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Original Article

# Comparative analysis of plasma total antioxidant capacity in patients with hyperglycemia and hyperglycemia plus dyslipidemia

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ABSTRACT

*Aims:* The aim of this study was to measure plasma total antioxidant capacity (TAC) level and superoxide dismutase (SOD) activity in order to assess the oxidative stress status and the antioxidant defense system in patients with hyperglycemia and both hyperglycemia and dyslipidemia.
*Materials and methods:* Sixty blood samples of hyperglycemia, 60 blood samples of both hyperglycemia and dyslipidemia and 60 blood samples of normoglycemia and normolipidemia (controls) were collected into study. All samples were measured for the levels of plasma TAC and SOD by colorimetric method using microtiter-plate reader.
*Results:* Plasma TAC significantly decreased in patients with hyperglycemia ($0.42 \pm 0.1$ mM) and both hyperglycemia and dyslipidemia $0.41 \pm 0.1$ mM) compared to those of controls ($0.47 \pm 0.14$) ($P < 0.05$), whereas plasma SOD significantly increased in patients with hyperglycemia ($81.0 \pm 17.9$ U/ml) and both hyperglycemia and dyslipidemia ($83.7 \pm 21.3$ U/ml) compared to those of controls ($73.7 \pm 17.4$ U/ml) ($P < 0.05$). However, the levels of plasma TAC and SOD had no significant difference between patients with hyperglycemia and both hyperglycemia and dyslipidemia ($P > 0.05$).
*Conclusions:* The present study showed the significant difference of plasma TAC and SOD levels in hyperglycemic patients with and without dyslipidemia compared to those of controls. There was no additive or synergistic effect in terms of decreased plasma TAC levels and elevated SOD activities between hyperglycemic patients with and without dyslipidemia.


## 1. Introduction

Hyperglycemia and dyslipidemia are groups of metabolic disorders characterized by elevated levels of plasma glucose [1] and by the lipid profiles of elevated levels of plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) including reduced high-density lipoprotein cholesterol (HDL-C) [2]. Diabetic hyperglycemia can lead to many complications including diabetic retinopathy, diabetic neuropathy, diabetic nephropathy and diabetic cardiovascular disease, whereas hypercholesterolemia can cause atherosclerosis [3]. High levels of glucose and/or cholesterol in blood may damage the cells through oxidative stress [4].

Studies have shown that hyperglycemia promotes the overproduction of reactive oxygen species (ROS) and induces oxidative stress causing the pathogenesis of diabetic complications [5]. However, oxidative stress can also decrease insulin sensitivity and damage the β-cells of pancreas resulting in the onset of diabetes mellitus [6]. The different cell types in hyperglycemia can also increase ROS production [6]. There are many evidences to show the correlation between diabetes and oxidative stress by measuring biomarkers [3]. Oxidative stress and activation of polyol pathway can be increased by hyperglycemia, which can provide inflammation and renal damage [7]. In addition to hyperglycemia, there have been reports that hypercholesterolemia may raise the risk for atherosclerosis [8]. Atherosclerosis involves in inflammatory and oxidative stress process on the arterial wall [9]. Oxidative stress and inflammation are interrelated because oxidative stress can cause inflammation; on the other hand inflammation can induce oxidative stress leading to cell injury [10]. Studies indicate that high levels of glucose and cholesterol lead to atherosclerotic vascular damage in patients with pathologic condition of insulin resistant [11]. The oxidation of low density lipoprotein (LDL) plays a vital role in atherosclerosis. High levels of oxidized LDL may damage blood

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vessel and lead to produce foam cells and plaque resulting in atherosclerosis [12].

Antioxidant defense mechanisms consist of both enzymatic and non-enzymatic systems which show working as a team to prevent and attenuate the harmful effects of oxidative stress [13]. The measurement of each antioxidant is complex and very time-consuming as well as high-cost experiments. For this reason, the measurement of total antioxidant capacity (TAC) in serum or plasma can provide information of antioxidant status in patients [14]. Studies on antioxidant systems, the enzymatic antioxidant system plays a major role in controlling ROS levels [15]. SOD is the first line of defense superoxide radicals. SOD catalyzes the conversion of superoxide radical to hydrogen peroxide which is then reduced to water by catalase and glutathione peroxidase [16].

In this work, we choose one of the antioxidant enzymes on the basis of data in scientific literature to evaluate the enzymatic antioxidant system. There are few studies to compare antioxidant status between patients with hyperglycemia and both hyperglycemia and dyslipidemia. In our study, we measured plasma TAC level and SOD activity to assess the oxidative stress status and the antioxidant defense system in patients with hyperglycemia and both hyperglycemia and dyslipidemia.

## 2. Materials and methods

### 2.1. Subjected and study design

A random sample of sixty patients (27 men, 33 women) with hyperglycemia (glucose > 120 mg/dL, HbA1c > 6%), average age 62.1 ± 10.5 years and normal lipid profiles (TC < 200 mg/dL, LDL-C < 140 mg/dL, TG < 150 mg/dL), and sixty patients (28 men, 32 women) with both hyperglycemia and dyslipidemia (glucose > 120 mg/dL, HbA1c > 6%, TC > 200 mg/dL, LDL-C >140 mg/dL, TG > 140 mg/dL), average age 59.8 ± 10.4 years, were collected for plasma measurements of TAC and SOD.

Samples of control consisted of 28 men and 32 women, average age 63.7 ± 9.6 years, showed normal blood glucose (glucose < 110 mg/dL)and lipid profiles (TC < 200 mg/dL, LDL-C < 140 mg/dL, TG < 150 mg/dL). The sample with upper limit of creatinine and bilirubin were excluded from the study.

### 2.2. Specimens

Blood samples were drawn from an antecubital vein after overnight fasting and collected into Li-heparin tubes. The plasma was separated from whole blood by centrifuging blood samples at 3000 xg for 20 min at 4 °C. The plasma was then collected and stored at −80 °C until analysis. Samples with hemolysis were excluded. This study was approved by Ethics Committee of Research Institute of Rangsit University.

### 2.3. Plasma TAC analysis

Total antioxidant capacity in plasma was determined using antioxidant assay kit (Sigma-Aldrich). The assay is based on the inhibition of the oxidation of ABTS (2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to a radical cation, $ABTS^{+}$. The antioxidants in the sample prevent $ABTS^{+}$ formation and the color intensity decreases proportionally depending on the amount of antioxidants. The absorbance was read at 405 nm using a microtiter-plate reader, EZ Read 2000 (Biochrom). All samples were assayed in duplicate following manufacturer's instruction manual. Plasma TAC value is quantified as Trolox equivalent antioxidant capacity and expressed in the millimolar (mM) concentration.

### 2.4. Antioxidant enzyme assay

SOD was selected as the parameter for assessment of the enzymatic antioxidant system. SOD in plasma was measured using SOD determination kit (Sigma-Aldrich). The reaction based on the inhibition of the production of a water-soluble formazan dye which is produced by WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, monosodium salt) and superoxide anion. The rate of inhibition was proportional to the concentration of SOD. The absorbance was read at 450 nm using a microtiter-plate reader, EZ Read 2000 (Biochrom). All samples were assayed in duplicate following manufacturer's instruction manual. 1 U of SOD activity is defined as the amount of SOD required to inhibit the reduction of a water-soluble formazan dye by 50% under the assay conditions.

### 2.5. Biochemical analysis

Plasma glucose, TC, LDL-C, HDL-C and TG levels were assayed by colorimetric method using commercially available kits (Roche Diagnostics, Switzerland). Glycated hemoglobin (HbA1c) was determined on the basis of the turbidimetric inhibition immunoassay for hemolyzed whole blood using kit and assayed as described by the supplier (Roche Diagnostics). All tests were done on Cobas C 501 analyzer (Roche Diagnostics, Switzerland).

### 2.6. Statistical analysis

Statistical analysis of the data was performed using Microsoft Excel version 2010 software (Microsoft Corporation, Redmond, WA, USA). All variables were expressed as mean ± SD (standard deviation). Differences between the three groups were compared for significant by Student's unpaired two-tailed *t*-test. All statistical tests were 2-sided at the 5% significant level.

## 3. Results

The characteristics of the study groups are shown in Table 1. No differences were observed regarding age, sex distribution in between groups. Whereas, glucose and HbA1c levels in patients with hyperglycemia and both hyperglycemia and dyslipidemia were significantly elevated compared to those of controls ($P < 0.001$). There have been significant increases in TC, LDL-C and TG and decrease in HDL levels in patients with both hyperglycemia and dyslipidemia in comparison to controls and hyperglycemic patients without dyslipidemia ($P < 0.001$). However, there were no significant differences in TC, LDL-C, HDL-C and TG levels between controls and hyperglycemic patients without dyslipidemia ($P > 0.05$).

Level of plasma TAC was found to be decreased in patients with hyperglycemia (0.42 ± 0.1 mM) and both hyperglycemia and dyslipidemia (0.41 ± 0.1 mM) compared to those of controls (0.47 ± 0.14 mM) (Table 2). As shown in Fig. 1, the analysis of TAC levels in the plasma revealed significant differences in hyperglycemic patients with and without dyslipidemia as compared to those of controls ($P = 0.023$ and 0.043 respectively). However, there was no significant difference in plasma TAC levels between hyperglycemic and both hyperglycemic and dyslipidemic groups ($P = 0.74$). SOD levels in the plasma of patients with hyperglycemia (81.0 ± 17.0 U/ml) and patients with both hyperglycemia and dyslipidemia (83.7 ± 21.3 U/ml) were significantly higher than those of controls (73.7 ± 17.4 U/ml) ($P = 0.21$ and 0.005 respectively) but there was no significant difference between hyperglycemic and both hyperglycemic and dyslipidemic groups ($P = 0.45$) (Fig. 2).