Plasma Lipid Peroxidation and Antioxidant Nutrients in Type 2 Diabetic Patients

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Background and Objective: *Observation shows diabetic patients to be more prone to oxidative stress because of hyperglycemia. The elevation of free radical production by this hyperglycemic production may exacerbate cardiovascular complication in diabetes. This study aims to investigate the oxidative stress related parameters in type 2 DM. Since the effects of glycemic control and cardiovascular complications in DM on these parameters has been not fully determined, the comparison between plasma MDA (malondialdehyde) and antioxidant nutrients with their age-matched normal healthy group may be used to determine the susceptibility of oxidative stress in this type of DM.*

Material and Method: *MDA and antioxidant nutrients (vitamin A, C, E and β-carotene) were analyzed in plasma of 19 subjects with poorly controlled type 2 DM (fasting plasma glucose [FPG] >180 mg/dl), 26 subjects with fairly controlled type 2 DM (FPG ≤ 180 mg/dl), and 20 subjects with type 2 DM complicated coronary heart disease (CHD) who were matched for age and gender. Twenty healthy subjects with normal plasma glucose level (FPG < 110 mg/dl) and matched for age and gender served as a control group. In all groups of DM these oxidative stress parameters were compared to a normal group.*

Results: *The plasma MDA levels were significantly higher in all types of DM compared to age-matched normal control. Plasma antioxidant vitamin C and E significantly lower only in poorly controlled and CHD complicated type 2 DM, respectively. The mean of plasma vitamin E level was lowest in type 2 DM complicated with CHD. No significant differences in both plasma vitamin A and β-carotene were noted between any types of DM and age-matched normal healthy group. The positive correlation between MDA and FPG was demonstrated in most group of patients with their normal subjects except in fairly controlled type 2 DM and negative correlation between vitamin E and FPG was also demonstrated in type 2 DM with CHD.*

Conclusion: *These findings suggested that diabetic patients were susceptible to oxidative stress and higher plasma glucose level had an association with free radical-mediated lipid peroxidation. The lowest level of vitamin E in type 2 DM complicated with CHD indicated that oxidative stress played an important role in cardiovascular complication and vitamin E supplementation may be necessary for treatment and prevention in this group of diabetics.*

Keywords: *Lipid peroxidation, Antioxidants, Atherosclerosis, Oxidative stress, Type 2 diabetes*

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The

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chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels. However, the major mortality of diabetic patients is often found in cardiovascular disease. The data from Framingham study showed that diabetic men

have a 50% increase in coronary heart disease (CHD) compared to non-diabetic men and women have a two-fold increase in the clinical expression of CHD compared to non-diabetic women. The sudden death is also 50 % more in diabetic men and in women and there is a three-fold increase in sudden death compared to non-diabetic women[1]. In addition to chronic hyperglycemia resulting in progression of disease, oxidative stress resulting from increased free radical formation and/or decreased antioxidants in the body also has a relation with long-term complications[2,3]. In diabetes, there are more mechanisms that induce oxidative stress than in normal individuals; glucose autoxidation, non-enzymatic glycation of protein, and polyol pathway. These pathways enhance generation of reactive oxygen species (ROS) that leading to tissue damage and cause several complex syndromes in diabetic patients such as cataracts, renal dysfunction, nerve damage, and atherosclerosis. Especially, atherosclerosis leading to the coronary heart disease (CHD) is the major cause of death among diabetics. Atherosclerosis is the thickening and rigidity of the artery and arteriole. Its pathogenesis begins with oxidation of low density lipoproteins (LDLs) by ROS. Increased lipid peroxidation (measured as levels of malondialdehyde or MDA) caused crosslink formation between single molecules of proteins and oxidation of LDL particles and led to the oxidized LDL formation. The oxidized LDLs cannot be recognized by LDL receptors and be scavenged by macrophages to generate foam cells. After these foam cells accumulation, fatty streak will form and progress to a fibrous plaque and finally to the atherosclerosis. Restriction of blood supply in the coronary arteries causes myocardial infarction and sudden death[4,5].

It seems that the oxidative stress associates with diabetes mellitus and its complications. In addition to lipid peroxidation, antioxidant nutrients such as vitamin E, vitamin C, vitamin A, and β-carotene are also involve in detoxification of the ROS. Vitamin E, A, and β-carotene are lipophilic antioxidants whereas vitamin C is hydrophilic antioxidant. Vitamin E function as a free radical chain breaker particularly it interferes with the propagation step of lipid peroxidation, whereas vitamin C can regenerate vitamin E and can act as antioxidant in its own right. The vitamin A and β-carotene have actions by quenching both singlet oxygen and other free radicals generated by photochemical reactions[6]. Both vitamin A and β-carotene are effective antioxidant only at low oxygen concentration.

In patients with type 2 diabetes, increased oxidative stress and lower concentrations of antioxidants have been reported but results were inconsistent. MDA level was found significantly higher while antioxidant defenses were reported significantly lower in patients of diabetes with[7-10] and without complications[11-15]. Others have reported no change in indices of oxidative stress[16-17]. Data on oxidative stress in patients of type 2 diabetes with and without complication is scant especially in Thai patients. Therefore, the study of the oxidative stress status may be the knowledge base for an understanding of the pathogenic mechanisms of cardiovascular complication in diabetes and may have important implications regarding antioxidant supplements in order to slow progression, select optimal therapies and prevent plaque complications and their consequences. The aim of the present study was undertaken to assess lipid peroxidation and antioxidant status in type 2 diabetic patients with and without CHD compared to normal controls.

Material and Method
1. Subjects
1.1. Control subjects
Twenty healthy subjects were a control group with FPG < 110 mg/dl. They were 12 males and 8 females. The ages ranged from 35 to 70 years old. The mean age average was 56.35 ± 1.62 years.

1.2. Type 2 diabetic patients
Type 2 diabetic patients were divided into 3 groups as followings:
1.2.1. Fairly controlled type 2 diabetic patients whose fasting plasma glucose is ≤ 180 mg/dl. This group contained 26 subjects included 13 males and 13 females. The ages ranged from 49 to 85 years old. The mean age average was 63.96 ± 1.69 years.
1.2.2. Poorly controlled type 2 diabetic patients whose fasting plasma glucose is > 180 mg/dl. This group had 19 subjects included 8 males and 11 females. The ages ranged from 39 to 83 years old. The mean age average was 62.37 ± 2.46 years.
1.2.3. Type 2 diabetes complicated with coronary heart disease (CHD). There were 20 subjects. They were 6 males and 14 females. The ages ranged from 31 to 76 years old. The mean age average was 60.50 ± 2.56 years.

All 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 and patients on insulin were excluded from the study.

Informed consent was obtained from all participants 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.

2. MDA assay in plasma

MDA in plasma was performed as described by Satoh[18]. In brief, plasma was mixed with 20% TCA and allowed to stand for 10 min. Then, 0.05 M H_2SO_4 and TBA were added. The mixture was mixed and place in a boiling water bath for 30 min. The resulting chromogen was extracted with n-butanol and centrifuged at 1871 x g for 10 min and measured against butanol blank at 532 nm excitation and 553 nm emission by spectrofluorometer.

3. Vitamin C determination in plasma

Plasma vitamin C determined as described by Mitacek[19]. Briefly, plasma was added to 10% TCA to precipitate protein. After centrifugation at 1871 x g for 10 min, clear supernatant was mixed with 4% dinitrophenylhydrazine. The mixture was incubated at 60° C for 45 min and chilled for 10 min. 65% H_2SO_4 was slowly mixed with the mixture and stand for 30 min. The absorbance was read at 520 nm against reagent blank.

4. Vitamin A, E, and β-carotene assay

These antioxidant vitamins were assay by HPLC[20]. In brief, plasma, α-tocopheryl acetate as in-ternal standard and ethanol was mixed for 15 sec. Then hexane was added and mixed vigorously for 2 min. The tube was centrifuged at 5198 x g, 4° C for 5 min. The hexane layer was transferred and evaporated under a stream of nitrogen gas. The lipid residue was dissolved in ethanol and injected into the Sphere clone 5 μ ODS, 250 x 4.60 mm of HPLC. The mobile phase was methanol: acetonitrile: chloroform (25: 60: 15) at a flow rate 1.5 ml/min. Vitamin A, E and α-tocopheryl acetate were detected at 290 nm and at 450 nm for β-carotene.

5. Statistical analysis

Results are expressed as mean ± SEM. Distribution of variables was tested for approximation to Gaussian distribution using kurtosis and skewness test. Data were compared by one-way analysis of variance (one-way ANOVA) using scheffe test for four matched groups. Pearson rank correlation test was used for testing correlation between variables. Statistical analysis was performed using SPSS 11.0 software (SPSS. Inc., Chicago. IL, USA).

Results

Table 1 showed the data of age, fasting plasma glucose, hematological data and lipid profile between the normal healthy group and the type 2 diabetic patients. This table showed that all subgroups of type 2 DM were significantly higher of FPG than normal subjects. The study of plasma lipid profile showed that the total cholesterol, LDL-cholesterol were not significantly

Table 1. Database of age, FPG and lipid profile in normal healthy subject and type 2 diabetic patients (Values are mean ± SEM)

Parameter	Normal (n=20)	Poorly controlled type 2 DM (n=19)	Fairly controlled Type 2 DM (n=26)	Type 2 DM with CHD (n=19)
Age (years)	56.35 ± 1.62	63.96 ± 1.69	62.3 ± 2.46	60.50 ± 2.56
FPG (mg/dl)	105.10 ± 3.05	245.00 ± 22.52[a]	128.50 ± 3.88[b]	167.26 ± 13.26[b]
Hb (g/dl)	14.27±0.38	11.13±.0.33[c]	12.61±0.51[a]	13.70±0.42
Hct (%)	43.56±1.09	46.66±1.80	45.00±1.46	36.50±1.38[b]
Total cholesterol(mg/dl)	217.10 ± 9.57	255.65 ± 13.05	229.87 ± 9.02	225.56 ± 13.90
LDL-cholesterol	134.24 ± 9.79	172.43 ± 12.38	156.65 ± 8.01	143.59 ± 13.55
Triglyceride(mg/dl)	133.31 ± 12.66	180.28 ± 22.59	153.58 ± 11.97	220.06 ± 20.32[b]
VLDL-cholesterol(mg/dl)	27.05 ± 2.25	36.06 ± 4.51	30.44 ± 2.44	43.51 ± 3.78[b]
HDL-cholesterol(mg/dl)	55.8 ± 2.69	47.16 ± 3.15	42.79 ± 2.42[c]	43.25 ± 1.97[b]

a = significance at p < 0.001 vs. normal
b = significance at p < 0.01 vs. normal
c = significance at p< 0.05 vs. normal

different from the normal healthy group. This was due to the treatment of cholesterol-lowering drug in these diabetic subjects. However, the increasing of trigly-ceride, VLDL-cholesterol and decreasing of HDL-cholesterol also found in type 2 DM complicated with CHD. The lower level of HDL may exacerbate the cardiovascular complication in this group of diabetes. 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.

Plasma malondialdehyde level

Plasma malondialdehyde (p-MDA) levels in these subgroups of type 2 DM (poorly controlled, fairly controlled and type 2 DM complicated with CHD) were significantly higher when compared to healthy normal group (p<0.05) as indicated in Table 2. However, when the comparisons were performed among these subgroups, significant difference was not found.

Plasma antioxidant nutrients level
Plasma vitamin C level

The vitamin C level only in poorly controlled type 2 DM was significantly lower (p<0.05) as compared with this normal group. However, no significant differences was observed between normal subjects and fairly controlled or type 2 DM with CHD (p=0.10 and p=0.13, respectively). Among each of type 2 DM subgroups, significant differences were not found: poorly controlled *vs* fairly controlled (p=0.89), poorly controlled *vs* type 2 DM with CHD (p=0.91) and fairly controlled *vs* type 2 DM with CHD (p=1.00) (Table 3).

Vitamin E status

Levels of plasma vitamin E in healthy normal group had no significant difference as compared to poorly controlled or fairly controlled DM patients (p=1.00 and p=0.92 respectively). However, when the plasma vitamin E levels were compared between this normal group and type 2 DM complicated with CHD it was found that the vitamin E level in normal group was significantly higher (p<0.001) as shown in Table 4. Among these subgroups of type 2 DM, the multiple comparisons were performed. The results showed that the vitamin E level in poorly controlled DM was not significantly different from that in controlled DM group (p=0.88) but there was significantly lower level of plasma vitamin E in type 2 DM with CHD than in poorly controlled or fairly controlled DM (p<0.001).

Table 2. Plasma malondialdehyde (p-MDA) (µmol/L) in normal subjects and type 2 diabetic patients (Values are mean ± SEM)

Subjects	p-MDA (µmol/L)
Normal (n=20)	2.29 ± 0.08
Poorly controlled type 2 DM (n=19)	3.44 ± 0.18[c]
Fairly controlled type 2 DM (n=26)	3.30 ± 0.18[c]
Type 2 DM with CHD (n=20)	3.45 ± 0.36[c]

c = significance at p < 0.05 vs. normal

Table 4. Plasma vitamin E (µg/ml) in normal subjects and type 2 diabetic patients (Values are mean ± SEM)

Subjects	Vitamin E (µg/ml)
Normal (n=20)	19.00 ± 1.18
Poorly controlled type 2 DM (n=19)	19.23 ± 1.40
Fairly controlled type 2 DM (n=26)	17.86 ± 1.15
Type 2 DM with CHD (n=20)	10.39 ± 0.97[a]

a = significance at p < 0.001 vs. normal

Table 3. Plasma vitamin C (µg/dl) in normal subjects and type 2 diabetic patients (Values are mean ± SEM)

Subjects	Vitamin C (µg/dl)
Normal (n=20)	1.16 ± 0.05
Poorly controlled type 2 DM (n=19)	0.85 ± 0.07[c]
Fairly controlled type 2 DM (n=26)	0.93 ± 0.05
Type 2 DM with CHD (n=20)	0.93 ± 0.09

c = significance at p < 0.05 vs. normal

Table 5. Plasma vitamin A (µg/dl) in normal subjects and type 2 diabetic patients (Values are mean ± SEM)

Subjects	Vitamin A (µg/dl)
Normal (n=20)	114.05 ± 6.95
Poorly controlled type 2 DM (n=19)	131.25 ± 8.46
Fairly controlled type 2 DM (n=26)	131.53 ± 5.57
Type 2 DM with CHD (n=20)	96.71 ± 7.27

Plasma vitamin A level

The plasma vitamin A level of healthy normal group showed no significant difference when compared with poorly controlled DM (p=0.43), fairly controlled DM (p=0.35) or type 2 DM with CHD (p=0.41). The level of plasma vitamin A in poorly controlled DM was also not significantly different from controlled DM (p=1.00) but as compared the type 2 DM with CHD to the poorly controlled or controlled DM, the CHD complicated type 2 DM had significantly less amount of vitamin A (p<0.05 and p<0.01, respectively) (Table 5).

Plasma β-carotene level

The plasma β-carotene level among normal healthy group were compared with each of type 2 DM subgroups (poorly controlled, fairly controlled and type 2 DM with CHD) showed no significant difference (p = 0.99, 0.85 and 1.00, respectively). When these subgroups were compared to each others, they also

showed no significant difference as shown in Table 6.

Correlation analysis

Results of Pearson correlation analysis between FPG and other laboratory parameters in type 2 diabetic mellitus patients and control group appear in Table 7. In correlation analysis of FPG to other parameters, no significant correlations were detected in fairly controlled DM except HDL-cholesterol.

Discussion

Peroxidation of polyunsaturated fatty acids in blood produces malondialdehyde (MDA), a secondary breakdown product of lipid peroxidation leading to oxidative damage. Oxidative damage to polyunsaturated lipids by free radical process is a widely accepted mechanism for cellular and tissue injury. The rise in MDA indicated that any oxidative stress incurred sufficiently cause of free radical-mediated peroxidation of lipid components in cell membrane[21]. Therefore, 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 plasma of all patients' groups comparing to their age-matched healthy subjects. However, the plasma MDA levels were not different among all patients' groups. These may be due to the enhancement of the plasma lipid peroxide removal by aldehyde dehydrogenase enzyme in liver mitochondria. This enzyme has function to destroy toxic aldehyde and pro-

Table 6. Plasma β-carotene (μg/dl) in normal subjects and type 2 diabetic patients (Values are mean ± SEM)

Subjects	β-carotene (μg/dl)
Normal (n=20)	22.06 ± 4.54
Poorly controlled type 2 DM (n=19)	20.52 ± 4.01
Fairly controlled type 2 DM (n=26)	17.53 ± 2.17
Type 2 DM with CHD (n=20)	21.98 ± 4.27

Table 7. Correlation analysis between FPG and other laboratory parameters in type 2 diabetic patients and control group

Parameters	Poorly controlled DM (n=39)	Fairly controlled DM (n=46)	DM with CHD (n=40)
Total cholesterol	0.41[a]	0.08	0.16
LDL-cholesterol	0.41[a]	0.16	0.10
VLDL-cholesterol	0.38[a]	0.16	0.38[a]
Triglyceride	0.39[a]	0.18	0.37[a]
HDL-cholesterol	-0.44[a]	-0.39[a]	-0.35[a]
p-MDA	0.70[c]	0.28	0.41[b]
vitamin C	-0.29	-0.27	-0.14
vitamin E	0.11	-0.19	-0.32[a]
vitamin A	0.13	0.24	-0.28
β-carotene	-0.09	-0.10	-0.004

a significant at p< 0.05 vs. normal
b significant at p< 0.01 vs. normal
c significant at p< 0.001 vs. normal

tects tissue aldehyde accumulation[22]. In addition, plasma MDA can be moderated by enhancement of the degradation of excretion[23]. These results demonstrated that diabetic patients were prone to accumulation of potentially harmful oxidative stress. These findings are consistent with the reports of the others[7,24]. The determinations of antioxidant vitamins to prevent lipid peroxidation were also performed in this study. Many researchers reported the role of antioxidant vitamins including vitamin C, vitamin E, vitamin A, and β-carotene to defense damage by ROS in human diseases such as cancer, inflammation, and arthritis. Diabetes mellitus is another interesting one and it is currently under study. Our work was designed to investigate the differences in these dietary vitamins among various condition of diabetic patients and normal subjects. We found that the antioxidant of plasma vitamin C level was significantly reduced only in poorly controlled type 2 patients. The other type 2 DM subgroups in this study also had lower vitamin C levels than in the normal group but failed to achieve significance at the 95% confidence interval. The active transport of vitamin C (ascorbic acid or AA) appears to be decreased by hyperglycemia[24] and insulin deficiency[25]. Hyperglycemia has also been shown to inhibit uptake of dehydroascorbic acid (DHA), the oxidized species of AA. A decreased ability of cells to take up and store AA would result in an increased urinary loss of this important antioxidant because extracellular AA could not be stored. The chronic high glucose levels could down-regulate and impair DHA uptake relevant in chronic poorly controlled hyperglycemic patients. This caused the decrement of AA[26]. For the mean value of plasma vitamin E, only type 2 DM with CHD of this study was shown to be significantly lower than that in the normal group whereas the other groups of patients were not. This result corresponded to the deleterious effect on vascular wall such as atherosclerosis in this group as reported by Miwa et al[27] and Diaz et al[28]. Horwitt et al[29] suggested that total lipid content has an influence on the plasma vitamin E level since vitamin E is mainly found in LDL particles. There is evidence that vitamin E : cholesterol ratio is a more reliable criterion for vitamin E status than plasma vitamin E alone[30]. This is because the use of this ratio can correct for conditions that result in increase plasma lipid levels[31]. Thus, vitamin E status should be observed and compared again with this ratio. Nevertheless, the similar result obtained from the investigation of plasma vitamin E ratio that only CHD patient's group had significantly decreased in the vitamin E ratio comparing to the normal

subjects (data was not shown). There are several reports that have been considered in the effect of low vitamin E level on sensitivity of red blood cells to oxidative damage. The depletion of vitamin E on the hematopoietic system of several species has shown the changes in red cell mass, size and increase in peroxidation of cell membrane[32,33]. The lower levels of vitamin E status and high levels of plasma MDA in CHD complicated type 2 patients may be supported by these evidences. In the observation of plasma vitamin A and β-carotene levels, it was demonstrated that no significant different in levels of these antioxidants were noted between any patients' groups and their normal groups. It may be proposed that vitamin A and β-carotene was not deficient in these patients corresponding to the other reports[34-36].

To prove the hypothesis whether hyperglycemia can induce the oxidative damage in diabetes mellitus, the association between fasting plasma glucose and either plasma MDA or vitamin E were investigated. The results in almost every patients' groups combined with their matched normal groups indicated that there were fair correlations between FPG and plasma MDA (r = 0.70; p<0.001 for poorly controlled type 2 DM, r = 0.41; p<0.01 for type 2 DM with CHD) except in the fairly controlled type 2 DM (r = 0.28; p>0.05). This correlation corresponded to the negative correlation of FPG and vitamin E in type 2 DM with CHD (r = -0.32, p< 0.05). The results of our study agree with the hypothesis that chronic hyperglycemia induces oxidative stress in diabetic patients[37].

In various studies, HbA$_{1c}$ level was noted as showing positive correlations with total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and negative correlation with HDL-cholesterol in DM patients[38-40]. Similar correlations were detected in fasting plasma glucose and total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, and HDL-cholesterol in poorly controlled type 2 DM patients, as reported in the literature.

Conclusion

In conclusion, our study supported the hypothesis that hyperglycemia activated cellular and tissue damage by oxidative stress. However, there were compensatory mechanisms for defense against the ROS. Normalization of oxidative stress was not achieved in the diabetic patients, even in the fairly controllable diabetics. Thus, supplementation of nutritional antioxidants may be useful to reduce the oxidative damage for treatment in diabetic patients.

References

1. Nesto RW. Diabetes and heart disease. In: Krall LP, editor. World book of diabetes in practice volume 3. Amsterdam: Elsevier; 1988: 256-9.
2. Gallou G, Ruelland A, Legras B, Maugendere D, Allanic H, Cloarec L. Plasma malondialdehyde in type I and type II diabetic patients. Clin Chim Acta 1993; 214: 227-34.
3. Vantyghem MC, Balduyck M, Zerimech F, Martin A, Douillard C, Bans S, et al. Oxidative markers in diabetic ketoacidosis. J Endocrinol Invest 2000; 23: 732-6.
4. Jakus V. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. Bratisl Lek Listy 2000; 101: 541-51.
5. Baynes JW, Thorpe SR. Glycoxidation and lipoxidation in atherogenesis. Free Radic Biol Med 2000; 28: 1708-16.
6. Bunker VW. Free radicals, antioxidants and ageing. Med Lab Sci 1992; 49: 299-312.
7. Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, et al. Enhanced serum levels of thiobarbituric-acid reactive substances in diabetes mellitus. Am J Med 1995; 98: 469-75.
8. Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci 1996; 90: 255-60.
9. Collier A, Rumley A, Rumley AG, Paterso JR, Leach JP, Lowe GD, et al. Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Diabetes 1992; 41: 909-13.
10. Kedziora-Kornatowska KZ, Luciak M, Blaszczyk Pawlak W. Lipid peroxidation and activities of antioxidant enzymes in erythrocytes of patients with non-insulin dependent diabetes mellitus with or without nephrophaty. Nephrol Dial Transpant 1998; 13: 2829-32.
11. Dincer Y, Alademir Z, Ilkova H, Akcay T. Susceptibility of glutathione and glutathione-related antioxidant activity to hydrogen peroxide in patients with type 2 diabetes: effect of glycemic control. Clin Biochem 2002; 297-301.
12. Vijayalingam S, Parthiban A, Shanmugasundaram KR, Mohan V. Abnormal antioxidant status in impaired glucose tolerance and non-insulin-dependent diabetes mellitus. Diabetic Med 1996; 13: 715-9.
13. Yoshida K, Hirokawa J, Tagami S, Kawakami Y, Urata Y, Kondo T, et al. Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione systhesis and efflux. Diabetologia 1995; 38: 201-10.
14. Ceriello A, Bortolotti N, Falleti E, Taboga C, Tonutti L, Crescentini A, et al. Total radical-trapping antioxidant parameter in NIDDM patients. Diab Care 1997; 20: 194-7.
15. Inouye M, Mio T, Sumino K. Glycated hemoglobin and lipid peroxidation in erythrocytes of diabetic patients. Metabolism 1999; 48: 205-9.
16. Nurooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritchler H, Rosen P, Halliwell B, et al. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia 1997; 40: 647-53.
17. Van der Jagt DJ, Harrison JM, Ratliff DM, Hunsaker LA, Van Der Jagt DL. Oxidative stress indices in IDDM subjects with and without long-term diabetic complications. Clin Biochem 2001; 34: 265-70.
18. Satoh K. Serum lipid peroxides in cerebro-vascular disorder. Clin Chim Acta 1978; 28: 37-43.
19. Mitacek EJ. Biochemistry laboratory manual. 2nd ed. New York: Burgess International, Increase; 1988: 135.
20. Miller KW, Yang CS. An isocratic high-performance liquid chromatrography method for the simultaneous analysis of plasma retinal, α-tocopherol and various carotenoids. Anal Biochem 1985; 145: 21-6.
21. Jenkin RR. Free radical chemistry relationship to exercise. Sports Med 1988; 5: 156-70.
22. Kretzcheman M, Muller D, Hubscher J, Marin E, Klinger W. Influence of aging, training and acute physical exercise on plasma glutathione and lipid peroxides in man. Int J sports Med 1991; 12: 215-22.
23. Partiban A, Vijayalingam S, Shanmugasundaram KR, Mohan R. Oxidative stress and the development of diabetic complications-antioxidants and lipid peroxidation in erythrocytes and cell membrane. Cell Biol Int 1995; 19: 987-93.
24. Beaudeux JL, Guillausseau PJ, Peynet J, Flourie F, Assayag M, Tielman D, et al. Enhanced susceptibility of low-density lipoprotein to in vitro oxidation in type 1 and type 2 diabetic patients. Clin Chim Acta 1995; 239: 131-41.
25. Moser U. Uptake of ascorbic acid by leukocytes. Ann NY Acad Sci 1987; 498: 200-15.
26. Verlangieri AJ, Sestio J. Effect of insulin on ascorbic acid uptake by heart endothelial cells: possible relationship to retinal atherogenesis. Life Sci 1981; 29: 5-9.

27. Ngkeekwong FC, NG LL Two distinct uptake mechanisms for ascorbate and dehydroascorbate in human lymphoblasts and their interaction with glucose. Biochem J 1997; 324(Pt 1): 225-30.

28. Miwa K, Igawa A, Nakagawa K, Hirai T, Inoue H. Consumption of vitamin E in coronary circulation in patients with variant angina. Cardiovasc Res 1999; 41: 291-98.

29. Diaz MN, Frei B, Vita JA, Keaney JFJ. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997; 337: 408-16.

30. Horwitt HK, Harvey CC, Dahm CH, Searcy MT. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Ann NY Acad Sci 1972; 203 233-6.

31. Dithurnham, Jadavies, Crump BJ, Situnayake RD, Davis M. The use of different lipids to express serum tocopherol : lipid ratios for the measurement of vitamin E status. Ann Clin Biochem 1986; 23: 514-20.

32. Brody T. Nutritional biochemistry. San Diego (CA): Academic Press; 1994: 459-76.

33. Kayden HJ, Bjoranson L. The dynamics of vitamin E transport in the human erythrocyte. Ann NY Acad Sci 1972; 203: 127-39.

34. Olmedilla B, Granado F, Gil-Martinez E, Blanco I. Reference levels of retinol, αtocopherol and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. Clin Chem 1997; 43: 1066-71.

35. Olmedilla B, Granado F, Southon S, Wright AJ, Blanco I, Gil-Mar tines E, et al. Baseline serum concentrations of carotenoids, vitamins A, E, and C, in control subjects from five European countries. Br J Nutr 2001; 85: 227-38.

36. Granado F, Olmedilla B, Botella F, Simal A, Blanco I. Retinol and α-tocopherol in serum of type 1 diabetic patients with intensive insulin therapy: a long term follow-up study. App Nutr Invest 2003; 19: 128-32.

37. Varvarovska J, Racek J, Stozicky F, Soucek J, Trefil L, Pamahacova R. Parameters of oxidative stress in children with type 1 diabetes mellitus and their relatives. J Diab Complic 2003; 17: 7-10.

38. Strobl W, Widhalm K, Schober E, Frisch H, Pollak A, Westphal G. Apolipoproteins and lipoproteins in children with type 1 diabetes: relation to glycosylated serum protein and HbA$_{1c}$. Acta Paediatr Scand 1985; 74: 966-71.

39. Andersen GE, Christiansen JS, Mortensen HB, Christiansen KM, Pedersen-Bjergaard L, Kastrup KW, et al. Plasma lipids and lipoproteins in type 1 diabetic children and adolescent in relation to metabolic regulation, obesity and genetic hyperlipoproteinemia. Acta Paediatr Scand 1983; 72: 361-5.

40. Ohta T, Nishiyama S, Nakamura T, Saku K, Maung KK, Matsuda I. Predominance of large low density lipoprotein particles and lower fractional esterefication rate of cholesterol in high density lipoprotein in children with insulin-dependent diabetes mellitus. Eur J Pediatr 1998; 157: 276-81.

ระดับไลปิดเปอร์ออกซิเดชั่นและสารอาหารแอนติออกซิแดนท์ในพลาสม่าของผู้ป่วยเบาหวานประเภทที่ 2

ธวัชชัย พีรพัฒน์ดิษฐ์, ณัฏฐ์ชัย ภัทร์ชนันท์, อธิป ลิขิตลิลิต, สายฝน ผลดี, ชาญ ศรีรัตนสถาวร

ภูมิหลังและวัตถุประสงค์: *ผู้ป่วยเบาหวานมักจะอ่อนไหวต่อภาวะออกซิเดทีฟสเตรสเนื่องจากการมีน้ำตาลในเลือด สูง การเพิ่มขึ้นของระดับน้ำตาลในเลือดเป็นสาเหตุสำคัญที่ทำให้มีการเพิ่มของอนุมูลอิสระ ซึ่งการเพิ่มขึ้นของอนุมูล อิสระนี้จะเร่งการเกิดโรคแทรกซ้อนโดยเฉพาะโรคที่เกี่ยวกับระบบหลอดเลือดในผู้ป่วยเบาหวาน ดังนั้นการศึกษานี้มี วัตถุประสงค์เพื่อตรวจหาภาวะออกซิเดทีฟสเตรสที่สัมพันธ์กับปัจจัยที่ทำให้เกิดโรคแทรกซ้อนในผู้ป่วยเบาหวาน ประเภทที่ 2 ซึ่งยังไม่มีการศึกษากันมากนัก ด้วยเหตุนี้การเปรียบเทียบระหว่างระดับของ MDA (มาลอนไดอัลดีไฮด์) และสารอาหารที่เป็นแอนติออกซิแดนท์ในพลาสม่ากับคนปกติที่มีอายุใกล้เคียงกันจึงถูกใช้เพื่อประเมินความว่องไวต่อ การเกิดภาวะออกซิเดทีฟในผู้ป่วยเบาหวานประเภทที่ 2 นี้*

วัสดุและวิธีการ: *วิเคราะห์ระดับของ MDA โดยใช้เครื่อง spectrofluorometer และสารอาหารแอนติออกซิแดนท์ (ไวตามิน A, C, E และ b-carotene) โดยใช้ HPLC ในพลาสม่าของผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลไม่ดี จำนวน 19 ราย (มี FPG >180 มก./ดล.), ในผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลได้ดีจำนวน 26 ราย (มี FPG <180 มก./ดล.) และในผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อน จำนวน 20 ราย และในคนปกติ (มี FPG <110 มก./ดล.) โดยมีอายุและเพศใกล้เคียงกัน ผลของการทดลองของผู้ป่วยเบาหวานประเภทที่ 2 ทุกกลุ่ม เปรียบเทียบกับคนปกติจะใช้ ANOVA test และสหสัมพันธ์ระหว่างระดับน้ำตาลในเลือดกับตัวแปรของภาวะ ออกซิเดทีฟสเตรสจะใช้ Pearson rank correlation coefficient*

ผลการศึกษา: *ระดับของ MDA ในพลาสม่าของผู้ป่วยเบาหวานประเภทที่ 2 จะเพิ่มสูงขึ้นอย่างมีนัยสำคัญในผู้ป่วย เบาหวานประเภทที่ 2 ทุกกลุ่มเมื่อเทียบกับคนปกติ ระดับของสารอาหารแอนติออกซิแดนท์ในพลาสม่ามีเฉพาะไวตามิน C ที่มีค่าลดลงอย่างมีนัยสำคัญในผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมระดับน้ำตาลไม่ดี ส่วนไวตามิน E มีค่าลดลง อย่างมีนัยสำคัญในผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อน สำหรับระดับของไวตามิน A และ β-carotene พบว่าไม่มีความแตกต่างกันในผู้ป่วยเบาหวานทุกกลุ่มกับคนปกติ สหสัมพันธ์ระหว่าง FPG และ MDA จะพบได้ในผู้ป่วยเบาหวานประเภทที่ 2 ทุกกลุ่ม ยกเว้นผู้ป่วยเบาหวานประเภทที่ 2 ที่ควบคุมน้ำตาลได้ดี ส่วน สหสัมพันธ์ระหว่าง FPG และ vitamin E จะพบได้ในผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อน เท่านั้น*

สรุป: *ผลการทดลองนี้แสดงให้เห็นว่าผู้ป่วยเบาหวานมีความว่องไวต่อภาวะออกซิเดทีฟสเตรส และระดับของกูลโคส ในพลาสม่าจะสัมพันธ์กับกระบวนการเกิดไลปิดเปอร์ออกซิเดชั่นที่เกิดจากอนุมูลอิสระ ระดับของไวตามิน อี ที่ต่ำที่สุดใน พลาสม่าในผู้ป่วยเบาหวานประเภทที่ 2 ที่มีโรคหลอดเลือดหัวใจแทรกซ้อน ชี้แนะว่าภาวะออกซิเดทีฟสเตรสแสดง บทบาทที่สำคัญในโรคแทรกซ้อนนี้และการเสริมไวตามิน อี อาจจะจำเป็นต่อการรักษาและการป้องกันโรคแทรกซ้อน ในกลุ่มผู้ป่วยเบาหวาน*