

Lipid Peroxidation and Antioxidant Enzyme Activities in Erythrocytes of Type 2 Diabetic Patients

Atip Likidlilid MSc*, Natchai Patchanans MSc*,
Thavatchai Peerapatdit MD**, Charn Sriratanasathavorn MD***

* Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

** Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

*** Her Majesty's Cardiac Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background and Objective: Although diabetes mellitus (DM) patients are claimed to be under oxidative stress because of prolonged exposure to hyperglycemia, the influence of glycemic control and cardiovascular complication in diabetes on oxidative stress parameters has not been fully studied. The present study aimed to investigate lipid peroxidation end product (malondialdehyde, MDA) and antioxidant enzymes in fairly controlled type 2 DM (fasting plasma glucose [FPG] \leq 180 mg/dl) or type 2 DM complicated with coronary heart disease (CHD) and poorly controlled type 2 DM (FPG > 180 mg/dl) in comparison to a normal healthy group (FPG < 110 mg/dl).

Material and Method: MDA and antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were determined in the red cell of 19 subjects with poorly controlled type 2 DM, 26 subjects with fairly controlled type 2 DM and 20 subjects with type 2 DM complicated with CHD who were matched for age and gender. Twenty healthy subjects with normal plasma glucose level and matched for age and gender were served as a control group. In all groups of DM these oxidative stress parameters were compared to a control group by one-way ANOVA test. Pearson rank correlation coefficient was used to compare the relationship between FPG and oxidative stress status in type 2 DM and normal controls.

Results: The red cell MDA levels were significantly higher in all types of diabetes compared to age-matched normal controls. The mean of red cell MDA level was highest in type 2 DM complicated with CHD. Red cell antioxidant enzyme activities were also significantly increased except for SOD and GPx activities in fairly controlled type 2 DM. The significant positive correlation between oxidative stress status (as MDA and CAT) and FPG was found in poorly controlled type 2 DM and type 2 DM complicated with CHD whereas in fairly controlled type 2 DM the significant positive correlation between CAT and FPG was only observed.

Conclusion: These findings strongly confirmed the evidence that diabetic patients were susceptible to oxidative stress and higher blood glucose level had an association with free radical-mediated lipid peroxidation. The highest level of MDA in type 2 DM complicated with CHD suggested that oxidative stress played an important role in the pathogenesis of cardiovascular complication. The results also showed the increase in antioxidant enzymes. These could probably be due to adaptive response to pro-oxidant in diabetic state. Hence, there seems to be imbalance between oxidant and antioxidant systems in type 2 diabetic patients.

Keywords: Antioxidant, Oxidative stress, Lipid peroxidation, Coronary heart disease, Type 2 diabetes

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Diabetes mellitus (DM) is a severe metabolic disease characterized by hyperglycemia and other metabolic disturbances resulted from impaired function in carbohydrate, lipid and protein metabolism⁽¹⁾. It is the most common endocrine disorder and represents one of the most serious clinical and public health

Correspondence to: Peerapatdit T, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. E-mail: sitpp@mahidol.ac.th

problem worldwide. Type 2 diabetes mellitus is caused by defective production of insulin in pancreatic β -cell in response to glucose metabolism and by dyslipidemia in the action of insulin on its target tissues (insulin resistance). The prevalence of DM, especially type 2 DM, has rapidly increased in industrialized and many developing countries including Thailand. It was estimated that DM will affect approximately 366 million people by 2030. This figure is expected to increase

from 4.1% in 2000 to 6.0% in 2030 in developing countries⁽²⁾.

The microvascular and macrovascular complications are the major cause of morbidity and mortality in patients with DM. However, diabetic patients often die from macrovascular disease and major mortality is the coronary heart disease (CHD)⁽³⁾. There is a correlation between chronic hyperglycemia and long-term complications in diabetes. It is possible that long term, high blood glucose levels in uncontrolled DM is the cause of glucose autooxidation, non-enzymatic protein glycation and activation of polyol pathway with increase oxidative stress^(4,5). Oxidative stress is the result of the imbalance in pro-oxidant/antioxidant ratio in favor of the former, potentially leading to macromolecules and cell dysfunction^(6,7). Enhanced oxidative stress contributes to the deterioration of pancreatic β -cell progressively due to glucose toxicity, which leads to severe impairment of glucose-stimulated insulin secretion, apparent degranulation of β -cells and decreased β -cell numbers, resulting in the pathogenesis of DM^(8,9). Type 2 DM is also associated with increased oxidative stress, which may contribute to microvascular and macrovascular complications⁽¹⁰⁾. In normal physiological conditions, there is a balance in the generation of reactive oxygen/nitrogen species (ROS/RNS) and antioxidant defense system to deactivate and protects organisms against ROS/RNS toxicity. In diabetes, impairment in the pro-oxidant/antioxidant balance can damage cellular macromolecules, leading to DNA and protein modification and lipid peroxidation. Hyperlipidemia has also been reported as one of the causative factors for increased lipid peroxidation in DM^(11,12). These pathways enhance tissue degeneration and cause several complex syndromes in diabetic patients such as cataracts, renal dysfunction, nerve damage and atherosclerosis. Especially, the atherosclerosis leads to the CHD which is the major cause of death among diabetics.

Although the underlying mechanisms of the pathogenesis of type 2 DM still remain to be examined, oxidative stress has been shown to be responsible, at least in part, for the progression of type 2 DM and its complications⁽¹³⁾. This is supported by increased oxidative damage to lipids and DNA and impaired antioxidants in these patients^(14,15), as well as the use of some antioxidants nutrients as adjuvants or dietary supplements in the treatment of type 2 DM⁽¹⁶⁻¹⁹⁾.

Apart from lipid peroxidation used as a marker for oxidative stress, a variety of natural antioxidants

exist to scavenge ROS and prevent oxidative damage to biological membranes. One group of these antioxidants is intracellular enzymatic action, which include superoxide dismutase (SOD), the enzyme which dismutates superoxide to hydrogen peroxide, and other two hydrogen peroxide-scavenging enzymes that is glutathione peroxidase (GPx) and catalase (CAT), which convert hydrogen peroxide to water. These antioxidant enzymes in blood have been cited as markers for vascular injury in type 2 DM^(20,21).

Several studies have been carried out to evaluate the ROS induced lipid peroxidation and the antioxidant status in type 2 DM but results were controversial. Lipid peroxidation end product namely malondialdehyde (MDA) level was found significantly higher^(22,23) while antioxidant enzymes were reported significantly increased⁽²⁴⁻²⁶⁾, decreased⁽²⁷⁻²⁹⁾ and unchanged^(30,31). However only a few data were currently available on the relationship between oxidative stress and diabetes in Thai type 2 DM. Hence, the aim of the present study was undertaken to evaluate oxidative stress by measuring MDA and antioxidant enzymes in patients with type 2 DM (with or without CHD complication) and effect of glycemic control compared with healthy individuals.

Material and Method

Subjects

Control subjects (non-diabetic group)

The criteria used for the selection for both diabetes and normal controls were performed by well established diagnostic criteria as recommended by World Health Organization^(32,33). Controls included healthy individuals who attended for a routine health check-up at the department of Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital. The ages ranged from 35 to 70 years. None of the healthy controls were taking any medication or dietary supplements. They were selected after physical examination (weight, height, and blood pressure measurements, chest X-ray, respiratory, and eye examination) and laboratory tests (complete blood count, blood urea nitrogen, creatinine, uric acid, fasting blood glucose, liver function tests, total cholesterol, triglyceride, and high density lipoprotein cholesterol). They had normal fasting plasma glucose with FPG < 110 mg/dL and no history of diabetes. Subjects with hypertension, diabetes mellitus, cardiovascular diseases, renal or hepatic diseases, inflammation, injury or trauma in the previous month were excluded from the present study.

Type 2 diabetic patients

Patients who were already diagnosed to have type 2 DM based on the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2003)⁽³⁴⁾. The participants were categorized into groups, namely:

1) Fairly controlled type 2 diabetic patients whose FPG is \leq 180 mg/dL. The ages ranged from 49 to 85 years old which all sex, age matched to the healthy controls.

2) Poorly controlled type 2 diabetic patients whose FPG is $>$ 180 mg/dL. The ages ranged from 39 to 83 years old which all sex, age matched to the normal healthy controls.

3) Type 2 diabetes complicated with CHD. The aged range of this group was from 31-76 years old. These were diagnosed according to clinical investigations, electrocardiogram (ECG) pattern and angiography which all sex, age matched to the healthy controls.

No diabetic subjects were taking medications other than oral anti-diabetic pills. Subjects who supplemented with antioxidants or with any renal dysfunction (*i.e.* raised blood urea and serum creatinine levels), with coexistent illness (*i.e.* infections), congestive heart failure, acute myocardial infarction, proliferative retinopathy, febrile illness, diabetic ketoacidosis, chronic diseases and patients on insulin, were excluded from the present study.

Informed consent was obtained from all participants who enrolled in the present study according to the ethical guidelines of the Helsinki declaration. The work was carried out with the approval of the ethical clearance committee of the Faculty of Medicine Siriraj Hospital, Mahidol University.

Sample collection and preparation

All participants were advised to take no medication on the morning of blood sample collection. A 5 mL of fasting blood sample was taken between 6.00 and 8.00 a.m. from all the participants. Blood was obtained from the antecubital vein after an overnight fast. The erythrocyte suspension was prepared according to the method of Beutler et al⁽³⁵⁾. It was immediately centrifuged under refrigeration at 3,000 x g for 10 min. Plasma and the buffy coat were carefully removed and the red cells were washed three times with cold phosphate buffer saline, pH 7.4 (5 mM phosphate buffer containing 0.15 M NaCl). The erythrocytes were then suspended in an equal volume of physiologic saline. Appropriately diluted hemolysates were then

prepared from erythrocyte suspension by the addition of cold distilled water for the evaluation of SOD, GPx, CAT and MDA. The plasma was used for the determination of other biochemical parameters.

Determination of erythrocyte lipid peroxidation (MDA)

MDA was performed as described by the method of Cynamon⁽³⁶⁾. In brief, red cell suspension was exposed to 1 mL of 0.75% H₂O₂ solution and incubated at 37°C for 1 hr. 1 mL of 28% trichloroacetic acid-arsinite solution was added and mixed well. The mixture was centrifuged at 1871 x g for 10 min. 1.5 mL of 1% thiobarbituric acid in 0.05 M NaOH was added into the supernatant to react with MDA to form a colored adduct. The mixture was placed in a boiling water bath for 15 min and absorption was read at 535 nm by Shimadzu UV 1601 spectrophotometer using 1,1,3,3-tetramethoxypropane as standard.

Determination of erythrocyte SOD activity

SOD assay was performed according to the instruction of Ransod reagent kit from Randox Laboratories Ltd, UK. The SOD activity was determined by inhibiting the SOD activity using xanthine and xanthine oxidase as a superoxide generator to react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to form a red formazan dye. The absorbance was read at 505 nm by spectrophotometer.

Determination of erythrocyte GPx activity

GPx assay was also performed according to the instruction of Ransel reagent kit form Randox Laboratories Ltd, UK. The GPx activity was determined by coupled enzyme reaction in which glutathione (reduced form) and NADPH reacts with cumene hydroperoxide to produce oxidized glutathione and cumene hydroxide. The decrease in absorbance of NADPH was measured at 340 nm by spectrophotometer.

Determination of erythrocyte CAT activity

CAT assay was performed as described by the method of Beutler⁽³⁷⁾. In brief, 1M Tris-HCl with 5 mM EDTA and 10 mM H₂O₂ was incubated at 37°C for 10 min. Then, diluted hemolysate (1:2,000) was added and measured absorbance at 230 nm for 3 min.

Determination of hemoglobin content

Hemoglobin was treated with Drabkin's reagent containing potassium ferricyanide, potassium

cyanide and potassium dihydrogen phosphate. The ferricyanide oxidized hemoglobin to methemoglobin which was converted to cyanmethemoglobin by the cyanide. Absorbance was measured at 540 nm⁽³⁸⁾.

Measurement of other biochemical parameters

Blood glucose, total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were determined by colorimetric enzymatic methods using commercial kits. Low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula: $LDL-C = TC - HDL-C - TG/5$ which is expressed as mg/dL⁽³⁹⁾. Very low-density lipoprotein cholesterol (VLDL-C) was estimated by using the following equation: $VLDL-C = TC - HDL-C - LDL-C$.

Statistical analysis

Results are expressed as mean \pm standard error of mean (SEM). Distribution of variables was tested for approximation to Gaussian distribution using kurtosis and skewness test. Data for four matched groups were compared by one-way analysis of variance (one-way ANOVA) followed by Scheffe test. Pearson rank correlation test was used for testing correlation coefficient (r) between variables by the bivariate correlation method. Statistical analysis was performed using Statistical Package for the Social Science (SPSS) software package version 17.0. A

probability of $p \leq 0.05$ was considered as a significant difference.

Results

Demographic and metabolic characteristics of the normal subjects and the three groups with type 2 DM are shown in Table 1. Demographic characteristics of the four groups did not show a significant difference. All subgroups of type 2 DM had significantly higher FPG than normal subjects. For hematological data, hematocrit (Hct) was significantly lower in type 2 DM complicated with CHD whereas hemoglobin (Hb) was significantly lower in both poorly controlled type 2 DM and fairly controlled type 2 DM. The study of plasma lipid profile showed that total cholesterol and LDL-cholesterol in fairly controlled type 2 DM and type 2 DM with CHD were not significantly different compared to the normal healthy group. This was due to the successive treatment of cholesterol-lowering drug in these diabetic subjects. However, the increasing of triglyceride, VLDL-cholesterol and decreasing of HDL-cholesterol also found in type 2 DM complicated with CHD. The atherogenic indices as indicated by TC/HDL-C and TG/HDL-C ratios showed a significant increase in all subgroups of type 2 DM when compared with the control group. However, it is still believed that absolute cholesterol and TG numbers are more useful to plan treatment than the TG/HDL-C ratio or any other cholesterol ratio. Thus, the lower level of HDL-C and

Table 1. Demographic and anthropometric characteristics of healthy normal subject and type 2 diabetic patients (values were mean \pm SEM)

| Variables | Normal | Poorly controlled type 2 DM | Fairly controlled type 2 DM | Type 2 DM with CHD |
|-------------------|--------------------|--------------------------------|--------------------------------|--------------------------------|
| Subjects (n) | 20 | 19 | 26 | 20 |
| Sex (male/female) | 12/8 | 13/13 (p* = 0.264) | 8/11 (p = 0.500) | 6/14 (p = 0.057) |
| Age (years) | 56.35 \pm 1.62 | 63.96 \pm 1.69 (p = 0.583) | 62.30 \pm 2.46 (p = 0.544) | 60.50 \pm 2.56 (p = 0.228) |
| FPG (mg/dL) | 105.10 \pm 3.05 | 245.00 \pm 22.52 (p < 0.001) | 128.50 \pm 3.88 (p = 0.016) | 167.26 \pm 13.26 (p = 0.001) |
| Hb (g/dL) | 14.27 \pm 0.38 | 11.13 \pm 0.33 (p < 0.001) | 12.61 \pm 0.05 (p = 0.007) | 13.70 \pm 0.42 (p = 0.376) |
| Hct (%) | 43.56 \pm 1.09 | 46.66 \pm 1.80 (p = 0.156) | 45.00 \pm 1.46 (p = 0.476) | 36.50 \pm 1.38 (p = 0.001) |
| TC (mg/dL) | 217.10 \pm 9.57 | 255.65 \pm 13.05 (p = 0.025) | 229.87 \pm 9.02 (p = 0.393) | 225.56 \pm 13.90 (p = 0.667) |
| LDL-C (mg/dL) | 134.24 \pm 9.79 | 172.43 \pm 12.38 (p = 0.020) | 156.65 \pm 8.01 (p = 0.140) | 143.59 \pm 13.55 (p = 0.874) |
| TG (mg/dL) | 133.31 \pm 12.66 | 180.28 \pm 22.59 (p = 0.062) | 153.58 \pm 11.97 (p = 0.381) | 220.06 \pm 20.32 (p = 0.001) |
| VLDL-C (mg/dL) | 27.05 \pm 2.25 | 36.06 \pm 4.51 (p = 0.068) | 30.44 \pm 2.44 (p = 0.456) | 43.51 \pm 3.78 (p = 0.001) |
| HDL-C (mg/dL) | 55.80 \pm 2.69 | 47.16 \pm 3.15 (p = 0.026) | 42.79 \pm 2.42 (p < 0.001) | 43.25 \pm 1.97 (p = 0.001) |
| TC/HDL-C | 4.05 \pm 0.26 | 5.82 \pm 0.39 (p = 0.007) | 6.49 \pm 1.13 (p = 0.035) | 5.27 \pm 0.36 (p = 0.016) |
| TG/HDL-C | 2.55 \pm 0.28 | 5.24 \pm 1.42 (p = 0.028) | 4.10 \pm 0.56 (p = 0.016) | 5.53 \pm 0.76 (p = 0.014) |

* Significant difference (p < 0.05) as compared to control subjects
SEM = standard error of mean

higher level of TG may exacerbate the cardiovascular complication in this group of diabetes (in particular, type 2 DM with CHD).

Oxidative stress and antioxidant status in study groups

Erythrocyte MDA level

The results of red blood cell malondialdehyde (MDA) level in normal healthy subjects and type 2 DM are shown in Table 2. The levels of MDA in poorly controlled, fairly controlled and type 2 DM with CHD were significantly higher than that in the normal group ($p < 0.001$, $p = 0.042$, $p < 0.001$, respectively). Moreover, type 2 DM with CHD had a significantly higher level of MDA than poorly controlled and fairly controlled type 2 DM ($p < 0.001$) while MDA between poorly and fairly controlled DM was not significantly different ($p = 0.45$).

Erythrocyte superoxide dismutase activity

The erythrocyte SOD activity in poorly controlled and type 2 DM with CHD groups were found to be significantly increased compared to the normal healthy group ($p = 0.012$, $p = 0.006$, respectively) as shown in Table 3. However, there was no significant difference between the fairly controlled type 2 DM and normal control group ($p = 0.090$). The comparisons among subgroups of type 2 DM were also performed but showed no significant difference: poorly controlled vs. fairly controlled ($p = 0.77$), poorly controlled vs.

type 2 DM with CHD ($p = 1.00$), fairly controlled vs. type 2 DM with CHD ($p = 0.62$).

Erythrocyte glutathione peroxidase activity

GPx activity in poorly controlled type 2 DM and type 2 DM complicated with CHD were significantly higher than that in normal healthy subjects ($p = 0.033$ and $p = 0.036$, respectively) (Table 4). However, no significant difference was shown when compared with fairly controlled type 2 DM ($p = 0.924$). The comparisons were also observed among these type 2 DM subgroups and showed no significant difference [poorly controlled vs. fairly controlled ($p = 0.10$), poorly controlled vs. type 2 DM with CHD ($p = 0.99$), fairly controlled vs. type 2 DM with CHD ($p = 0.21$)].

Erythrocyte catalase activity

The Erythrocyte catalase (CAT) activity was significantly higher in poorly controlled, fairly controlled type 2 DM and type 2 DM with CHD than that in healthy normal subjects ($p < 0.001$) (Table 5). The present result also demonstrated that in multiple comparisons among these subgroups of type 2 DM, they showed no significant difference in the CAT activities [poorly controlled vs. fairly controlled ($p = 0.094$), poorly controlled vs. type 2 DM with CHD ($p = 0.704$), fairly controlled vs. type 2 DM with CHD ($p = 0.623$)].

Table 2. Erythrocyte malondialdehyde (MDA) ($\mu\text{mol/g Hb}$) in normal healthy subjects and type 2 diabetic patients (values were mean \pm SEM)

| Subjects | MDA ($\mu\text{mol/gHb}$) | p-value* |
|--------------------------------------|-----------------------------|----------|
| Normal (n = 20) | 0.24 \pm 0.01 | |
| Poorly controlled type 2 DM (n = 19) | 0.39 \pm 0.03 | <0.001 |
| Fairly controlled type 2 DM (n = 26) | 0.35 \pm 0.01 | 0.042 |
| Type 2 DM with CAD (n = 20) | 0.57 \pm 0.02 | <0.001 |

* Significant difference ($p < 0.05$) as compared to control subjects

Table 3. Erythrocyte superoxide dismutase (SOD) activity (U/gHb) in normal healthy subjects and type 2 diabetic patients (values were mean \pm SEM)

| Subjects | SOD (U/gHb) | p-value* |
|--------------------------------------|-----------------------|----------|
| Normal (n = 20) | 1,543.00 \pm 95.08 | |
| Poorly controlled type 2 DM (n = 19) | 2,217.11 \pm 155.57 | 0.012 |
| Fairly controlled type 2 DM (n = 26) | 2,018.96 \pm 102.05 | 0.090 |
| Type 2 DM with CAD (n = 20) | 2,263.32 \pm 177.27 | 0.006 |

* Significant difference ($p < 0.05$) as compared to control subjects

Table 4. Erythrocyte glutathione peroxidase (GPx) activity (U/gHb) in normal healthy subjects and type 2 diabetic patients (values were mean \pm SEM)

| Subjects | GPx (U/gHb) | p-value* |
|--------------------------------------|------------------|----------|
| Normal (n = 20) | 23.59 \pm 2.18 | |
| Poorly controlled type 2 DM (n = 19) | 37.75 \pm 3.65 | 0.033 |
| Fairly controlled type 2 DM (n = 26) | 26.59 \pm 2.54 | 0.924 |
| Type 2 DM with CAD (n = 20) | 35.93 \pm 4.20 | 0.036 |

* Significant difference ($p < 0.05$) as compared to control subjects

Table 5. Erythrocyte catalase activity ($\times 10^4$ U/g Hb) in normal healthy subjects and type 2 diabetic patients (values were mean \pm SEM)

| Subjects | CAT ($\times 10^4$ U/gHb) | p-value* |
|--------------------------------------|----------------------------|----------|
| Normal (n = 20) | 14.38 \pm 0.63 | |
| Poorly controlled type 2 DM (n = 19) | 26.59 \pm 2.67 | <0.001 |
| Fairly controlled type 2 DM (n = 26) | 21.60 \pm 0.99 | <0.001 |
| Type 2 DM with CAD (n = 20) | 24.14 \pm 0.54 | <0.001 |

* Significant difference ($p < 0.05$) as compared to control subjects

Table 6. Correlation coefficient analysis between FPG and other biochemical parameters in type 2 diabetic patients with control group

| Parameters | Poorly controlled type 2 DM (n = 39) | Fairly controlled type 2 DM (n = 46) | Type 2 DM with CHD (n = 40) |
|----------------|---|---|--------------------------------|
| TC (mg/dL) | 0.414 (p = 0.009) | 0.093 (p = 0.541) | 0.229 (p = 0.156) |
| LDL-C (mg/dL) | 0.417 (p = 0.008) | 0.171 (p = 0.256) | 0.194 (p = 0.231) |
| TG (mg/dL) | 0.387 (p = 0.015) | 0.164 (p = 0.238) | 0.377 (p = 0.017) |
| VLDL-C (mg/dL) | 0.381 (p = 0.017) | 0.178 (p = 0.276) | 0.374 (p = 0.017) |
| HDL-C (mg/dL) | -0.441 (p = 0.005) | -0.385 (p = 0.008) | -0.347 (p = 0.028) |
| TC/HDL-C | 0.392 (p = 0.014) | 0.284 (p = 0.056) | 0.454 (p = 0.003) |
| TG/HDL-C | 0.365 (p = 0.022) | 0.203 (p = 0.177) | 0.376 (p = 0.017) |
| MDA | 0.399 (p = 0.012) | 0.255 (p = 0.087) | 0.481 (p = 0.002) |
| SOD | 0.333 (p = 0.038) | 0.279 (p = 0.061) | 0.121 (p = 0.458) |
| GPx | 0.097 (p = 0.557) | -0.045 (p = 0.768) | 0.049 (p = 0.764) |
| CAT | 0.406 (p = 0.010) | 0.343 (p = 0.020) | 0.496 (p < 0.001) |
| Hb (g/dL) | -0.537 (p < 0.001) | -0.013 (p = 0.930) | 0.272 (p = 0.090) |
| Hct (%) | -0.064 (p = 0.697) | 0.046 (p = 0.763) | -0.196 (p = 0.226) |

Pearson correlation coefficient analysis

Results of Pearson correlation coefficient analysis among FPG, oxidative stress status and other biochemical parameters in type 2 DM patients with normal controls appear in Table 6. In linear correlation analysis of FPG to all parameters, the result showed significant positive correlations with total cholesterol ($r = 0.414$, $p = 0.009$), LDL-C ($r = 0.417$, $p = 0.008$),

VLDL-C ($r = 0.381$, $p = 0.017$), TG ($r = 0.387$, $p = 0.015$), TC/HDL-C ($r = 0.392$, $p = 0.014$), TG/HDL-C ($r = 0.365$, $p = 0.022$), MDA ($r = 0.339$, $p = 0.012$), SOD ($r = 0.333$, $p = 0.038$) and CAT ($r = 0.406$, $p = 0.010$) and a negative association with HDL-C ($r = -0.441$, $p = 0.005$) in poorly controlled type 2 DM. Similarly, the significant positive correlations with VLDL-C ($r = 0.374$, $p = 0.017$), TG ($r = 0.377$, $p = 0.017$), TC/HDL-C ($r = 0.454$, $p =$

0.003), TG/HDL-C ($r=0.376$, $p=0.017$), MDA ($r=0.481$, $p=0.002$) and CAT ($r=0.496$, $p<0.001$) and a negative association with HDL-C ($r=-0.347$, $p=0.028$) were found in type 2 DM with CHD. For fairly controlled type 2 DM, the significant positive correlation was only CAT ($r=0.343$, $p=0.020$) whereas a negative correlation was HDL-C ($r=-0.385$, $p=0.008$).

Discussion

In the present study, the authors found that patients with CHD were accompanied with low HDL-C and high TG values. The low level of HDL-C, which exerts anti-atherogenic and antioxidative effects when present in sufficient amounts, is a key feature for oxidative stress status⁽⁴⁰⁾. The reduced HDL-C level is often accompanied by elevation in plasma TG level⁽⁴¹⁾, and associated with increased cardiovascular disease risk^(42,43) which corresponded to the present study. In type 2 DM, the increased secretion of apolipoprotein B (apoB) as a result of fatty acid synthesis in the liver^(44,45), a protein identified as a key component of the VLDL assembly process, leads to increase plasma level of TG and reduce level of HDL-C. In addition, the elevation of free fatty acid and glucose in DM can decrease activity of lipoprotein lipase, a pivotal enzyme in the removal of these lipoproteins from the circulation, that control the TG-rich lipoproteins and HDL particles⁽⁴⁶⁾. As a result, hypertriglyceridemia usually accompanies with decreased HDL-C, which is also a prominent feature of plasma lipid abnormalities seen in diabetic subjects⁽⁴⁷⁻⁴⁹⁾, especially in type 2 DM with CHD. Moreover, Boizel et al found that the ratio of TG/HDL-C may be related to the process involved in small dense LDL pathophysiology which is the best predictor of CHD⁽⁵⁰⁾. The present results suggested that hypertriglyceridemia and decreased HDL-C level contribute to the development of atherosclerosis and increased the risk of CHD, which were in accordance with other studies^(42,43).

De Zwart et al has proposed that oxidative stress may be associated with the pathogenesis of diabetes and its complications⁽⁵¹⁾, particularly CHD^(52,53). The rise in MDA indicated that any oxidative stress incurred sufficiently could cause free radical-mediated peroxidation of lipid component in cell membranes. Thus, MDA is a good indicator for evaluating oxidative stress in degenerative diseases like diabetes mellitus. The present study showed that MDA was increased significantly in red cells of all patients' groups comparing to their aged-matched healthy subjects. In addition, type 2 DM complicated

with CHD had significantly higher red cell MDA than the others, suggesting that diabetic patients with CHD were more prone to accumulation of potentially harmful by oxidative stress than diabetic patients without CHD. This is consistent with the reports of others^(22,23,54). The present results suggested that the increasing of MDA in type 2 DM with CHD will mediate the cross-link between protein to form MDA modified lipoprotein adduct that can trigger the atherogenesis in these patients as shown by the discovery of de Olivera et al⁽⁵⁵⁾. In addition, the positive correlation between MDA and FPG was found in poorly controlled type 2 DM and type 2 DM complicated with CHD ($r=0.399$, $p=0.012$; $r=0.481$, $p=0.002$, respectively) but not in fairly controlled type 2 DM ($r=0.255$, $p=0.087$). This relationship also suggested that high levels of glucose may mediate the free radical production to induce lipid peroxidation in diabetes.

In the present study of antioxidant enzyme activities, the poorly controlled type 2 DM and type 2 DM with CHD showed significant elevation in SOD enzyme activity whereas in fairly controlled type 2 DM, SOD activity was also increased but did not reach the significant level when compared to the normal group. This indicated that oxidative stress induced by high level of glucose may increase superoxide radical production within diabetic patients which was not found in fairly controlled DM. This finding was in agreement with the other evidences^(24,56) which SOD activity decreased but did not reach normal levels by glucose control and insulin therapy. Nevertheless, Dominguez et al⁽²⁸⁾ also demonstrated that the SOD activity in onset type 1 diabetic patients were higher than that in normal subjects and after follow-up in this patient group, the SOD activity was decreased but still higher than normal.

The CAT activities in all patients' groups were also significantly elevated compared to their healthy matched groups. This suggested that adaptive mechanisms for the increased production of hydrogen peroxide (H_2O_2) by promoting CAT activity also occurred. This reflects the overwhelming adaptive response to the challenges of oxidative stress in the diabetic state with and without complications. The present study in glutathione peroxidase (GPx), a selenium-dependent enzyme, found that poorly controlled type 2 DM and type 2 DM with CHD had significantly higher GPx activities than the sex, age matched normal group. The increment of erythrocyte GPx activity in the present study may be explained by which the GPx is a selenium-dependent enzyme and

it would appear an adaptive process of combating excessive peroxidative damage. Consequently, diabetic tissue retains selenium and thereby increases GPx activity in the cells⁽²⁵⁾. Similar result was reported by Gebre-Medhin et al⁽⁵⁷⁾ who observed the increased levels of plasma selenium in diabetic children. However, a selenium turnover study is required to confirm whether a compensatory mechanism sets in for saving tissue selenium levels for increase the GPx activity in diabetes.

In the present study, the increased levels of MDA and antioxidant enzymes clearly show that diabetic patients were exposed to an increased oxidative stress via lipid peroxidation. The antioxidant enzymes play an important role in scavenging ROS produced under oxidative stress. However, conflicting reports on activities of these antioxidant enzymes (SOD, CAT and GPx) have been observed. The SOD activity has been shown to be increased^(24,28,54,58-60), decreased^(25,27,33,61) and unchanged^(30,31,62). The controversy also rises about the GPx [activity increased^(25,27,29), decreased^(28,63-65), unchanged^(24,31,62,65, 66)] and CAT enzyme [activity increased^(26,66-68), decreased^(25,29,69,70), unchanged^(31,66)]. Ookawara et al⁽⁷¹⁾ suggested that decreased or unchanged in these enzyme activities may be caused by the glycation or ROS induced fragmentation by autoxidation of these enzymes and made them to be impaired. In addition, the patients in this and other studies were already on either monotherapy or combination of antidiabetic therapy. It is well known that most of the oral hypoglycemic drugs also have potent antioxidant effects⁽⁵⁴⁾. This may be one of the reasons that why most of the antioxidant enzyme activities in various literature reviews were inconsistent. The correlations between fasting plasma glucose and oxidative stress status is supported by our results demonstrating an association between blood levels of glucose and oxidative stress status such as MDA level and CAT activity in poorly controlled type 2 DM and type 2 DM with CHD. Only CAT activity showed significant positive relationship in fairly controlled type 2 DM. No significant associations were observed with SOD and GPx activities in type 2 DM with CHD, although the p values of the activities did approach statistical significance. This present study suggested that the enzymes were possibly associated with the progressive glycation caused by high glucose level. Approximately 50% of red cell SOD in patients with type 2 DM has been reported to be glycated, resulting in the decreased activity^(72,73). Therefore, the present results may be inferred that these two antioxidant

enzyme activities in type 2 DM may be glycated in the level that could not diminish their activities until reaching the significant level. For CAT activity, there was fair correlation between FPG and CAT activity in all subgroups of type 2 DM suggested that CAT may tolerate autoxidation induced fragmentation by H₂O₂ in diabetes. This is consistent with the high affinity (low Km) of this enzyme that makes it function efficiently to remove high concentration of H₂O₂ from its environment. This finding was in agreement with Rice-Evans et al⁽⁷⁴⁾. Thus, the present study depicted that the antioxidant enzyme activities in these diabetic patients were elevated but could not protect the patients from oxidative damage since lipid peroxidation (defined by erythrocyte MDA levels) still occurs at a very high level compared to the normal subjects which corresponded to the previous reports⁽⁷⁵⁻⁷⁷⁾.

Conclusion

In conclusion, the present study supported the hypothesis that hyperglycemia activated cellular and tissue damage by oxidative stress but the compensatory mechanisms for defense against the ROS to normalize oxidative stress was not achieved in the diabetic patients even in the fairly controllable diabetics. Consequently, glucose control could affect the smallest change in lipid peroxidation and antioxidant enzyme activities. Moreover, this oxidative stress increased with poor glycemic control and type 2 DM with CHD. As a result, patients with hyperglycemia, dyslipidemia and oxidative stress present a high risk for CHD and need early aggressive intervention in type 2 DM.

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ระดับไลปิดเปอร์ออกซิเดชัน และการทำงานของแอนติออกซิแดนซ์เอนไซม์ในเม็ดเลือดแดงของ ผู้ป่วยเบาหวานประเภทที่ 2

อธิป ลิขิตลิลิต, ณัฐรัชย์ ภัทรชนันท์, ธวัชชัย พิรพัฒน์ดิษฐ์, ชาญ ศิริรัตนสถาวร

ภูมิหลังและวัตถุประสงค์: การมีระดับน้ำตาลในเลือดสูงมักเป็นสาเหตุสำคัญที่ทำให้มีการเพิ่มขึ้นของอนุมูลอิสระที่เรียกว่าภาวะออกซิเดทีฟสเตรส ซึ่งอนุมูลอิสระที่เพิ่มขึ้นนี้จะไปทำลาย β cell ของตับอ่อน หรือ ไปทำปฏิกิริยากับสารต่าง ๆ ในร่างกาย ทำให้โรคเบาหวานมีอาการรุนแรงขึ้นและเกิดโรคแทรกซ้อน โดยเฉพาะโรคที่เกี่ยวข้องกับระบบหลอดเลือดหัวใจ ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อตรวจหาภาวะออกซิเดทีฟสเตรส ที่สัมพันธ์กับปัจจัยที่ทำให้เกิดโรคแทรกซ้อนในผู้ป่วยเบาหวานประเภทที่ 2 โดยการเปรียบเทียบระหว่างระดับของ MDA (มาลอนไดอัลดีไฮด์) และการทำงานของแอนติออกซิแดนซ์เอนไซม์ในเม็ดเลือดแดง เทียบกับคนปกติที่มีเพศและอายุใกล้เคียงกันเพื่อประเมินความเสี่ยงต่อการเกิดภาวะออกซิเดทีฟสเตรส และโรคแทรกซ้อน ในผู้ป่วยเบาหวาน ประเภทที่ 2

วัสดุและวิธีการ: ระดับ MDA และการทำงานของแอนติออกซิแดนซ์เอนไซม์ superoxide dismutase (SOD), glutathione peroxidase (GPx) และ catalase (CAT) จะถูกวิเคราะห์โดยใช้เครื่อง spectrophotometer ในเม็ดเลือดแดงผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลไม่ดีจำนวน 19 ราย (มี FPG > 180 มก./ดล.), ในเม็ดเลือดแดงผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลได้พอสมควรจำนวน 26 ราย (มี FPG \leq 180 มก./ดล.) และในเม็ดเลือดแดงผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อนจำนวน 20 ราย เปรียบเทียบกับคนปกติจำนวน 20 ราย (มี FPG < 110 มก./ดล.) โดยมีอายุและเพศใกล้เคียงกัน ผลของการทดลองใช้ ANOVA test ในการเปรียบเทียบผู้ป่วยเบาหวานประเภทที่ 2 ทุกกลุ่มกับคนปกติ และสัมประสิทธิ์ของสหสัมพันธ์ระหว่างระดับน้ำตาลในเลือดกับตัวแปรของภาวะออกซิเดทีฟสเตรสจะใช้ Pearson rank correlation coefficient

ผลการศึกษา: ระดับของ MDA ในเม็ดเลือดแดงของผู้ป่วยเบาหวานประเภทที่ 2 จะเพิ่มสูงขึ้นอย่างมีนัยสำคัญในผู้ป่วยเบาหวานประเภทที่ 2 ทุกกลุ่มเมื่อเทียบกับคนปกติ ยิ่งไปกว่านั้นยังพบว่าระดับของ MDA มีค่าสูงสุดในผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อน สำหรับการทำงานของแอนติออกซิแดนซ์เอนไซม์พบว่าจะมีค่าสูงขึ้นอย่างมีนัยสำคัญทั้งในผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลไม่ดี และผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหัวใจแทรกซ้อน ยกเว้นผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลได้ พบว่าการทำงานของแอนติออกซิแดนซ์เอนไซม์คือ SOD และ GPx จะไม่แตกต่างจากคนปกติ ขณะที่ CAT จะสูงกว่าคนปกติอย่างมีนัยสำคัญ ส่วนสัมประสิทธิ์ของสหสัมพันธ์ระหว่าง FPG กับภาวะออกซิเดทีฟสเตรส พบว่าระดับของ MDA และการทำงานของเอนไซม์ CAT จะมีความสัมพันธ์ในเชิงบวกในผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลไม่ดี และผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อน ขณะที่ผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลได้ พบว่ามีความสัมพันธ์ในเชิงลบระหว่าง FPG และการทำงานของเอนไซม์ CAT เท่านั้น

สรุป: ผลการทดลองนี้แสดงให้เห็นว่าผู้ป่วยเบาหวานประเภทที่ 2 อยู่ในภาวะที่ว่องไวต่อการเกิดออกซิเดทีฟสเตรส ซึ่งสัมพันธ์กับระดับน้ำตาลในเลือด และจากการที่ระดับของ MDA ที่สูงมากในผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อน แสดงว่าภาวะออกซิเดทีฟสเตรส แสดงบทบาทที่สำคัญในการเกิดภาวะแทรกซ้อนดังกล่าว นอกจากนี้การทำงานของแอนติออกซิแดนซ์เอนไซม์ที่เพิ่มสูงขึ้นในผู้ป่วยเบาหวาน แสดงให้เห็นว่าผู้ป่วยเบาหวานมีการปรับการทำงานของแอนติออกซิแดนซ์เอนไซม์เพื่อตอบสนองต่อภาวะออกซิเดทีฟสเตรส
