The Importance of Uric Acid and Catalase as antioxidants in Diabetic Subjects

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ABSTRACT

This study was designed to show the hazards of hyperglycemia on antioxidants level in diabetic subjects (type 2) and put lights on the importance of uric acid as scavenging compound in diabetic subjects.

The study was conducted at the Department of Physiology, College of Medicine in Al Mustansyria University. A total of 30 healthy individuals were enrolled as a control group in this study with 33 diabetic subjects of type 2 DM.

Uric acid and catalase enzymes were measured in both groups. The level of catalase was significantly lower in diabetic subjects than in control $p < 0.01$. Also the uric acid was significantly lower in diabetic subjects than the controls $P < 0.05$.

It has been regarded that hyperglycemia is the principle factor in reducing the level of uric acid and catalase enzyme in the plasma of our diabetic subjects which both are regarded as part of defense mechanism against the increase in oxidative stress associated with hyperglycemia.

Key words: Uric acid, catalase, hyperglycemia, & antioxidant

INTRODUCTION

Hyperglycemia, the primary laboratory manifestation of Diabetes Mellitus has been accepted as being essential for the development of diabetic complications. Many evidences have indicated that the same biochemical pathway strictly associated with hyperglycemia (Non-enzymatic glycosylation, glucose autoxidation and polyol pathways) can increase the production of free radicals. (1)

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Antioxidant defense system appears to be compromised in diabetic subjects. It has been demonstrated that scavenging of free radicals can be reduced by Super Oxide Radicals and lack of Glutathaion and ascorbic acid, which are associated with diabetes. (2) Reduction of other antioxidant, such as vitamin E, uric acid and decreased activity of catalase are also found in

diabetes.[3] The mechanism by which the antioxidant reservoir is reduced is not clear.

Protein damage due to the protein/glycoprotien disruption may be mechanically in origin, the process that lowers the activity of primary antioxidant enzymes. [4]

Oxidative stress in biological system results from an imbalance between the production and removal of reactive oxygen species. Such imbalance leads to increase in reactive intermediate formation in the body, which in turn leads to cellular damage. [5]

Beta cells are prone to be destroyed by free radicals because of the low antioxidant enzyme.[6] There are two proposed mechanisms by which immune cell could damage the beta cells of the pancreas: [7]

1. Infiltration of microphages produces superoxide as primary source of free radicals. The superoxide can be further converted to more active radical, Hydroxyl radicals, which attack cellular membrane and cause DNA breakage.[8] The consequences of the DNA breakage leads to cell death as the cells failed to repair the damage; also the activation of DNA repair enzyme, especially the activation of poly (ADP-ribose) synthetase, deplete the DNA levels in cells, inhibiting proinsulin synthesis and increasing the sensitivity of free radicals. [9]

2. Cytokines are released by T cells, and microphages, in the insulin secreting cells and induce the formation of inter cellular free radicals causing selective damage to Beta cells. [10]

	Blank (ml)	Test (ml)
Phosphate buffer	1.0	-
Diluted Hemolysate	2.0	2.0
Hydrogen Peroxide	-	1.0

Uric acid is the end product of Purines metabolism,[11] and it is regarded as free radicals scavenger.[12] Uric acid (UA) is produced by the oxidation of hypoxanthine and xanthine by xanthine oxidase and dehydrogenase enzymes.[13]

However, under selected circumstances, UA at physiological pH is able to stimulate the oxidation of LDL triggered by metals. [14] In addition, when the human plasma is exposed to NO^{-3} (nitrate), UA seems to be a major protective antioxidant.[15] Catalase enzyme is one of the hydroperoxidase enzymes. It is hemoprotein containing 4 heme groups.

It is found in the blood, bone marrow, mucus membrane, kidney and liver.[16] It is also found in peroxisomes in most tissues [17], its function is assumed to be the

destruction of H_2O_2 by the following reactions:

$$2H_2O_2 \xrightarrow{CAT} 2H_2O+O_2$$

$$2H_2O+SH_2 \xrightarrow{CAT} 2H_2O+S$$

- *Were (S) is anyone of a number of hydrogen, donating substrates.*

Catalase in erythrocytes may help to protect them against H_2O_2 generated by dismutation of O_2 generated by hemoglobin auto-oxidation. Since H_2O_2 diffuses rapidly, erythrocytes can also protect other tissues against oxidative damage by absorbing H_2O_2. [18]

Catalase is a potent enzyme; it has two enzymatic functions depending on the concentration of H_2O_2 available. If the steady-state concentration of H_2O_2 in the system is high the enzyme acts catalytically e.g. remove H_2O_2 to form H_2O and O_2.

However, at low concentration of H_2O_2 and in the presence of suitable hydrogen donor (e.g., Methanol, Ethanol) catalase acts proximally, it removes H_2O_2 and oxidizes its substrate. Catalase present in the peroxisomes of aerobic cells, serves to protect the cell from the toxic effects of H_2O_2. Although it is clear that all catalases share a general structure, some differ in the number and identity of domains. [19] The aim of the study is to measure the level of the free radicals in healthy individuals and diabetic subjects indire scavenger's defense system, and to study the importance of uric acid and catalase enzyme in diabetic subject.

MATERIALS & METHODS

The study was conducted at the Department of Physiology, College of Medicine in Al-Mustansiriya University.

A total of 30 healthy subjects were taken as a control group and their age was ranging from 25 to 60 years (mean $\pm$SD = 46.4 $\pm$6) all were normo-glycemic (FBG, mean $\pm$SD = 97.6 $\pm$9.37 mg/dl), and OGTT was performed for all according to WHO criteria for the diagnosis and classification of Diabetes Mellitus 1997.

33 diabetic patients with type II DM were enrolled in this study. The age of those patients was ranging from 38 to 65 years (mean $\pm$SD = 50.3 $\pm$6.3) with their mean value of fasting plasma glucose (FPG, mean $\pm$SD=167.36 $\pm$33.mg/dl), and duration of the disease is from 2-10 years.

Measurements:

- The reagents for the measurement of catalase were:

1. 50 mM (pH=7.0) Phosphate buffer solution.

2. 30 mM H_2O_2: prepared by diluting 0.34 ml of 30% H_2O_2 with phosphate buffer solution to 100ml volume.

ϖ The Procedure was as follows:

- Catalase activity was measured by combining (0.4 ml) of packed red cell with (1.6 ml) of H_2O, freeze and thawed three times to form hemolysate.
- Hemolysate (0.1ml) was mixed with (0.1ml) H_2O then (20µL) of this added to (10 ml) of 50 mM phosphate buffer (pH=7.0). After rapid mixing the reaction rate (Decomposition of H_2O_2) was determined from absorbance change at 240 nm after 15 and 30 seconds.

ϖ The Catalase activity was calculated as follows:

$$K = 0.I53 \log \frac{\text{Abs. at 15 sec.}}{\text{Abs. at 30 sec.}}$$

$$\text{Kgm Hb} = \frac{K}{\frac{\text{Xg Hb}}{\text{ml}} \times \frac{0.1}{0.2} \times \frac{0.02\text{ml}}{10} \times 2}$$

- *Where K is the first order constant.*

ϖ Determination of Uric Acid in Serum: Uric acid was assayed by using of enzymatic colormetric Kit from Biomagreb, and it was oxidized by uricase to allantoine and hydrogen peroxide, according to th equations:

Uric acid + O_2 + H_20 → Allantoine + $C0_2$ + H_2O_2 → $2H_2O_2$ + 4-Amenophenazone peroxidase + 2, 4 Dichloro-phenol-sulfate → Quinoneimine + $4H_20$

ϖ The reagents used were:

Reagent (R1)	Phosphate buffer PH=7.4	50 mml/L
Reagent (R2)	Uricase	70u/L
	Peroxidase	660 u/L
	4-Amino phenazone	1 m mol/L
Reagent (R3)	Uric acid (standard)	6 mg/dL

Dissolve the content of one bottle R2 with (30 mL) of R1; this working reagent is stable for 3 weeks at 2-8°c, 7 days at 20-25°c.

ϖ Procedure:

	Blank	Standard	Sample
Working reagent	1ml	1ml	1ml
Standard	-	20µl	-
Sample	-	-	20µl

•Mix, incubate 5min. at 37°c or 10min, at 20-25°c, the color is stable for 30 min.

•Read the absorbance at 510 nm wavelength.

ϖ Calculation:

$$\text{Uric acid concentration} = \frac{\text{Abs. Sample}}{\text{Abs. Sample}} \times n$$

- Where n=6mg/dl

The statistical Analysis: The results were presented as mean ± standard deviation (SD) and percentage whenever possible.

Statistical analysis were performed using both student's t-test and correlation test taking $P<0.05$ as the lowest limit of significance. Analysis was performed using Microsoft Excel Package (2003).

RESULTS

Our study has showed that the level of CAT was (5.71 +_ 0.45 K/gm Hb) in the control group while the level was significantly lower in diabetic patients than control (3.98_+ 0.41 k/gm Hb) ($P<0.01$). Also it was found that the level of UA was 4.76±0.36 mg/dl in control group which is significantly higher than diabetic patients (3.93±0.31 mg/dl, $P<0.05$).

DISCUSSION

μ Catalase enzyme activity:

The level of the CAT was found to be significantly lower in diabetic subjects than control group ($P< 0.01$). Our results are in harmony with many recent studies.[20, 21 & 22] As CAT controls the level of H_2O_2 and preventing it from reaching toxic level that could induce oxidative damage at cellular sites.[23] The low levels of CAT found in our study is attr

responsible for the production of high levels of H_2O_2 leading to higher consumption of CAT.

Our way of analyzing the data is identical with what mentioned by other authors,[18] also Watanab [24] had attributed the reduction of CAT to the higher levels of H_2O_2 produced by poly-morphonucler leukocytes in type II DM.

μ Serum uric acid:

The level of uric acid was found to be significantly lower in diabetic subjects than controls ($P<0.05$). We think that hyperglycemia can induce this reduction in the uric acid level due to its osmotic diuresies mechanism.

Same results were reported by Erderberg *et al,* [25] they think that hyperglycemia can induce this reduction in the UA level due to its osmotic diuresies mechanisms, while Gonzales-Scililia *et al*[26] had stated that hyperfilteration in renal glomerului contributes to hyperurocoseuria in diabetic subjects, also we can add that the reduction in uric acid level may be due to increased oxidative stress in diabetes which will consume a large amount of uric acid, and in this concept we are in harmony with Sinclair *et al,* [3] who regarded the principle mechanism for reducing UA in diabetes is due to hyperglycemia which cause a

reduction of the antioxidant system and increase in oxidative stress.

Diabetic subjects have a lower antioxidant level (Catalase and UA) as compared with control subjects and hyperglycemia play the main role in this difference.

We emphasis that UA can play an important role as free radical scavenging compound that is important in reducing the hazardous effect of hyperglycemia in DM. So the physicians should be aware about this role and try to keep it within the normal limit and not to be in rash to lower its level to the minimal lower value, whenever its value reaches the upper normal limits.

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