Int J Clin Lab Res 25: 162–164, 1995

International Journal of Clinical Laboratory RESEARCH

Plasma and urine malondialdehyde levels in non-insulin-dependent diabetic patients with and without microalbuminuria

T. Özben, S. Nacitarhan, N. Tuncer

Department of Biochemistry, Faculty of Medicine, Akdeniz University, TR-07060 Antalya, Turkey

Received: 20 April 1995 / Accepted: 15 June 1995

Summary. Malondialdehyde, a marker of lipid peroxidation, was measured as thiobarbituric acid-reactive substance in 78 non-insulin-dependent diabetic patients and 28 healthy subjects. Patients were divided into groups and subgroups according to the presence of microalbuminuria and other complications. Plasma and urine malondialdehyde concentrations were significantly higher in patients with and without microalbuminuria than in controls. In contrast to urine malondialdehyde, plasma malondialdehyde levels were significantly higher in microalbuminuric diabetes than in the normoalbuminuric diabetic group. There was no correlation between malondialdehyde concentration and glycemic control. This study confirmed the existence of lipid peroxidation disorders in diabetic patients.

Key words: Malondialdehyde – Lipid peroxidation – Non-insulin-dependent diabetes mellitus – Microalbuminuria – Diabetic nephropathy

Introduction

The shorter life expectancy of diabetic patients is due to vascular complications [1]; lipid peroxidation may be involved in the development of these complications. Free radicals are reactive chemical species with unpaired electrons which can react with virtually all cell components. The potential consequences of these reactions include oxidation and peroxidation of membrane lipids, denaturation of proteins, generation of chemotactic factors, impairment of collagen synthesis, disruption of membrane permeability, and increased inflammatory cell infiltration [2]. As a consequence, free radical activity has been implicated in inflammation, aging, malignant change, and vascular damage [3, 4]. Diabetics are more prone to develop renal diseases than the normal population [5]. An early marker for the development of diabetic nephropathy is increased excretion of albumin, so called microalbuminuria [6–8]. Excretion of other proteins and sialic acid sometimes precedes the onset of microalbuminuria [9, 10]. Abnormal lipid peroxidation may be detected in diabetic patients [1, 2, 11–13]. However, the role of oxygenated free radicals in long-term vascular complications is still hypothetical. In this study, we measured malondialdehyde (MDA) concentrations as thiobarbituric acid-reactive substances (TBARS) in diabetic patients with and without microalbuminuria and compared these with a control group. The results were analyzed in relation to the presence or absence of vascular complications other than microalbuminuria.

Correspondence to: T. Özben

Materials and methods

Subjects. Seventy-eight non-insulin-dependent diabetic patients (26 with normoalbuminuria, 52 with microalbuminuria) and 28 age- and sex-matched controls were studied. Informed consent was obtained from all subjects and the study was approved by the local medical ethics committee. The diabetic group consisted of 33 males and 45 females (mean age ± SD, 54.2 ± 9.9 years) and was classified according to the criteria established by the National Diabetes Data Group [14], 18 patients were maintained on insulin, 52 on oral hypoglycemic agents, and 8 on diet alone; 45 microalbuminuric and 15 normoalbuminuric diabetic patients had one or more complications, such as hypertension, hyperlipidemia, retinopathy, cataract, or neuropathy. The patients were in good or fair metabolic control on examination. All patients had normal, sterile urines and normal renal function, as evaluated by plasma creatinine and urea levels.

After an overnight fast, blood samples were drawn with an anticoagulant from an antecubital vein without stasis, and plasma was either immediately analyzed or frozen at −20 °C. All hemolyzed specimens were discarded. Urine samples were collected from 8 a.m. to 10 a.m. on 3 consecutive days and filtered through filter paper before analysis. In preliminary studies on 24-h and 2-h urine samples, a satisfactory correlation ($r = 0.78$, $P < 0.001$) was found and thus 2-h samples were used.

Methods. Urine albumin concentration was measured by an immunochemical technique. Patients with an albumin excretion rate between 20 µg/min and 200 µg/min were classified as microalbuminuric. We modified the method of Ohkawa et al. [15] for the determination of urinary MDA levels. The plasma MDA level was determined by the method of Sato et al. [16]. The TBARS concentration was derived from a standard MDA curve obtained by hydrolysis of 1,1,3,3-tetra-ethoxypropane. The recovery of MDA added to plasma was 92% ± 3%. The coefficient of variation was 4.2%. Plasma fructosamine levels were measured by the method of Baker et al. [17] using a commercial kit from Boehringer-Mannheim (Germany).

Concentrations of fasting glucose, urea, creatinine, cholesterol, triglyceride, and high-density lipoprotein (HDL)-cholesterol [after

Table 1. Summary of data from control subjects and diabetic patients[a]

	Diabetic normo-albuminuric patients (n = 26)	Diabetic micro-albuminuric patients (n = 52)	Control subjects (n = 28)
Duration of diabetes (months)	44.2 ± 43.6	111.3 ± 95.9**	–
Urine			
AER (μg/min)	16.5 ± 6.6	78.1 ± 23.2*·**	1.1 ± 0.8
MDA (nmol/ml)	5.3 ± 2.0*	5.9 ± 2.6*	4.0 ± 0.7
Creatinine (mmol/l)	12.4 ± 8.2	12.4 ± 6.6	9.8 ± 2.1
Glucose (mmol/l)	1.9 ± 0.5*	6.5 ± 1.3*·**	–
Plasma			
MDA (nmol/ml)	5.9 ± 1.4*	7.0 ± 2.3*·**	4.9 ± 0.8
Glucose (mmol/l)	9.2 ± 4.2*	11.5 ± 4.7*·***	4.6 ± 0.5
Fructosamine (μmol/l)	350.6 ± 12.8*	379.9 ± 11.3*	272.5 ± 13.8
Urea (mmol/l)	6.7 ± 1.9	8.5 ± 3.4	5.7 ± 1.7
Creatinine (mmol/l)	79.6 ± 17.7	97.2 ± 35.4	88.4 ± 17.7
Cholesterol (mmol/l)	5.2 ± 1.0	5.8 ± 1.9	4.2 ± 0.9
Triglyceride(mmol/l)	1.7 ± 0.7	2.2 ± 0.7	1.1 ± 0.5
HDL-cholesterol (mmol/l)	0.6 ± 0.2	0.7 ± 0.3	1.0 ± 0.3
LDL-cholesterol (mmol/l)	3.8 ± 0.9	3.7 ± 1.4	2.6 ± 0.8
VLDL-cholesterol (mmol/l)	0.8 ± 0.3	1.5 ± 0.2	0.5 ± 0.2

* $P < 0.001$, Micro- and/or normoalbuminuric diabetic patients vs. control subjects; ** $P < 0.001$, *** $P < 0.005$, microalbuminuric vs. normoalbuminuric diabetic patients

[a] All results are expressed as mean ± SD; AER, Albumin excretion rate; MDA, malondialdehyde; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein

Table 2. Data from normo- and microalbuminuric diabetic patients in relation to the presence or absence of other complications

	Duration of diabetes (months)	AER (μg/min) (μ/min)	Urine MDA (nmol/ml)	Plasma MDA (nmol/l) (nmol/ml)	Plasma glucose (mmol/l)	Plasma fructosamine (μmol/l)
Normoalbuminuric diabetic patients (n = 26)	44.2 ± 43.6	16.5 ± 6.6	5.3 ± 2.0	5.9 ± 1.4	9.2 ± 4.2	350.6 ± 12.8
With complications (n = 15)	43.3 ± 33.5	16.3 ± 7.4	5.5 ± 2.1	6.2 ± 1.2	9.3 ± 3.9	352.9 ± 15.6
No complications (n = 11)	45.3 ± 56.4	18.2 ± 5.6	5.3 ± 1.8	5.6 ± 1.6	9.9 ± 5.0	348.8 ± 8.8
Microalbuminuric diabetic patients (n = 52)	111.3 ± 95.9	78.1 ± 23.2	5.9 ± 2.6	7.0 ± 2.3	11.5 ± 4.7	379.9 ± 11.3
With complications (n = 45)	113.1 ± 100.4	80.2 ± 23.1	5.8 ± 2.7	7.0 ± 2.4	11.5 ± 4.7	363.9 ± 10.6*
No complications (n = 7)	99.4 ± 64.1	71.4 ± 22.5	6.2 ± 2.6	6.6 ± 1.7	11.1 ± 5.3	482.7 ± 10.9

* $P < 0.05$, Microalbuminuric diabetic patients with complications vs. those without complications

precipitation of very low-density lipoprotein (VLDL)- and low-density lipoprotein (LDL)-cholesterol with phosphotungstic acid and magnesium ions] in plasma were measured using Dart reagents on the Dacos autoanalyzer (Coulter Electronics, Hialeah, USA). LDL-cholesterol was calculated from the Friedewald formula: LDL-cholesterol = Total cholesterol – (HDL-cholesterol + Triglyceride/5). VLDL-cholesterol was calculated from the formula: VLDL-cholesterol = Triglyceride/5 [18].

Statistical analysis. The means and standard deviations were calculated for each parameter. Comparisons between patient groups and controls were made with the Newmann-Keul's test. Correlations between variables were assessed by the linear regression method.

Results

Results are summarized in Table 1 and 2. In both diabetic groups, plasma and urinary MDA levels were significantly higher than in controls ($P<0.001$). In contrast to urine MDA levels, plasma MDA levels were significantly higher in microalbuminuric diabetics than in normoalbuminuric diabetics ($P<0.001$). When both diabetic groups were divided into two subgroups according to the presence or absence of complications, there were no significant differences in plasma and urine MDA levels between the subgroups. There was no correlation between plasma and urine MDA levels in both patient groups and controls ($P>0.05$). In addition, in both patient groups the plasma MDA level did not correlate with other measured parameters, including plasma glucose, fructosamine, cholesterol, triglyceride, HDL- and LDL-cholesterol, albumin excretion rate, and duration of diabetes. There was a weak correlation between plasma MDA and VLDL-cholesterol levels only in microalbuminuric diabetic patients.

Discussion

Previous studies have shown elevated levels of MDA in both type 1 and type 2 diabetic patients [1, 2, 16, 19, 20]. In our study we also observed significantly higher plasma

and urine MDA levels in type 2 diabetic patients with or without microalbuminuria than in controls. In microalbuminuric diabetics, the plasma MDA level was significantly higher than in normoalbuminuric diabetics, but the urine MDA level was similar. There have been no previous reports of urine MDA levels in diabetic patients, so we were unable to compare our results with others.

The presence of complications had no effect on plasma and urine MDA levels in either diabetic group. Sato et al. [16] reported a significant increase in MDA in type 2 diabetes with vascular disease. However, these authors did not specify the type of complications. Kaji et al. [21] also failed to mention the presence or absence of vascular complications in their diabetic population. Gallou et al. [1] found no difference in TBARS concentration between type 1 diabetics with microangiopathy and type 1 diabetic patients without vascular complications. These results are consistent with ours.

The exact mechanism by which elevated blood glucose leads to membrane lipid peroxidation in diabetic subjects is not known. It can be explained by insulinopenia-induced release of free radicals from metabolic pathways (hexose monophosphate shunt, β-oxidation of free fatty acids) [22–24] or by hyperglycemia-stimulated platelet aggregation which leads to lipoperoxide synthesis (thromboxane, prostaglandins, and other molecules) [19, 24].

The decrease in activity of tissue antioxidant enzymes reported in diabetes mellitus also explains an increase in lipoperoxidation products in diabetic patients [21]. Recent in vitro studies in a cell-free buffer have shown that glucose can enolize and thereby reduce molecular oxygen under physiological conditions, yielding α-keto-aldehydes, hydrogen peroxide, and free radical intermediates [25, 26]. Oxygen radicals formed cause peroxidative breakdown of phospholipid fatty acids and accumulation of MDA [25, 27, 28]. Peroxidative lipid damage in the membrane may have a role in the increased coagulability, altered phospholipid organization, and cellular damage known to occur in other tissues of diabetic patients. The carbonyl groups of MDA may bind to the amino groups of the proteins and phospholipids, forming Schiff's bases and causing their destabilization and rendering them more susceptible to various stress factors.

In conclusion, this study has shown an increase in plasma and urinary MDA in diabetic patients with and without microalbuminuria. Further research is required to clarify this multifactorial process and to elucidate the role of lipid peroxides in the pathogenesis of diabetic complications.

References

1. Gallou G, Ruelland A, Legras B, Maugendre D, Allannic H, Cloarec L. Plasma malondialdehyde in type 1 and type 2 diabetic patients. Clin Chim Acta 1993; 214:227.
2. Collier A, Rumley A, Rumley AG, Paterson JR, Leach JP, Lowe GDO, Small M. Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Diabetes 1992; 41:909.
3. Dormandy TL. An approach to free radicals. Lancet 1983; II:1010.
4. Stringer MD, Görög PG, Freeman A, Kakker W. Lipid peroxides and atherosclerosis. BMJ 1989; 298:281.
5. Clamp JR, Hartog M, Shelley JH. Carbohydrate containing materials in urine from normal and diabetic subjects. Clin Sci 1979; 56:193.
6. Baggio B, Briani G, Cicerello E, Gambaro G, Bruttomesso O, Tiengo A, Borsatti A, Crepaldi G. Urinary glycosaminoglycans, sialic acid and lysosomal enzymes increase in nonalbuminuric diabetic patients. Nephron 1986; 43:187.
7. Mogensen CE. Prediction of clinical diabetic nephropathy in IDDM patients. Alternatives to microalbuminuria. Diabetes 1990; 39:761.
8. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1982; I:1430.
9. Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem. 1995; 32:303.
10. Cheung CK, Cockram CS, Yeung VTF, Swaminathan R. Urinary excretion of transferrin by non-insulin-dependent diabetics: a marker for early complications? Clin Chem 1989; 35:1672.
11. Lyons TS. Oxidized low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes? Diabetes Med 1991; 8:411.
12. Jennings PE, Jones AF, Florkowski CM, Lunec J, Barnett AH. Increased diene conjugates in diabetic subjects with microangiopathy. Diabetes Med 1987; 4:452.
13. Jos J, Rybak M, Patin PH, Robert JJ, Boitard C, Thevenin R. Etude des enzymes antioxydantes dans le diabete insulino-de'pendant de l'nfant et de l'adolescent (in French). Diabete Metab 1990; 16:492.
14. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28:1039.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351.
16. Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohishi N, Yagi K. Lipid peroxide level in plasma of diabetic patients. Biochem Med 1979; 21:104.
17. Baker JR, Metcalf PA, Johnson RN, Newman D, Rietz P. Use of protein based standards in automated colorimetric determinations of fructosamine in serum. Clin Chem 1985; 31:1550.
18. Tietz NW, ed. Textbook of clinical chemistry. Philadelphia: Saunders; 1986:786, 805–806, 854, 873–885.
19. Noberasco G, Odetti P, Boeri D, Maiello M, Adezati L. Malondialdehyde (MDA) level in diabetic subjects. Relationship with blood glucose and glycosylated hemoglobin. Biomed Pharmacother 1991; 45:193.
20. Altomare E, Vendemiale G, Chicco D, Procacci V, Cirelli F. Increased lipid peroxidation in type 2 poorly controlled diabetic patients. Diabete Metab 1992; 18:264.
21. Kaji H, Kurasaki M, Ito K, Saito K, Saito T, Niioka T, Kojima T, Ohsaki Y, Ide H, Tsuji M, Kondo T, Kawakami Y. Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non-insulin dependent) diabetic women. Klin Wochenschr 1985; 73:765.
22. Kitohara M, Eyre HS, Lynch ER, Rollison ML, Hill HR. Metabolic activity of diabetic monocytes. Diabetes 1980; 29:251.
23. Wohaieb SA, Godin DV. Alterations in free radical tissue – defense mechanism in streptozotocin-induced diabetes in rat. Diabetes 1987; 36:1014.
24. Dousst JC, Trouilh M, Foglietti MJ. Plasma malonaldehyde levels during myocardial infarction. Clin Chem Acta 1983; 129:319.
25. Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 1989; 38:1539.
26. Wolff SP, Dean RT. Glucose autoxidation and potein modification: the potential role of autoxidative glycosylation in diabetes. Biochem J 1987; 245:243.
27. Carrell RW, Winterbourn CC, Rachmilewitz EA. Activated oxygen and haemolysis. Br J Haematol 1975: 30:259.
28. Clark IA, Cowden WB, Hunt NH. Free radical-induced pathology. Med Res Rev 1985; 5:297.