Lipid Hydroperoxide, Malondialdehyde and Total Antioxidant Capacity as Predictors for Oxidative Stress in Type 2 Diabetes Mellitus

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List of abbreviation

T2D; type 2 diabetes mellitus, CAD; cardiovascular disease, ROS; reactive oxygen species, TC; total cholesterol, HDL-C; high density lipoprotein, LDL-C; low density lipoprotein, MDA; malondialdehyde, MDA; thiobarbituric acid substance, LOOH ; lipid hydroperoxide, TAC; total antioxidant capacity

Abstract

Objective: Lipid hydroperoxide (LOOH) and malondialdehyde (MDA), products of lipid peroxidation, are occurring cellular processes and involved in cell adhesion and proliferation, inflammatory responses, aging, and death. Lipid peroxidation plays an important role in the premature development of atherosclerosis. We aim to determine the LOOH, MDA, and total antioxidant capacity (TAC) as the markers of oxidative stress in type 2 diabetes (T2D) patients.

Materials and Methods: The study included 206 participants stratified as 123 T2D (with 37 males and 86 females) and 83 healthy controls (with 18 males and 65 females) randomly selected from Phitsanulok residents. MDA, LOOH, TAC levels and others biochemical markers were measured from the blood samples of these participants.

Results: LOOH and MDA levels were significantly higher ($P<0.05$), and the TAC was lower in T2D patients. There were associations between elevated LOOH (OR=12.86; 95% CI 5.23-31.63), MDA (OR=8.54; 95% CI 3.15-23.18), hypertriglyceridemia (OR=3.96; 95% CI 1.49-10.48), abdominal obesity (OR=4.05; 95% CI 1.58-10.35) and T2D adjusting for age and gender. Increased LOOH and MDA levels, and decreased TAC levels are indicated oxidative stress occurred in T2D patients.

Conclusion: Our results revealed that T2D patients had elevated oxidative stress enzymes that may cause oxidative damage. Therefore, LOOH, MDA and TAC assays are the good markers for predicting oxidative stress in T2D patients.

Keywords: Lipid hydroperoxide, Malondialdehyde, Total antioxidant capacity, Type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus, according to Diabetes Association defines diabetes as being present if one of three criteria are present: 1) a casual plasma glucose of > 200 mg/dL (11.1 mmol/L) in someone with symptoms of diabetes; 2) fasting (eight-hour) plasma glucose of > 126 mg/dL (7.0 mmol/L); or 3) two-hour plasma glucose of > 200 mg/dL (11.1 mmol/L) as part of an oral glucose tolerance test, with a glucose load of 1.75 g/kg to a maximum of 75 gm [1]. Hyperglycemia results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in variety of tissues. Oxidative modification of low density lipoprotein cholesterol (LDL-C) and cellular lipids contribute to foam cell formation, endothelial dysfunction and destructive inflammation process associated with atherosclerosis[2]. Lipid peroxidation is generated from polyunsaturated fatty acids and all cellular lipids, probably contributes to the initiation, promotion, and/or progression of many human diseases including diabetes, atherosclerosis, rheumatoid arthritis, and liver disease [3, 4]. There are various plasma markers of lipid peroxidation including lipid hydroperoxides (LOOH), malondialdehyde (MDA), conjugated dienes, and $F_2\alpha$-isoprostanes, represent end products of the lipidperoxidation cascade. Several studies have consistently reported increased oxidative stress [5, 6] as well as decreased antioxidative defense mechanisms [6-9] in diabetic patients.

Type 2 diabetic patients (T2D) have been generally described as being under enhancedoxidative stress[10].We aim to determine LOOH, MDA, and TAC levels as the markers of oxidative damage and the association of these biochemical markers in T2D patients.

Materials and methods

Subjects: 206 participants in this study were stratified as 123 T2D (mean age=59.45 ± SD = 10.03 yr, with 37 males and 86 females) and 83 healthy control subjects (mean age=58.69 ± SD = 8.27 yr, with 18 males and 65 females), randomly selected from Phitsanulok residents who came for their health check up in our service project during July 2008 March 2009. The inclusion criteria of T2D in the present study included (i) history of hyperglycemia more than 5 years (ii) without acute illness and clinical signs of ischemia, myocardial infarction, unstable angina or stroke and (iii) no changes in treatment over 30 days prior to inclusion in this study. Healthy controls were from general population in the same district. Inclusion criteria for healthy controls included: absence of history of CVD, absence of hypertension, no condition limiting mobility, life-treatening diseases, or other disease or condition that would impair compliance. Exclusion criteria were smoking and intake of antioxidant supplementation in the past 2 months prior to their inclusion in the study. All T2D patients were regularly treated with glycemic lowering drugs, and lipid lowering drug. The Ethics Committee of the Naresuan University approved the study protocol. All participants gave written informed consent.

Anthropometric measurement

Waist circumference (WC) of these volunteers was measured at the midpoint between the rib cage and the top of the lateral border of the

iliac crest during minimal respiration. The blood pressure measurements were obtained in seated position for 5 min after resting. Measurements were made twice on all participants at 5 min intervals with a digital blood pressure monitor, ES-P 110 (Terumo cooperation, Japan). The average of the two measurements was used for data analysis.

Blood sampling

Venous blood was taken in the morning after an overnight fast for at least 8-12 hours. The serum was separated and kept frozen at -70 °C until assayed.

Glucose and lipid profiles assays

Fasting plasma glucose (Glu), serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) were measured by using enzymatic procedure with a Hitachi 912 autoanalyzer (Roche Diagnostic, Switzerland). We calculated low density lipoprotein cholesterol (LDL-C) with Friedewald's formula in specimens with TG levels <400 mg/dl.

LOOH assay

The method for LOOH assay was as described previously[11,12]. Hydroperoxide content was determined using a molar absorption coefficient of $4.3 \times 10^4 M^{-1} \bullet cm^{-1}$ or by reference to a cumene hydro peroxide standard curve. We also confirmed the serum signal authenticity as a ferric-xylenol Orange complex can be established by scanning from 500 nm to 600 nm and identifying the absorbance peak at 560 nm. The within- run and between-run coefficient of variation for lipid hydroperoxide assay in control material assay was 4.11% and 5.16% (n=10).

MDA assay

MDA level was determined by using the TBARs assay, a spectroscopic techniques as our previous report[3]. The method is based on the formation of red (pink) chromophore following the reaction of TBA with MDA and the other breakdown products of peroxidized lipids called MDA. One molecule of MDA reacts with 2 molecules of TBA to yield a pink pigment with absorption maximum at 532 nm.

Total antioxidants capacity assay (TAC)

The assay is based on the reaction of metmyoglobin with hydrogen peroxide to form ferryl myoglobin, a free radical species. A chromogen 2,2'-amino-di-[3-ethylbenzthiazole sulphonate] is incubated with ferryl myoglobin to produce a radical cation which has a relatively stable blue-green color that can be measured at 600 nm. Antioxidants in the added serum can suppress this color production to a degree proportio al to their concentration. The assay was calibrated using 6-hydroxy-2, 5, 8-tetramethylchroman-2-carboxylic acid (Trolox), and results were expressed as mmol/l trolox equivalent[13]. The within run coefficient of variation for the TAC assay in control material assay was 4.8% (n=10).

Statistical analysis

The values are expressed as mean and standard deviation. Differences in the mean of each variable were calculated using t-tests. Multiple logistic regression analysis was performed to test the mediating effect of T2D on the associations of elevated LOOH, MDA, hypertriglyceridmia, and abdominal obesity. Bivariate correlation between LOOH, MDA and TAC with the other variables was done by using Pearson correltion. The cutoff point levels were obtained from receiver operating characteristic (ROC) curve. All analyses were carried out using SPSS computer program version 13.0 (SPSS Inc., Chicago, IL).

Results

Clinical characteristics of the T2D patients and healthy controls are shown in Table 1. Both LOOH and MDA levels were significantly higher *(P<0.001)* and TAC was also significantly lower *(P<0.001)* in T2D. All the conventional cardiovascular risk factors (blood pressure, WC, Glu, TC, and TG) were also significantly higher *(P<0.05)* and HDL-C level was lower in T2D patients (Table 1). Excepted LDL-C wasn't significantly different. Odds ratio (OR) of T2D patients associated with elevated lipid hydroperoxide was 12.86 (95% CI, 5.23-31.63), elevated MDA 8.54 (95% CI, 3.15-23.18), hypertriglyceridemia 3.96 (95% CI,1.49-10.48), and abdominal obesity 4.05 (95% CI, 1.58-10.35) adjusted by age and gender in the present study (Table 2).

LOOH was significantly correlated with WC and Glu (r= 0.370 and 0.236, *P<0.05)* and negatively correlated with HDL-C (r= -0.244, *P*=0.007) were observed in T2D patients. A significantly negative correlation between MDA and HDL-C (r=-0.241, *P*<0.05) was observed. TAC was not correlated with any variables that may act as independent marker (Table 3).

ROC curve analysis of those LOOH, MDA, and TAC assays were shown in Fig 1. The cutoff levels of LOOH, MDA, and TAC for prediction of oxidative stress in present study were 6.01 U/ml (with sensitivity and specificity of 85.4 and 84.3%), 3.50 μmol/l (with sensitivity and specificity of 74.0 and 83.1%), and 0.503 Trolox units (with sensitivity and specificity 71.1 and 99.9%), respectively.

Table 1 General characteristic of healthy control subjects and type 2 diabetes mellitus patients

	Healthy control (n=83)	Type 2 diabetic patients (n=123)	P-value
Age (yr)	55.93±12.01*	59.45±10.03*	0.560
Systolic BP (mmHg)	122.88±13.84	136.80±19.30	<0.001
Diastolic BP (mmHg)	74.02±9.08	80.45±11.76	<0.001
BMI (kg/m^2)	22.86±3.94	25.54±3.98	<0.001
WC (cm)	80.72±9.64	96.31±20.21	<0.001
Glucose (mg/dl)	95.46±9.10	124.44±38.97	<0.001
Total cholesterol (mg/dl)	201.81±28.93	227.47±57.06	<0.001
Triglycerides (mg/dl)	103.81±46.33	191.56±152.19	<0.001
HDL-C (mg/dl)	71.84±14.79	60.87±16.14	<0.001
LDL-C (mg/dl)	108.25±27.85	119.30±50.21	0.07
Lipid Hydroperoxide (U/ml)	4.01±2.03	8.50±2.99	<0.001
MDA (μ mol/l)	2.87±0.96	5.94±3.17	<0.001
TAC (Trolox unit)	0.534±0.018	0.393±0.018	<0.001

* Mean±SD

BP; blood pressure, BMI; body mass index, WC; waist circumference, HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, LOOH; lipid hydroperoxide, MDA; malondialdehyde, TAC; total antioxidant capacity

Table 2 Odds ratios and 95% conference interval (CI) of T2D patients for elevated lipid hydroperoxide, MDA, hypertriglyceridemia, and abdominal obesity model, adjusted for age and gender

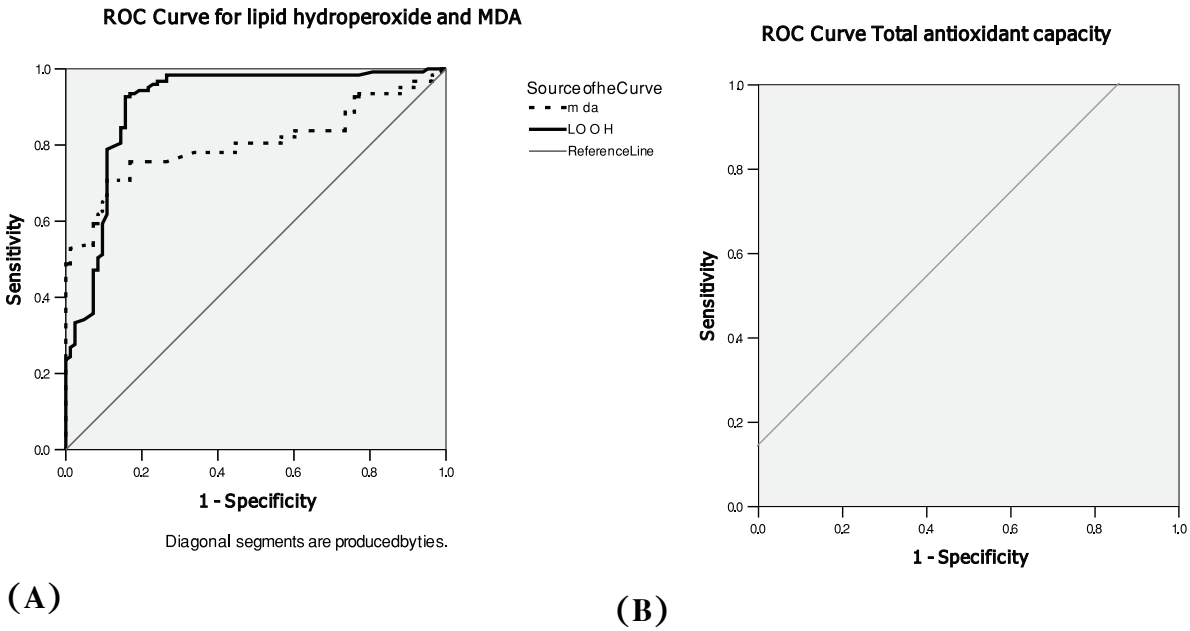
Test variable(s)	OR	95% confidence interval		P-value
		Lower bound	Higher bound	
Elevated LOOH	12.86	5.23	31.63	<0.001
Elevated MDA	8.54	3.15	23.18	<0.001
Hypertriglyceridemia	3.96	1.49	10.48	0.006
Abdominal obesity	4.05	1.58	10.35	0.004

LOOH; lipid hydroperoxide, MDA; malondialdehyde

Table 3 Bivariate correlation between LOOH, MDA and other variables

LOOH (U/ml) with	**r**	**P-value**
WC (cm)	0.370	<0.001
Glu (mg/dl)	0.236	0.009
HDL-C (mg/dl)	-0.244	0.007
MDA (µmol/l) with	**r**	**P-value**
HDL-C (mg/dl)	-0.241	0.007

LOOH; lipid hydroperoxide, MDA; malondialdehyde, WC; waist circumference, Glu; glucose, HDL-C; high density lipoprotein cholesterol



(A) (B)

Fig 1. ROC curves for (A) lipid hydroperoxide (LOOH), MDA and (B) total antioxidant of T2D in the type 2 diabetes patients

LOOH; lipid hydroperoxide, MDA; malondialdehyde, TAC; total antioxidant capacity

Discussions

Our results showed that T2D patients had significantly elevated triglyceride levels, and low HDL-C levels. There was no significant difference in LDL-C levels, and elevated lipid peroxidation markers. The pathogenesis of diabetic dyslipidemia suggests that insulin resistance has a central role in the development of this condition[14-16]. There were associations between increased triglycerides levels, decreased level of HDL-C and normal or slightly elevated LDL-C levels and increased the concentration of small dense LDL-C particles[17]. In general, it is assumed that increased lipid peroxidationis the result of increased oxidative stress[3,18,19]. Measurement of LOOH and the products of lipid peroxidation, is used to indicate in vivo or in vitro oxidative damage[3,18,19].

Dyslipidemia in T2D patients were elevated risk for coronary heart disease. That characterized by elevated serum triglyceride and decreased serum HDL-C concentrations are usually accompanied by the presence of small dense LDL particles as the atherogenic lipoprotein phenotype. This phenotype prevalence and concomitance with the elevation of oxidative stress may suggest a higher overall burden of atherosclerotic disease[13]. There is increasing evidence that oxidative stress plays an important role in the premature development of atherosclerosis[20, 21]. Our results showed that T2D patients had increased TG levels and decreased HDL-C levels, abdominal obesity, and increased in LOOH and MDA levels (oxidative stress). Both LOOH and MDA were products of lipid peroxidation. LOOH was significantly correlated with WC and Glu, both of which were the components of the metabolic status in patients with increasing oxidative stress. Our study, both LOOH and MDA were negatively correlated with HDL levels. These may be associated with the antioxidant and anti-ahtherogenic properties of HDL[22] that inhibited oxidative modification of LDL, which plays a central role in the initiation and propagation of atherosclerosis [23, 24].

In the demonstration of Nourooz-zadeh et al[25]. showed the major generation of hydroperoxides was LDL fraction. Yoshida et al.[26] demonstrated that the oxidative susceptibility of LDL in diabetic patients was increased as compared with that in healthy control subjects. Moreover, proinflammatory activities relevant to early events in atherogenesis appear to be manifested, at least by mean of lipid peroxide-(and oxidized LDL)-mediated expression of vascular adhesion molecules in endothelial cells[27] and other genes regulating inflammation[28]. Several studies have shown that oxidative modification of LDL may promote fatty streak formation and early lesion of atherosclerosis. Once elevated oxidative stress are occursc oncomitance with decreased TAC, accelerated atherosclerosis may be present. Thus, antioxidant might potentially be useful in preventing or delaying development of atherosclerosis as shown in rabbits[29], and a few epidemiologic studies have shown a favorable effect of high plasma vitamin E levels on cardiovascular morbidity and mortality in human subjects[30, 31]. As in our data were showed that TAC in T2D patients was significantly decreased ($P<0.001$) than healthy controls.

These raise the question of whether antioxidants treatments can be effective to prevent or delay the onset of diabetic complications. Antioxidants such as vitamin C and E, lipoic acid, antioxidative enzymes, delacetylcystein, and others has been reported to prevent hyperglycemia-induced biological changes such as cytokine induction, matrix synthesis, and cellular growth and turnover[32, 34].

The limitations of the present study included all of the methods used to assess oxidative stress in this study are indirect. Furthermore, the clinical utility of these assays is questionable due to required time for sample preparation and analyses. There is also a lack of standardization of these methods potentially explaining different outcomes on sample population groups. For example, sample handle is extremely important for measures of oxidative stress. If samples are not immediately centrifuged and store at -70°C lipids have been shown to auto-oxidized. Furthermore, samples which have been freeze thawed can also undergo lipid auto-oxidation. Oxidative modification of lipid associated with LDL and cellular constituents contribute to endothelial dysfunction and inflammatory pathway is associated with atherosclerosis[34].

In conclusion, our results revealed that T2D patients were elevated in oxidative stress that may cause oxidative damage. Therefore, LOOH, MDA and TAC assays are the good markers for predicting or monitoring oxidative stress in T2D.

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