysate where the labile fraction is eliminated, hemoglobins were retained by a cationic exchange resin. Hemoglobin A_{1c} (HbA_{1c}) was specifically eluted after washing away the hemoglobin A_{1a+b} fraction (HbA_{1a+b}), and was quantified by direct photometric reading at 415 nm.

GSP Measurement

We used thiobarbituric acid colorimetric method to measure GSPs. This method measures 5-hydroxy methylefurfural (HMF) produced by hexose dehydration reaction in the presence of boiling oxalic acid (15). To achieve a standard result, GSP concentration was stated as nmol HMF/mg protein.

Plasma MDA Measurement

We used modified colorimetric method to measure MDA concentration in plasma. This method was based on MDA reaction with thiobarbituric acid (TBA) and resulting MDA–TBA_2 complex was quantified by photometric reading at 535 nm (16).

Fasting Blood Sugar Measurement

Blood glucose concentration was measured enzymatically using glucose test kit (Parsazmoon Company, Tehran, Iran).

Urinary and Serum Creatinine Measurement

Urinary and serum creatinine was measured specrophotometrically according to the formation of color complex between creatinine and alkaline picrate (17).

Total Serum Protein Measurement

Biuret method was used to measure serum protein level (Parsazmoon Company, Iran).

Statistics

Differences in measured parameters between normal and diabetic subjects were assessed by *t*- test. The relationships between parameters were analyzed using linear regression analysis. A probability of 0.05 was set as the level of statistical significance.

RESULTS

Table 1 shows mean value of clinical markers in patients with Type 1 diabetes and control subjects. Urinary level of 8-OHdG was significantly higher in patients with Type 1 diabetes. We also found significantly higher level of HbA_{1c}, GSP, and MDA in patients with Type 1 diabetes.

Urinary level of 8-OHdG was significantly correlated with HbA_{1c} in diabetic group ($r = 0.41$, $p < 0.05$); this relationship is depicted in Figure 1. Serum glucose concentrations in diabetic patients was correlated with GSP and MDA ($r = 0.35$, $p < 0.05$ and $r = 0.39$, $p < 0.05$, respectively). Figure 2 shows the regression plot correlation between serum glucose and MDA. In control group the correlation between serum glucose and GSP was statistically significant ($r = 0.45$, $p < 0.05$), but we did not find significant correlation between serum glucose and MDA in this group. There was no significant correlation between serum glucose concentrations and HbA_{1c} in patients with Type 1 diabetes.

DISCUSSION

There are evidences indicating oxidative stress role in endothelial damage, pathogenesis, and complications of diabetic patients (18). In this study we focused on specific marker of oxidative damage to DNA in urine that is called 8-OHdG. We studied HbA_{1c}, GSP, and MDA concentration as markers for glycemic control, serum protein glycation, and lipid peroxidation, respectively. Levels of 8-OHdG and other modified bases as measured in DNA isolated from cells represent a dynamic equilibrium between rates of oxidative DNA damage and rates of repair of that damage. It follows that levels of oxidized bases can change not only because of changes in the rate of oxidative DNA damage, but also because of alterations in the rate of repair (19). Instead of measuring damage in specific cells, which concomitant problems such as artifact formation, a whole body burden of oxidative stress may be assessed by the measurement of urinary excretion of 8-hydroxydeoxyguanosine. 8-OHdG is considered a typical marker of DNA damage and repair, because guanine is the most fragile nuclear base, is unaffected by diet and is excreted to urine separately from other metabolic processes (20).

Earlier studies confirmed the association between oxidative DNA damage and the complications of diabetes, so that urinary 8-OHdG levels were significantly higher in diabetic patients with nephropathy and retinopathy (21). In our study urinary levels of 8-OHdG were significantly higher in diabetic patients (without complications). Therefore, probably diabetic patients

TABLE 1. Clinical Markers of Diabetic Patients and Healthy Subjects

Factor	Healthy subjects	Diabetic patients
n	48	32
F/M	26/22	18/14
Age (year)	19.83 ± 3.15	19.03 ± 5.18
Fasting blood glucose (mg/dl)	77.56 ± 5.84	291.4 ± 83.19*
Glycosylated hemoglobin (HbA_{1c})	5.16 ± 0.5	9.05 ± 1.47*
Glycated serum protein (nmol HMF/mg protein)	0.32 ± 0.12	0.79 ± 0.24**
Plasma malondialdehyde (μmol/l)	0.29 ± 0.07	0.73 ± 0.24**
Urinary 8-OHdG (ng/mg cr)	9.9 ± 3.87	16.77 ± 5.56**
Serum creatinine (mg/dl)	0.55 ± 0.09	0.55 ± 0.11
Urine creatinine (mg/dl)	84.22 ± 22.5	85.53 ± 20.18
Serum total protein (g/dl)	7.41 ± 0.6	7.04 ± 0.45

Data are presented as mean ± SD, *$P < 0.001$, **$P < 0.05$.

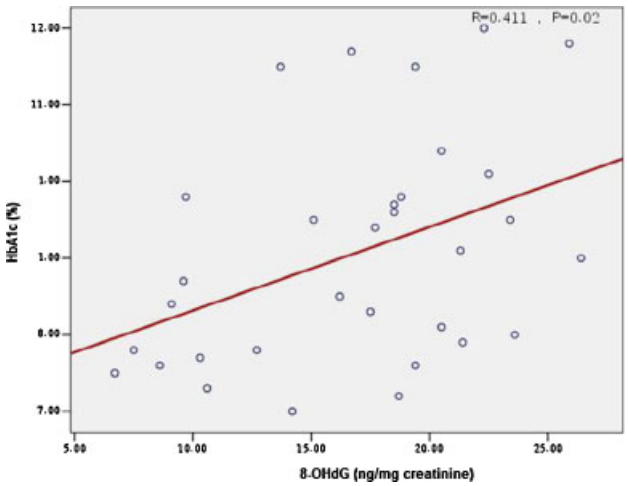

Fig. 1. Relationship between urinary 8-hydroxydeoxyguanosine (8-OHdG) concentration and glycated hemoglobin (HbA_{1c}) in diabetic patients ($n = 32$).

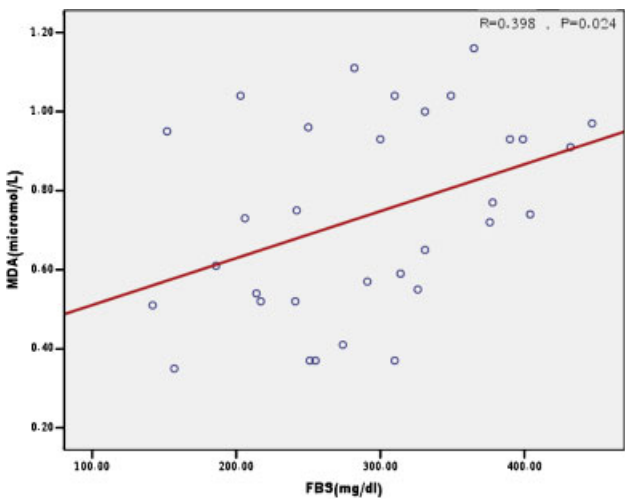

Fig. 2. Relationship between fasting blood glucose (FBS) and malondialdehyde (MDA) in diabetic patients ($n = 32$).

(even without complications) have elevated risk of mutagenesis.

Our results showed increased concentrations of HbA_{1c} in diabetic patients. Nowadays HbA_{1c} measurement is used as a preferred standard to evaluate glycemic control and an indicator to predict diabetes complications. Our chromatographic method for HbA_{1c} confirmed the results of Matteucci et al. study by Bio Rad Diamat automatic analyzer (22).

We found elevated concentrations of GSP in diabetic patients. As serum albumin turnover is slower than hemoglobin, the intensity of serum proteins glycation (especially albumin) is an indicator of glycemia in a period of few weeks. In the other hand, in the presence of blood abnormalities (such as hemolytic anemia), when HbA_{1c} measurement faces some errors, GSP measurement will be valuable. Earlier studies also established elevated concentrations of GSP in diabetic patients (23).

The results of the present research indicate higher concentration of plasma MDA in patients. The anticoagulant used during blood sampling, the type and strength of the acid used in the pretreatment procedure, and the duration of heating affect the amount of adduct produced. These facts, combined with differences in selectivity with the numerous analytical methods make the interpretation of previously reported plasma MDA results difficult. However, our finding of significantly increased plasma MDA in diabetics is supported by the findings of Griesmacher et al. and Ruiz et al. (24,25). We found a significant correlation between urinary 8-OHdG and HbA_{1c} in diabetic patients, suggesting a probable relationship between oxidative damage to DNA and AGE formation. This means that glycemic control in patients with Type 1 diabetes may reduce oxidative damage level to DNA. However, in some earlier studies, this kind of correlation has not been established (10). Therefore, we can conclude there are possibly other factors affecting DNA oxidation that make definite judgment tricky.

We did not find any correlation between fasting blood glucose concentration and HbA_{1c} in diabetic patients suggesting high variation of blood glucose level in our patients.

The obtained data indicated that blood glucose level was significantly correlated with plasma MDA. Therefore, hyperglycemia probably affects lipid peroxidation by free radical production in diabetic patients. Increased urinary OHdG in diabetic subjects that was found in this study supports the previous findings indicating DNA damage in these patients (5,10–11). Excess generation of mitochondrial reactive oxygen species (ROS) owing to hyperglycemia leads to activating stress-sensitive pathways such as NF-kB, p38, MAPK, and Jak/STAT, polyol and hexosamine pathway, PKC, and AGEs (26). Enhanced production of AGEs, sorbitol, and proinflammatory cytokines exert a positive feedback on ROS and reactive nitrogen species (RNS). Increase in ROS and RNS can potentiate LDL oxidation, lipid peroxidation, protein nitration, and DNA damage (26). Chemically OHdG is generated by either hydroxyl radical or singlet oxygen attach at C8 position of guanine (27). Malondialdehyd, lipid hydroperoxide and lipoperoxides are produced during oxidative damage to lipids and are known as lipid peroxidation indices in plasma (28). These events are known as factors causing diabetic vascular disease (26).

In conclusion, the obtained results support that oxidative stress in Type 1 diabetic patients can lead to damage to DNA, and diabetic complications may start

at early stages of disease. Therefore, urinary 8-OHdG can be a useful marker of oxidative stress assessment and glycemic control should be emphasized to prevent diabetic complications.

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