

## Evaluation of Serum Uric Acid and Glutathione Levels in Diabetic Patients and Healthy Subjects

Mahmood Bagherion<sup>1\*</sup>, Durdi Qujeq<sup>2</sup>, Gholam Reza Bakhshi Khaniniki<sup>3</sup>

1- MS. Department of Biochemistry, College of Science, Payame Noor University, Mazandaran, Iran.

2- Department of Biochemistry and Biophysics, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.

3- Payame Nour University, Tehran, Iran.

### \*Correspondence:

Mahmood Bagherion, MS. Department of Biochemistry, College of Science, Payame Noor University, Mazandaran, Iran

**Email:** mahmood\_bagherion@yahoo.com

**Tel:** (98) 111 225 3468

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### Abstract

**Objective:** It is well known that diabetes is one of the main health challenges. According to the evidence presented in recent years by investigators, serum uric acid has emerged as a possible risk factor for diabetes mellitus. The aim of the present study was to compare the mean serum uric acid and the antioxidant activity of glutathione levels in patients with diabetes and the control group.

**Materials and Methods:** This cross-sectional study carried out on 30 diabetic patients (51.3 ±13.8 years, 15 males and 15 females) as well as 30 non-diabetic controls (54.2 ±13.4 years, 15 males and 15 females). The uric acid and glutathione levels were measured by Uricase-PAP method and glutathione the butler (DTNB) methods, respectively. Analysis was performed using SPSS version 20. Statistical significance was defined as  $P<0.05$ .

**Results:** Mean serum uric acid levels in diabetic patients (2.87±0.63 mg/dl) was lower than the control group (3.76±0.7 mg/dl). Also, mean total glutathione in diabetic group (181.64±38.7 μmol/l) was lower than the control group (273.6±39.28 μmol/l). Differences in uric acid and glutathione levels between the two groups were significant ( $P<0.05$ ).

**Conclusion:** This study indicated that serum uric acid and glutathione levels changed in diabetic patients compared to healthy control subjects. It may be related to the biochemical interaction between serum glucose and purine metabolism, with increased excretion of uric acid during hyperglycemia and glycosuria. The present study revealed that evaluation of the levels of biochemical factors in diabetic patients at different stages of the disease, can be helpful in their condition.

**Keywords:** Diabetes, Glutathione, Glucose, Uric acid

## Introduction

Previous reports have suggested that diabetes mellitus is a major worldwide health problem characterized by chronic hyperglycemia (1), peripheral vascular diseases (2), nephropathy, neuropathy and

retinopathy (3,4). Several experimental studies have investigated that excessive urine production with a compensatory thirst are main signs of diabetes mellitus (5). It was also noted that diabetes mellitus is characterized by

disturbances of carbohydrate, lipid and protein metabolism (6). It is important to note that changes in lifestyle over the recent years have resulted in a dramatic increase in the incidence of diabetes (7,8).

Uric acid is formed by the breakdown of purine nucleotides (9). Previous reports have suggested that high serum levels of uric acid are strongly associated with prevalent health conditions such as obesity and diabetes (10). Some investigators have suggested that there is an association between high serum uric acid and insulin resistance, but the mechanism that high serum uric acid is a risk factor for diabetes has long been a matter of debate. In fact, hyperuricemia was presumed to be a consequence of insulin resistance (11). However, a prospective follow-up study showed that high serum uric acid is associated with higher risk of diabetes (12). This relationship is such that the increased blood glucose inhibits uric acid reabsorption in the proximal tubule and results in altered uric acid levels.

It is well established that free radicals play an important role in the pathogenesis of many chronic diseases, including diabetes (13). Previous reports have suggested that in healthy subjects, antioxidant compounds counter the effects of free radicals (14). It is important to note that antioxidants are produced either endogenously or are derived from dietary sources (15). A growing of evidence suggested that diabetes is a metabolic disorder and is generally accompanied by increased levels of free radicals and decreased concentration or activity of antioxidants (16). It is well accepted that Glutathione (GSH), Tri peptide is an important antioxidant (17). Glutathione is an essential component of the cells. With low glutathione levels, cells cannot perform many of their functions properly. Although glutathione have dozens of roles in the metabolism, its major functions can be summarized in four areas: 1) It is the major antioxidant produced by the body; 2) Our immune systems depend on a steady supply of glutathione; 3) It is important in detoxifying

many substances, including heavy metals, breaking down products of cigarettes and automobile exhaust, many cancerogen agents, and a multitude of pollutants and toxins we encounter on a daily basis; and 4) The major source of energy produced in our cells is derived from tiny structures called mitochondria.

It is well documented that diabetic individuals have an increased level of oxidative stress and free radical formation in their tissues. By the same token, their blood and tissues are marked by critically low glutathione levels. A reasonable assumption is that the increased oxidative stress depletes the tissues of glutathione, the latter being the major intracellular antioxidant responsible for neutralizing the free radicals. There is good evidence that a weakened glutathione antioxidant system is responsible, at least in part, for the observed cardiovascular disease seen in diabetics and a role for supplementation with antioxidants has been proposed.

The aim of this study was to compare the levels of uric acid and glutathione in diabetic patients and healthy controls.

## Materials and Methods

In this cross-sectional study 60 subjects (age range 30-80 years; 30 diabetic patients and 30 healthy subjects) were selected by random sampling methods. Exclusion criteria included use of drugs affecting the lipid biochemical markers, chronic liver or kidney disorders, smoking, and insulin injection. Informed consent was obtained from each participant.

## Sample collection

Sample preparation was carried out as quickly as possible. After overnight fasting, 10 ml of peripheral blood was drawn. Blood samples were collected in two types of tubes: the first tube for serum separation and the second tube containing EDTA for plasma separation. Plasma samples were separated from cells by centrifugation at 3000 rpm for 10 min, and the remaining blood was washed three times with

9 g/L NaCl solution. Cell membranes were removed by centrifugation at 1200 rpm for 5 min at 4°C. The hemolysates were then used to determine glutathione levels. Serum was separated by centrifugation with coagulated blood at 1000 rpm for 10 min at 4°C. Body mass index (BMI) was defined as weight (kg) divided by height squared (m<sup>2</sup>).

### Assay for Biochemical Parameters

The measurements of biochemical parameters including fasting blood sugar (FBS), total cholesterol (Total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein (VLDL-C), triglycerides (TG), uric acid and glutathione were done by spectrophotometric methods.

Fasting plasma glucose was measured using glucose oxidase by a Pars Azmoon kit (Pars Azmoon Co., Tehran, Iran) and serum uric acid levels were assessed by Uricase (URICASE/PAP) using a UV detector at a wavelength of 640 nm. Glutathione levels were also determined using dinitrobenzoic acid (DTNB) as the dye at a wavelength of 412 nm.

### Statistical Analysis

The data were assessed for statistical significance using student t-test statistics. Results were expressed as mean  $\pm$  standard error. Paired sample t-test was performed by SPSS software (SPSS for windows, version 19). A *P*-value  $< 0.05$  was used as the criterion for a statistically significant difference.

### Results

Sixty subjects including 30 patients with diabetes (15 men, 15 women) and 30 healthy subjects (15 men, 15 women) participated in this study. Baseline demographic and clinical characteristics of the population are shown in Table 1. There were no significant differences with regard to age, weight, and BMI between diabetic patients and healthy controls.

Levels of glucose, triglycerides and VLDL-C in diabetic patients were higher than controls.

**Table 1. Demographic characteristic of diabetic patients and healthy subjects.**

Variable	Controls	Diabetics	<i>P</i> -value
Age (year)	13.4 $\pm$ 54.2	13.08 $\pm$ 51.3	0.88
Weight (kg)	12.3 $\pm$ 73.8	13.6 $\pm$ 76.4	0.09
BMI (kg/m <sup>2</sup> )	4.8 $\pm$ 27.2	8.3 $\pm$ 27.2	0.98

Data are expressed as Mean  $\pm$  SD.

However, there was no difference between diabetic patients and healthy subjects for Total-C, HDL-C and LDL-C. Laboratory characteristics of diabetic patients and normal subjects are presented in Table 2. As shown in Table 3, significant lower levels of uric acid and glutathione were found in diabetic patients compared to healthy individuals.

### Discussion

According to our results, this study suggests that serum uric acid and glutathione levels are altered in diabetic patients compared to healthy control subjects. Also, the mean level of uric acid was lower in diabetic patients compared with healthy control subjects. This association may be related to the biochemical interaction between serum glucose and purine metabolism (17). A plausible mechanism for the observed results of an inverse association between increasing serum uric acid and diabetes mellitus may be related to the inhibition of uric acid reabsorption in the proximal tubule by high glucose levels in diabetic individuals. Many investigators have reported that there is a positive association

**Table 2. The mean and standard deviation of the laboratory variables in diabetic patients and control subjects.**

Variable	Controls	Diabetics	<i>P</i> -value
FBS (mg/dl)	89.36 $\pm$ 12.01	187.16 $\pm$ 49.51	$< 0.01$
TG (mg/dl)	208 $\pm$ 18.3	273 $\pm$ 39.14	$< 0.05$
Total-C (mg/dl)	214.3 $\pm$ 14.4	214.7 $\pm$ 31.5	NS
VLDL-C (mg/dl)	35.1 $\pm$ 3.7	58.6 $\pm$ 8.4	$< 0.05$
LDL-C (mg/dl)	121 $\pm$ 13.9	146 $\pm$ 11.8	NS
HDL-C (mg/dl)	33.2 $\pm$ 2.6	38.9 $\pm$ 5.9	NS

Data are expressed as Mean  $\pm$  SD.

**Table 3. The mean and standard deviations of variables uric acid and glutathione level in diabetic patients and control groups**

Variable	Controls	Diabetics	<i>P</i> -value
UA (mg/dl)	3.76 $\pm$ 0.7	2.87 $\pm$ 0.63	$< 0.05$
GSH ( $\mu$ mol/l)	181.64 $\pm$ 38.7	273.6 $\pm$ 39.28	$< 0.05$

Data are expressed as Mean  $\pm$  SD.

between elevated serum uric acid levels and diabetes (18,19), whereas some other studies have not reported such an association (20). Also, some studies reported that serum uric acid is inversely associated with diabetes mellitus (21,22). The exact reason for why previous studies found a positive relation between uric acid and diabetes is not clear. Most of these studies were limited by small sample sizes. However, extensive experimental studies are required in large number of samples to establish relation between uric acid and diabetes.

Recent evidence has highlighted that the reactive oxygen species (ROS) are formed under normal physiological conditions (23). Many investigators reported that the generation of reactive ROS is increased in diabetes (24). It has previously been reported that free radicals are formed in diabetes (25). It is well known that abnormally high levels of free radicals can lead to the damage of cellular organelles in diabetes mellitus (26). It should be noted that the increase in blood glucose

level and decreased insulin level depends upon the degree of  $\beta$ -cell destruction (27). Increased level of glycosylated hemoglobin has been observed in the diabetic patients (28).

In our study, a significant decrease of GSH was observed in the plasma of diabetic patients. Glutathione, is an important antioxidant (29). Reduced glutathione normally plays the role of an intracellular radical scavenger (30). A marked decreased plasma level of reduced glutathione is reported in diabetic patients. The results of our study are in agreement with other studies (31-34). GSH systems may have the ability to manage oxidative stress with adaptational changes in enzymes regulating GSH metabolism.

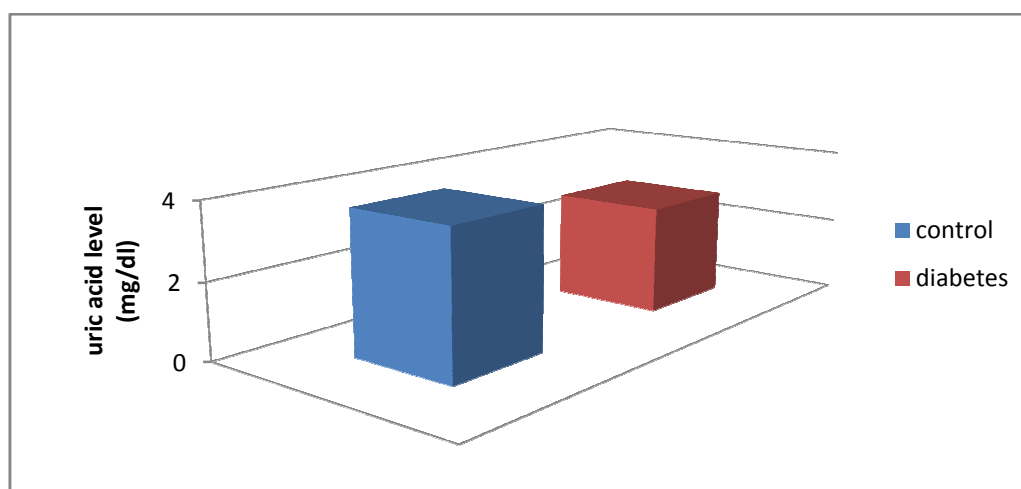
According to the results of this study, the mean glutathione was lower in diabetic patients compared with healthy subjects. Previously, investigators reported that in diabetic groups, concentrations of GSH were lower compared with healthy controls (35). There are evidences showing, a negative relationship between fasting blood glucose and

**Table 4. Mean serum uric acid and glutathione level in study groups with respect to age and sex.**

Age group	Controls				Diabetes			
	Male		Female		Male		Female	
	UA	GSH	UA	GSH	UA	GSH	UA	GSH
31 – 40	3.76	286	3.65	317.3	3.5	209.6	3.99	212.1
41 – 50	4.18	286.5	4.27	305	3.05	174.1	2.02	167.3
51 – 60	4.4	271	3.19	269	3.3	175.5	2.66	216
61 – 70	3.48	228	3.65	229.3	2.39	129.6	2.6	172
71 – 80	3.66	264	4.43	241	3.55	243	2.22	152

UA: Uric Acid, GSH: Glutathione

UA and GSH are presented as mg/dl and  $\mu$ mol/l, respectively.



**Figure 1. Levels of serum uric acid in diabetics and controls**

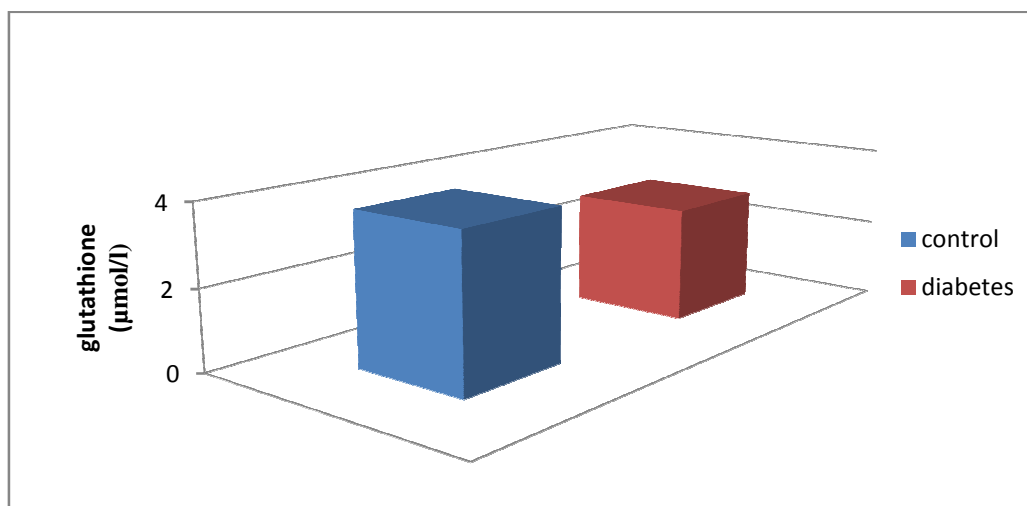


Figure 2. Levels of total glutathione in diabetics and controls.

serum levels of GSH in diabetic patients (36).

### Conclusion

Our present findings, taken together with previous results, indicated that the serum uric acid level was lower in diabetics than the controls. The uric acid may serve as a potential biomarker of deterioration of glucose metabolism. On the other hand, the glutathione level was lower in diabetes mellitus than

controls. Oxidative stress plays an important role during diabetes. Glutathione that work as an inhibitor in the destructive effects of oxidation is needed for improving or stopping the development of diabetes.

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