Plasma Lipid Peroxide and Antioxidant Levels in Diabetic Patients

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Abstract

The present study was to investigate the levels of plasma lipid peroxide products including malondialdehyde (MDA) and conjugated dienes (CD), and antioxidants including enzyme superoxide dismutase, glutathione peroxidase, catalase, plasma vitamin E and vitamin C in diabetic patients. Fifty-eight diabetic subjects; 16 males and 42 females, aged 30-75 years, were recruited. Eighteen of them had diabetes and forty of them had diabetes with hyperlipidemia. Twenty-seven healthy subjects, 8 males and 19 females, aged 30-75 years, were used as the control group.

The results showed that the concentrations of plasma MDA in diabetic patients with or without hyperlipidemia tended to be increased when compared to the controls but there were no significant differences. The CD values were increased significantly in both diabetic groups when compared with control subjects. Significantly elevated levels of plasma MDA and CD were found in diabetic patients with hypertriglyceridemia (>150 mg%). This increment did not change the antioxidant status in both enzymes and vitamins except that the plasma vitamin E levels and the ratios of tocopherol: cholesterol were increased significantly. An increase of lipid peroxide in plasma may be one important factor in the development of vascular complication and atherosclerosis seen in diabetic patients.

Oxygen free radicals are highly reactive compounds with unpaired electrons capable of initiating oxidation. They are continuously generated by normal metabolic pathways as a physiological process(1). Reactive free radicals formed within cells can oxidize biomolecules including protein, deoxyribonucleic acid and lipid which is the most susceptible, thus can lead to tissue injury and cell death. Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids and their esters and are capable of further lipoperoxide production by a free radical chain reaction(2). The

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oxidative damage to these biologic macromolecules has been suggested to contribute to many human degenerative diseases such as atherosclerosis, certain types of cancer and cataract[3].

Diabetes mellitus is a complex syndrome involving severe insulin dysfunction in conjunction with gross abnormality in glucose homeostasis and lipid metabolism[4]. Lipid peroxide levels in diabetic plasma have been found to be significantly higher than in healthy individuals[5]. Also Sato et al[6] reported an increase in thiobarbituric acid reaction in these patients especially in poorly controlled diabetics and diabetics with angiopathy[7]. This elevation has been considered as a cause of the degeneration of organ or tissue. It was also considered that the lipid peroxide formed in the primary site would be transferred *via* blood to other organs or tissues where the damage would be provoked by the propagation of lipid peroxidation. Oxygen free radical damage is usually prevented by a series of enzymatic defences such as superoxide dismutase, glutathione peroxidase and catalase and by a series of vitamins such as vitamin C, vitamin E and ß-carotene[8]. In addition, various sources of free radicals may modulate oxidative stress in diabetes including non enzymatic glycosylation of proteins and monosaccharide autooxidation, polyol pathway activity, indirect production of free radicals through cell damage from other causes and reduced antioxidant reserve[9-11]. There are scant reports about the antioxidant reserve in this disease. Hence, we attempted to measure lipid peroxidative products together with the antioxidant status including enzymes and vitamins in diabetic patients.

SUBJECTS AND METHOD

This study was conducted on 58 diabetic patients, 16 males and 42 females, 30-75 years of age, attending the medical clinic of Ramathibodi Hospital. Most of them (43 persons) who had hemoglobin A1c levels more than 8 per cent were considered as poorly controlled diabetes. Forty of them were hyperlipidemia. Twenty-seven healthy subjects; 8 males and 19 females, aged 30-75 years, were used as the control group. None of the subjects in this study had smoked, drunk alcohol or taken vitamins within 2 months before the study.

Fasting samples of venous heparinized blood were obtained from all subjects. Plasma was separated by centrifugation and immediately frozen at -80°C for analysis of lipid peroxide including malondialdehyde (MDA)[12,13] and conjugated diene (CD)[14], vitamin E (VE)[15] and vitamin C (VC)[16]. The erythrocytes were washed twice with equal volume of 0.9 per cent NaCl and centrifuged. After the final wash, packed cells were lysed with deionized water by 1:1.5 and stored at -80°C for analysis of superoxide dismutase (SOD)[17], glutathione peroxidase[18] and catalase[19]. Both plasma and red blood cell suspension were analyzed within 2 weeks.

Statistical analysis

All data were expressed as mean ± SD. Comparison between the two groups was performed by unpaired student *t* test. Significant level was set at $p<0.05$.

RESULT

The clinical characteristics of control and diabetic subjects are shown in Table 1. The mean ages did not differ among the three groups. The mean weights of diabetic patients with or without hyperlipidemia were significantly higher than that of the controls at $p<0.0001$ and $p<0.003$ respectively. The results in Table 2 show that the concentrations of plasma glucose were significantly higher in both diabetic groups. However, the levels of serum cholesterol, triglyceride and LDL-cholesterol increased significantly in diabetic with hyperlipidemia patients but HDL-cholesterol levels were decreased. As indicated in Table 3, the concentrations of plasma MDA in diabetics tended to be increased compared to the controls but there was no significant difference. The CD values were increased significantly in both diabetic groups when compared with the control subjects. Significantly elevated levels of plasma MDA and CD were found in diabetic patients with hypertriglyceridemia (>150 mg%) as shown in Table 4. However, the antioxidant levels including SOD, glutathione peroxidase, catalase and VC did not differ among the three groups except plasma VE levels as well as the ratios of tocopherol to cholesterol which were significantly increased in the diabetic group with hyperlipidemia. (Tables 3, and 4)

DISCUSSION

A major limitation in this area of research has been the absence of an accurate and reliable

Table 1. Clinical characteristics of control and diabetic subjects.

	Controls (n = 27)	Diabetics (n = 18)	Diabetics with hyperlipidemia (n = 40)
Age (yrs)	49.3 ± 10.2	55.2 ± 13.0	53.4 ± 10.6
Sex, male / female	8 / 9	6 / 12	10 / 30
Weight (kgs)	57.7 ±10.3	70.7 ± 15.2 a1	71.4 ± 12.8 a2
Height (cms)	158.1 ± 6.13	160.0 ± 8.8	157.8 ± 6.5
Duration of diabetes (yrs)	-	7.9 ± 5.3	9.1 ± 7.2
Treatment, OHA* /OHA with insulin	-	18 / 0	35 / 5

a1, a2 Significant difference compared to controls at $p < 0.003$, < 0.0001 respectively.
* Oral hypoglycemic agents

Table 2. Hematological and biochemical measurements in control and diabetic subjects.

Parameter	Controls (n = 27)	Diabetics (n = 18)	Diabetics with hyperlipidemia (n = 40)
Hct (%)	41.5 ± 3.60	41.7 ± 3.44	41.4 ± 4.21
Hb (g%)	13.4 ± 1.30	13.3 ± 1.10	13.6 ± 1.52
Glucose, mmol / L	5.13 ± 0.48	10.30 ± 3.97[a]	11.17 ± 3.57 [a]
Cholesterol, mmol / L	5.36 ± 0.79	5.18 ± 0.59	6.47 ± 0.98 [b]
Triglyceride, mmol / L	1.29 ± 0.59	1.27 ± 0.32	2.41 ± 1.20 [b]
HDL - cholesterol, mmol / L	1.48 ± 0.38	1.39 ± 0.35	1.24 ± 0.26 [c]
LDL - cholesterol, mmol / L	3.51 ± 0.74	3.10 ± 0.61	4.12 ± 0.98 [c]

[a] significant difference at $p < 0.0001$, compared to controls.
[b, c] significant difference from the control and diabetic group at $p < 0.0001$, 0.01 respectively.

Table 3. Plasma lipid peroxide and antioxidant levels in control and diabetic subjects.

Parameter	Controls (n = 27)	Diabetics (n = 18)	Diabetics with hyperlipidemia (n = 40)
MDA*, μmol / L	1.16 ± 0.29	1.22 ± 0.40	1.37 ± 0.69
CD**, OD / ml	0.45 ± 0.09	0.51 ± 0.08 a1	0.65 ± 0.20 a2
Vitamin C, mg%	1.09 ± 0.37	0.86 ± 0.39 [b]	1.02 ± 0.41
Vitamin E, μmol / L	33.0 ± 6.20	32.70 ± 5.02	46.40 ± 13.23 [c]
Vitamin E / Chol, mg / g	6.78 ± 1.16	6.97 ± 1.21	8.11 ± 2.50 [c]
Glu. Peroxidase ***, IU / gHb	24.74 ± 6.12	28.72 ± 7.94	27.21 ± 5.32
SOD****, U /g Hb	2512 ± 208	2549 ± 307	2628 ± 314
Catalase, IU x 10^4 / gHb	11.72 ± 1.88	10.71 ± 1.73	11.58 ± 2.52

* Malondialdehyde, ** Conjugated diene, *** glutathione peroxidase, **** Superoxide dismutase
a1, a2 Significant difference at $p < 0.0025$, 0.0001 respectively, as compared to controls
[b] Significant difference from the control at $p < 0.05$
[c] Significant difference from the control and diabetic group at $p < 0.005$

measurement of free radical activity. Oxygen free radicals react with lipids to produce lipid peroxide product. Measurement of lipid peroxide concentrations by malondialdehyde thiobarbituric acid (MDA-TBA) assay is the most widely recognised measure of free radical activity used in clinical research. Conjugated diene (CD), an intermediate of fatty acid oxidation, was also examined in this study. Our finding indicated that plasma MDA levels had a tendency to be increased but not sig-

Table 4. Plasma lipid peroxide and antioxidant levels in control subjects and diabetic patients with hypertriglyceridemia (> 150 mg%).

Parameter	Controls (n = 27)	Dibetics with hypertriglyceridemia (n = 28)
MDA, μmol / L	1.16 ± 0.29	1.53 ± 0.73 [a]
CD, OD / ml	0.45 ± 0.09	0.70 ± 0.20 [b]
Vitamin C, mg%	1.09 ± 0.37	1.13 ± 0.39
Vitamin E, μmol / L	33.50 ± 6.20	49.33 ± 13.26 [b]
Vitamin E / Chol, mg / g	6.78 ± 1.16	8.70 ± 2.54 [c]
Glu. Peroxidase, IU / gHb	24.74 ± 6.12	26.79 ± 6.00
SOD, U /g Hb	2512 ± 208	2609 ± 340
Catalase, IU x 104 /g Hb	11.72 ± 1.88	11.61 ± 2.79

a, b, c significant difference at $p < 0.025$, 0.0001, 0.001 respectively, compared to controls.

nificantly different in the diabetic group. Concomitantly, the values of CD were increased (Table 3). Significantly elevated levels of MDA and CD were found in plasma of diabetic patients with hypertriglyceridemia (Table 4) and there was a positive correlation between MDA and CD ($r=0.38$, $p=0.047$). However, the report of Sinclair[20] found that TBA reacting concentrations did not differ between control subjects and diabetic patients with or without microangiopathy. Their patients had normal lipidemia. Most investigators reported the evidence of increased lipid peroxidation[6,7] and other free radical reaction products in diabetes[21,22]. Epidemiological studies suggested that the level of lipid peroxides in human plasma is associated more with hypertriglyceridemia and vascular itself rather than directly with diabetes[23]. The changes in lipid peroxidation in this study did not change the antioxidant levels such as SOD, glutathione peroxidase, catalase and VC except that plasma VE levels as well as the ratio of tocopherol to cholesterol were increased significantly in the diabetic group with hyperlipidemia. This increment was in agreement with a previous report[24]. Moreover, Vatassery et al demonstrated an increase in concentrations of tocopherol in platelets. The tocopherol : cholesterol ratio is usually a more reliable criterion for vitamin E status than tocopherol alone[25]. In view of the known ability of VE to inhibit platelet aggregation[26], it seems likely that the reported increase in VE in diabetics with hyperlipidemia may be an attempt to compensate for increased adhesiveness of diabetic patients.

Lipid peroxidation has been implicated in vascular endothelial damage[23]. It is intimately involved in prostaglandin biosynthesis, stimulating both cyclooxygenase and thromboxane synthesis and simultaneously inhibiting prostacyclin production resulting in enhanced platelet aggregation[27]. It is possible that VE excess in diabetics with hyperlipidemia plays a protective role against the tendency for increased platelet aggregability in diabetes. Hence, an increase of lipid peroxide products was important in the development of atherosclerosis that may be a cause of the increased morbidity and mortality found in diabetes.

We conclude that diabetic patients with hypertriglyceridemia who have high levels of lipid peroxide are the high risk group for development of atherosclerosis. Although our finding demonstrated that this group did not alter antioxidant status in both enzymes and vitamins, we recommended that they should increase their intake of antioxidant vitamins or supplement of antioxidant vitamins especially vitamin C which may prove to be effective in decreasing vascular complication and also attenuate the pathogenesis of these diseases at advancing age.

SUMMARY

Our finding indicated that diabetic patients with hypertriglyceridemia had increased levels of lipid peroxides including MDA and CD. This increment did not change the antioxidant status in both enzymes and vitamins except plasma VE levels and the ratios of tocopherol : cholesterol increased significantly. An increase of lipid pero-

xide in plasma may be one important factor in the development of vascular complication seen in diabetic patients.

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การศึกษาระดับไลปิดเปอร์ออกไซด์และแอนติอ็อกซิแดนท์ในพลาสมาของผู้ป่วยเบาหวาน

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ได้ทำการศึกษาระดับไลปิดเปอร์ออกไซด์และแอนติอ็อกซิแดนท์ในพลาสมาของผู้ป่วยเบาหวานจำนวน 58 ราย เป็นเพศชาย 16 ราย เพศหญิง 42 ราย อายุ 30–75 ปี และศึกษาในคนปกติจำนวน 27 ราย เป็นเพศชาย 8 ราย เพศหญิง 19 ราย อายุ 30–75 ปี จากการศึกษาพบว่าผู้ป่วยเบาหวานที่มีระดับไตรกลีเซอไรด์ในซีรัมสูง (> 150 มก%) จะมีระดับ MDA และ CD ในพลาสมาสูงกว่าคนปกติ โดยไม่มีผลต่อการเปลี่ยนแปลงของระดับแอนติอ็อกซิแดนท์ ได้แก่ SOD, glutathione peroxidase, catalase, และ VC ยกเว้นระดับพลาสม่า VE และ สัดส่วนของ tocopherol ต่อ cholesterol ที่พบว่ามีการเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ซึ่งการเพิ่มขึ้นของพลาสมาไลปิดเปอร์อ็อกไซด์น่าจะเป็นปัจจัยอันหนึ่งที่ทำให้เกิดภาวะแทรกซ้อนเกี่ยวกับผนังหลอดเลือดในผู้ป่วยเบาหวานได้

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