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Association of Uric Acid with Antioxidant Capacity of Plasma in Patients with Type 2 Diabetic Nephropathy

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Abstract

Background: The role of attenuation in antioxidant capacity or expansion of pro-oxidant/oxidant is well known for the development of stress oxidative. The aim of this study was to evaluate the plasma antioxidant capacity along with uric acid in patients with diabetic nephropathy (DN+) and without diabetic nephropathy (DN-). **Materials and Methods:** The research population included 88 patients with DN, 66 patients without DN and 54 healthy people who were matched for age, gender, and body mass index (BMI). In all groups, total antioxidant capacity of plasma by the ferric reducing antioxidant power (FRAP) assay and serum uric acid by commercial kit, respectively.

Results: The mean age of patients in DN+, DN- and control groups were 59.3± 9.4, 60 ±11.2, and 54.6±6.9 years, respectively. Plasma antioxidant capacity was higher in patients with DN+, (1589± 330µ mol/l Fe2+) and DN- (1344± 347 µ mol/l Fe2+) than that in healthy controls (1187 ± 271 µ mol/l Fe2+) ($P<0.001$). The mean plasma uric acid in patients with DN+ was significantly higher (8.7±1.3 mg/dl) compared with DN- (7.3±1.2 mg/dl) ($P<0.01$), and significantly lower in control group (4.1±1.4 mg/dl) ($P<0.001$).

Conclusions: According to our results, despite innate antioxidant activity of uric acid and increase of total antioxidant capacity and concentration of uric acid in diabetic patients with or without nephropathy, it can not compensates the severity of oxidative stress. Further studies are required to determine the value of other antioxidant factors in increasing the power of total antioxidant capacity.

Keywords: Diabetes Mellitus; Nephropathy; Antioxidant; Uric acid

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Introduction

The World Health Organization has reported that the total prevalence of diabetes has been estimated to increase, from 4% in 1995 to 5.4% in 2025 (1). One study in Iran showed that 7.7% of Iranian adults (n = 2 million) and 16.8% (n = 4.4 million) suffer from diabetes and impaired fasting glucose, respectively (2). Diabetes mellitus (DM) is characterized by increase of glucose in plasma caused by dysfunction or production of insulin. A chronic condition of diabetes leads to failure and malfunctions of various organs and is the cause of retinopathy, neuropathy andnephro-leads to failure and malfunctions of various organs and is the cause of retinopathy, neuropathy and nephropathy (3). Hyperglycemia is one of the factors that produce a status of constant and progressive vascular wall injury, evidenced by endothelial dysfunction (4).

Some researchers believe that in diabetes (type 1 and type 2), increased flow of glucose, and free fatty acids are linked with increased mitochondrial reactive oxygen species (ROS) production (5).

Hyperglycemic stimulates the production of ROS such as superoxide, hydrogen peroxide and hydroxyl radical during a number of enzymatic and

nonenzymatic pathways. These consist of glucose oxidative phosphorylation, the polyol pathway, advanced-glycation end products, outflow during mitochondrial respiratory chain, and NADPH oxidase activation (5). ROS can causes oxidation of lipids, DNA and proteins that lead to cell damage (6, 7). Oxidative stress is one of several mechanisms that have a role in pathogenesis of type 2 diabetes. It shows a state of imbalance between oxidants and antioxidant defense system.

On the other hand, attenuation in the endogenous ROS scavenging defense mechanisms may lead to unsuccessful scavenging of ROS, resulting in oxidative damage and tissue injury (8). Since pancreatic B-cells have little expression of antioxidant enzymes, they will be sensitive to cytotoxic effect of oxidative stress. This seems to clarify the progressive deterioration of B-cell function in type 2 diabetes (9).

However, it is still unclear whether in general oxidative stress or some associated factors that is an initiating or progressing origin or it is now the consequence of diabetic nephropathy in type 2 diabetes. There is a range of defense mechanisms to keep cells from oxidative damage. Scavenging of radicals, production of secondary radicals in following reactions and repair of tissue damage are inhibited by antioxidants.

Therefore, it would be better to assay the net antioxidant power in valuable test like FRAP and one of biochemical factors (uric acid) that has an important power in defense or promotion of oxidative stress.

The aim of this study was to identify antioxidant capacity of plasma and difference of uric acid level, in type 2 diabetes with and without nephropathy and comparing them with healthy controls.

Materials and methods

All patients had type 2 diabetes among whom there were 88 with nephropathy and 66 without nephropathy. The diagnosis of diabetic nephropathy was based on the rate of 30 to 300 mg of albumin per 24-hour urine or proteinuria which protein excretion was greater than 500 mg per day. Patients were excluded from the study if they had microscopic hematuria, urinary tract infection or history of non diabetes-related renal disease, such as obstructive uropathy and taking any medications that might affected serum uric acid concentrations (eg, uric acid–lowering agents or diuretics).

The control group consisted of 54 healthy volunteers matched for age, gender and body mass index (BMI).

Fasting blood samples were collected (5 ml) from participants by a venipuncture into vacutainers tube with heparin anticoagulant. The blood was centrifuged at 3000 rpm for 10 min, and then plasma was separated and stored at -80 °C until required for analysis.

Ferric-Reducing antioxidant power (FRAP) assay

This assay was performed using TPTZ reagent as described by Benzie and Strain (10). This method measures the ability of the antioxidants contained in the sample to reduce ferric-tripiridyltriazine (Fe3+-TPTZ) to a ferrous form (Fe2+) which absorbs light at 593 nm. In this reaction, the Fe3+ binds to a reagent, i.e., TPTZ that is then converted to Fe2+-TPTZ complex in presence of plasma antioxidant factors. The ferro and ferric-iron form complex with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against µM/l concentration of Fe (II) standard solution. 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl3, 6H2O solution were mixed for preparation of stock. FRAP reagent was prepared right away before analysis by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl3, 6H2O solution. 50 µl of plasma was mixed with 1.8 ml of the FRAP reagent and was incubated at 37 ºC for 30 min in the dark condition. Then readings of the colored products (ferrous tripyridyltriazine complex) were determined at 595 nm against distilled water blank. FeSO4-7H2O (100 - 1000 µM) was used for calibration. Results were expressed as µM Fe2+/ ml plasma.

Uric acid assay

Plasma uric acid concentrations were measured by enzymatic method (uricase-peroxidase) using commercially available kit (pars azmon).

	DN- (n= 66)	DN + (n=88)	Control (n=54)	p-Value
Female, n (%) Male, n (%)	47 (71.2) 19(28.8)	54 (61.4) 34(38.6)	23 (42.6) 31 (57.4)	0.255
Age (years)	60.1±11.2	59.3±9.4	54.6±6.9	0.63
BMI (Kg/m2)	30.7±6.3	30.1±7.5	30.5±5.4	0.59

DN-, diabetes patients without nephropathy
DN+, diabetes patients with nephropathy

Statistical Analysis

All analyses were performed with the SPSS version 17.0 (SPSS Inc, Chicago, IL, USA) and expressed as mean±SD. Unpaired Student t-test or ANOVA were conducted to assess statistical significance of differences between patients or control group. $P<0.05$was considered statistically significant.

Ethics Statement

The study was approved by the Mazandaran University

of Medical Sciences Ethics Committee.

Results

Demographic results are shown in table 1. No significant difference was found between patients and healthy subjects.

Plasma antioxidant capacity was considerably higher in patients with DN+ (1589 ± 330 µ mol/l Fe2+) and DN- (1344± 347 µ mol/l Fe2+) than in healthy controls (1187± 27.1µ mol/l Fe2+) (P<0.001). It alsoincreased by progression of diabetes (DN+ versus DN- , P<0.01) (Figure 1).

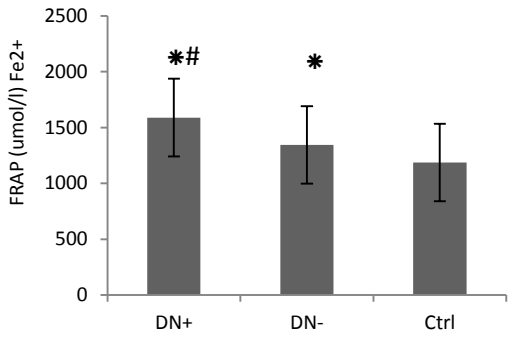

Figure 1. Plasma antioxidant capacity in patients with diabetes and control subjects. *P < 0.01, compared with control subjects; # P < 0.01, compared DN+ with DN-; DN-, diabetes patients without nephropathy; DN+, diabetes patients with nephropathy; Ctrl, control group.

As shown in Figure2. Plasma uric acid concentration averaged 19% higher in DN+ than in DN- . In other words uric acid level was significantly higher in patients with DN+ (8.7±1.4 mg/dl) compared with DN- (7.3±1.2), (P<0.01), but substantially lower in control group (4.1±1.3 mg/dl) (P<0.01).

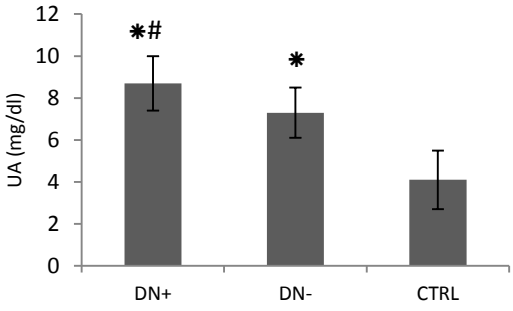

Figure 2. Plasma uric acid in patients with diabetes and control subjects. *P < 0.01, compared with control subjects; # P < 0.01 compared DN+ with DN-; DN-, diabetic patients without nephropathy; DN+, diabetic patients with nephropathy; CTRL, control group

Discussion

Oxidative stress, in spite of being plentiful in active pathological circumstances could have a consequential part in the pathogenesis of diabetes, atherosclerosis, anemia and malnutrition. It is a double aged blade which transpires in humans as a major part of host defense mechanism (11).

In accordance with the upshots of the researches, total antioxidant Ccapacity (TOC) which can be assessed by FRAP essay is proved to be of great benefit when it comes to comprehending the capability and conditions of the biological system in the oxidative stress. Reduction of ferric ions by uric acid (60%), protein (10%), bilirubin (5%), ascorbic acid (15%), α-tocopherol (5%) and others (5%) (10) are the central principles of FRAP assay.

Uric acid is most abundant of antioxidant in plasma (12) with a concentration to its solubility (120-450 µmol/L).Uric acid considered to be potent scavenger of free radicals. Recent researches corroborate the fact that among miscellaneous oxidant/antioxidant factors, uric acid is a strong predictor of impaired function in patients with Type 2 diabetes in spite of the fact that the antioxidant impact of UA exhibits that it may have therapeutic influences.

The fact of the matter is that uric acid has free extreme scavenging capability (13). Despite this issue, uric acid cannot be regarded adequate to safeguard diabetic patients against the enhanced oxidative stress by itself, since there are other combinations contributing to it. It is proven that the uric acid level is considerably higher in diabetic patients, especially in those with nephropathy.

Several mechanisms might be responsible for production of uric acid, by consequent procedures: 1) increased intake of foods that have purines, alcohol, and fructose, which create UA (14, 15); 2) impaired renal function and renal microvascular disorder, which can raises UA production by xanthine oxidoreductase. Oxidoreductase activity also contributes to macrophage foam cell formation and inflammation, and macrophage activation and/or decrease UA clearance (16, 17); 3) hyperinsulinemia, which raises renal UA reabsorption (18). It seem that an increase in UA concentration should be a protective mechanism to attenuate the adverse effects of oxidative stress but in diabetic patients this property of UA cannot compensate all the adverse events and probably causes impaired renal function. Within the serum, uric acid has dual function. It can react with a variety of oxidants and operate as an antioxidant. It has been suggested that uric acid acts as a peroxynitrite scavenger while it may also stimulate lipid peroxidation. (19, 20).

Furthermore, uric acid can stimulate oxidative stress (21, 22), thus causing preglomerular arteriolar damage. Instead, in these circumstances, when serum uric acid is transported into vascular smooth muscle cells, it could cause impaired NO production (23, 24),

inducing endothelial dysfunction and promoting the development of diabetic nephropathy (25). This damage can change glomerular haemodynamics and leads to chronic renal disorder. A recent study has indicated that the use of allopurinol, a xanthine oxidase inhibitor, in individuals with hyperuricemia was associated with slower progression of renal disease (26). Also, a study on the consequence use of allopurinol in type 2 diabetic patients with macro-albuminuria showed that lowering UA levels significantly decreased urinary proteinuria (27).

In conclusion, our results suggest that despite innate antioxidant activity of uric acid and increase of total antioxidant capacity and concentration of uric acid in diabetic patients with or without nephropathy, it cannot compensate the severity of oxidative stress alone. Therefore, it should be used alongside allopurinol, and known antioxidant drugs or nutriments to improve the severity of illness.

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Authors' contributions

ZM Study concept and design, supervised of the study, prepared the manuscript; AV laboratory test consultant and acquisition of data; M help in selecting and collecting of samples; MSH provided assistance for experiments; MS analysis and interpretation of data.

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Conflict of interest

There is no conflict of interest by authors.

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