Stiffening of connective tissue in elderly diabetic patients: relevance to diabetic nephropathy and oxidative stress

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Summary. Limited joint mobility seen in diabetes mellitus is thought to be the result of stiffening of periarticular connective tissue, which is presumably derived from increased cross-linking of collagen related to advanced glycation end products. In this study the extent of the stiffening of connective tissue was measured by the passive extension angle of the metacarpophalangeal joints in 205 elderly diabetic patients. Association with diabetic nephropathy, with which advanced glycation end products have recently been demonstrated to increase, and metabolic abnormalities were also considered. The angle of the metacarpophalangeal joints was significantly correlated with age ($r = -0.24$, $p < 0.01$), and was significantly smaller in men than in women ($p < 0.01$). The angle demonstrated a decrease in association with diabetic retinopathy and nephropathy, and only the association with nephropathy was significant ($p < 0.05$). The angle was weakly, but significantly, correlated with serum thiobarbituric acid reactants as a measure of lipid peroxides ($r = -0.15$,

$p < 0.05$), triglyceride ($r = -0.20$, $p < 0.01$) and HDL cholesterol ($r = 0.19$, $p < 0.01$), but not with blood glucose ($r = 0.02$), HbA_{1c} ($r = 0.06$) or duration of diabetes ($r = -0.05$). In addition, the angle in 14 non-diabetic patients on haemodialysis was significantly ($p < 0.05$) smaller than that in age- and sex-matched normal subjects. Thus, it was indicated that the stiffening of connective tissue was associated with diabetic nephropathy, serum lipid peroxide and dyslipidaemia. Stiffening of connective tissue seems to be more affected by oxidative stress than non-enzymatic glycation per se. Factors contributing to the stiffening of connective tissue, such as male sex, ageing, serum lipid peroxide, high triglyceride and low HDL cholesterol resemble those associated with arteriosclerosis.

Key words: Limited joint mobility, stiffening of connective tissue, diabetic nephropathy, non-enzymatic glycation, lipid peroxide, dyslipidaemia, oxidative stress.

Limited joint mobility (LJM) thought to be the result of stiffening of periarticular connective tissue has been demonstrated to occur frequently in patients with Type 1 (insulin-dependent) [1–7] and Type 2 (non-insulin-dependent) diabetes mellitus [8–12]. Although the pathogenesis of such stiffening of connective tissue in diabetes is not clearly understood, increased cross-linking of collagen related to advanced glycation end products (AGEs) must be considered [13–15]. Recently, it has been reported that AGEs on collagen increase in association with renal functional impairment [16, 17].

Several studies have demonstrated that LJM is associated with diabetic retinopathy, but its association with diabetic nephropathy has not been clearly proven [2, 3, 8–11]. In these studies the assessment of hand LJM was based on the method described by Rosenbloom et al. [3]. This method assesses the presence of LJM by measuring whether the interphalangeal joints of the hands could be extended to 180 degrees, that is, if fixed flexion defor-

mities of the fingers were seen. However, if stiffening of periarticular connective tissue is attributed to increased cross-linking of collagen, the extent of the limitation of joint mobility should be continuous. In the present study we assessed the extent of the stiffening of connective tissue by the passive extension angle of the metacarpophalangeal (MP) joints, and thereby found interesting associations of the angle of the MP joints with renal functional impairment and metabolic abnormalities in elderly diabetic patients. Stiffening of connective tissue seems to be associated with oxidative stress rather than non-enzymatic glycation per se.

Subjects and methods

Two hundred and five elderly diabetic patients (87 men, 118 women) with a mean age of 60.2 ± 0.8 years were studied whose age at onset of diabetes was confirmed as 20 years or older. Fifty-two patients

Fig. 1. The method for measuring the angle of the metacarpophalangeal joints. The upper picture is from a healthy woman, the lower from a woman with diabetes. Both are 36 years old

were treated with diet alone, 111 with sulphonylurea therapy and 42 with insulin (6 were Type 1 (insulin-dependent) diabetic patients). Diabetic retinopathy was classified into three grades: grade 1 was assigned to patients with no retinopathy, grade 2 to those with simple retinopathy and grade 3 to those with proliferative retinopathy. Diabetic nephropathy was also classified into three grades: grade 1 was assigned if albumin levels in random urine samples were less than 30 mg/g creatinine, grade 2 if the albumin levels were 30 mg/g creatinine or more, and grade 3 if serum creatinine levels were higher than the normal upper limit of $106 \mu\text{mol/l}$ for men or $89 \mu\text{mol/l}$ for women. Fourteen normal subjects, 14 non-diabetic and 10 diabetic patients undergoing haemodialysis, matched for age and sex, were also studied. None of the subjects, but one non-diabetic and three diabetic patients on haemodialysis were receiving lipid-lowering drugs. Patients with rheumatoid arthritis were excluded. All subjects gave their informed consent to this study.

After an overnight fast, blood samples were taken for measurements of plasma glucose, HbA_{1c} , serum creatinine, thiobarbituric acid reactants (TBAR), total cholesterol, HDL cholesterol and triglycerides. In the patients without overt proteinuria, determined by Albustix testing (Ames Division, Miles Laboratories, Elkhart, Ind., USA), the levels of albumin and creatinine were measured in a random urine sample. Blood pressure was measured in the supine position. Blood pressure was measured in patients on antihypertensive therapy without discontinuation of the antihypertensive drug.

The extent of the stiffening of connective tissue was assessed by the passive extension angle of the MP joints before the biochemical data were obtained. As demonstrated in Figure 1, the angle of the MP joints was measured while subjects lifted their hand as far as possible off the table with the fingers fixed flat on the table. The mean coefficient of variation of measurements by three examiners was 8.5%. The mean of the angles measured on the right and left hands in individual subjects was used for assessment. The mean coefficient of variation of the angles on the right and left hands was 11.5% in 14 normal subjects. Only one patient showed complete fixed flexion deformity of the MP joints, with an angle of 0 degrees.

Plasma glucose concentrations were measured by the hexokinase method, and HbA_{1c} by HPLC [18]. Serum and urinary creatinine concentrations were measured by Jaffe's reaction method [19]. Total cholesterol and triglyceride concentrations were measured by enzymatic methods, and HDL cholesterol was similarly measured enzymatically after the precipitation of VLDL and LDL by dextran sulphate [20]. LDL cholesterol was calculated ac-

cording to Friedewald et al. [21]: $\text{LDL cholesterol} = \text{total cholesterol} - (\text{HDL cholesterol} + 0.2 \times \text{triglyceride})$. The triglyceride levels in this study were less than 4.1 mmol/l . Serum TBAR levels were determined by the method of Yagi [22] with malondialdehyde as a standard using a commercial kit (Wako Pure Chemical Industries, Osaka, Japan). The intra- and inter-assay coefficients of variation were 2.3% and 5.1%, respectively. Urinary albumin concentrations were measured using a commercial RIA kit (Diagnostic Products Corp., Los Angeles, Calif., USA).

Statistical analysis

Data are presented as means $\pm$ SD. Statistical analysis was performed by using Student's *t*-test between two groups, analysis of variance with the Ryan's method between three groups, Pearson's correlation coefficient, and a chi-square test where appropriate. A *p* value of less than 0.05 was considered to be significant.

Results

In all 205 diabetic patients, the angle of MP joints was significantly correlated with age ($r = -0.24, p < 0.01$), and was significantly smaller in men than in women (41.0 ± 17.5 vs 51.9 ± 18.3 degrees, $p < 0.01$) with no significant difference in age between the sexes (58.4 ± 12.2 vs 61.6 ± 10.6 years). The clinical characteristics and the mean angles of the MP joints in the patients after classification into three groups according to the grade of diabetic retinopathy or nephropathy are shown in Tables 1 and 2.

When classified by the grade of retinopathy, the mean angle of the MP joints tended to be smaller in patients assigned grades 2 and 3 than in patients assigned grade 1, but no significant differences were seen between the three grades. Age, body mass index and serum TBAR were not significantly different, but significant differences were seen in duration of diabetes and HbA_{1c} as shown in Table 1. On the other hand, when classified by grade of nephropathy, the mean angle of the MP joints was found to decrease significantly ($p < 0.05$) in the order from grade 1 to 3. The mean age was significantly ($p < 0.01$) older in patients with grade 3 than in patients with grades 1 and 2. There were also significant differences in

Table 1. Clinical characteristics and the angles of the metacarpophalangeal (MP) joints in diabetic patients classified into three groups by the grade of diabetic retinopathy

	Diabetic retinopathy		
	Grade 1	Grade 2	Grade 3
<i>n</i>	135	43	27
Male/Female	57/78	17/26	13/14
Age (years)	59.6 ± 11.7	62.8 ± 11.5	59.3 ± 9.6
Duration of diabetes (years)	7.7 ± 6.2	12.1 ± 7.0^a	14.0 ± 7.5^a
HbA_{1c} (%)	6.5 ± 1.5	7.6 ± 1.5^a	7.5 ± 1.5^a
Serum TBAR ($\mu\text{mol/l}$)	3.3 ± 1.0	3.4 ± 0.8	3.4 ± 1.0
Angle of MP joints (degrees)	49.5 ± 19.2	42.9 ± 16.7	43.1 ± 17.9

^a $p < 0.01$ vs grade 1.

Means $\pm$ SD are shown. Grade 1 was assigned to patients with no retinopathy; grade 2, to patients with simple retinopathy; grade 3, to patients with proliferative retinopathy. TBAR, Thiobarbituric acid reactants

Table 2. Clinical characteristics and the angles of the metacarpophalangeal (MP) joints in diabetic patients classified into three groups by the grade of diabetic nephropathy

	Diabetic nephropathy		
	Grade 1	Grade 2	Grade 3
<i>n</i>	105	80	20
Male/Female	43/62	34/46	10/10
Age (years)	58.6 ± 11.5	60.5 ± 10.5	67.8 ± 11.9 ^{b,d}
Duration of diabetes (years)	8.7 ± 6.4	9.5 ± 6.9	13.8 ± 8.7 ^{b,d}
Fasting plasma glucose (mmol/l)	8.3 ± 2.3	9.9 ± 2.5 ^b	8.3 ± 2.0 ^d
HbA _{1c} (%)	6.4 ± 1.5	7.4 ± 1.6 ^a	6.9 ± 1.3 ^{a,c}
Serum triglyceride (mmol/l)	1.3 ± 0.6	1.5 ± 0.8 ^a	1.7 ± 0.7 ^a
Serum TBAR (μmol/l)	3.2 ± 0.8	3.6 ± 1.1 ^a	3.4 ± 0.7
Serum creatinine (μmol/l)	76.3 ± 11.3	78.6 ± 12.8	141.4 ± 42.2 ^{b,d}
Angle of MP joints (degrees)	51.2 ± 18.4	44.6 ± 17.6 ^a	37.3 ± 20.0 ^{a,c}

^a $p < 0.05$, ^b $p < 0.01$ vs grade 1; ^c $p < 0.05$, ^d $p < 0.01$ vs grade 2. Means ± SD are shown. Grade 1 was assigned if albumin levels in random urine samples were less than 30 mg/g creatinine; grade 2, if albumin levels were more than 30 mg/g creatinine; grade 3, if serum creatinine levels were higher than the normal upper limit of 106 μmol/l for men or 89 μmol/l for women. TBAR, Thiobarbituric acid reactants

duration of diabetes, fasting plasma glucose, HbA_{1c}, serum triglyceride, TBAR and creatinine as shown in Table 2.

Table 3 shows the correlation coefficients among the angle of the MP joints, clinical features and biochemical parameters in all diabetic patients. The angle of MP joints was significantly correlated with age, serum TBAR, HDL cholesterol and triglyceride, but not with fasting plasma glucose, HbA_{1c} or duration of diabetes. Serum TBAR was significantly correlated with LDL cholesterol, HDL cholesterol and triglyceride.

Table 4 shows the clinical characteristics and the mean angles of the MP joints in age- and sex-matched normal subjects, non-diabetic and diabetic patients on haemodialysis. The mean angle of the MP joints was significantly ($p < 0.05$) smaller in non-diabetic and diabetic patients on haemodialysis than in normal subjects. The angle tended to be smaller in diabetic patients on haemodialysis than in non-diabetic patients on haemodialysis, but no significant

difference was observed. Serum TBAR levels were higher in non-diabetic and diabetic patients on haemodialysis than in normal subjects.

Discussion

Since LJM seems to affect diabetic patients with a pre-pubertal onset of diabetes more severely than those with a postpubertal onset [5], only patients whose onset of diabetes was after 20 years of age were considered in this study. The passive extension angle of the MP joints has been used as an index of LJM with an angle of the MP joints of less than 45 or 60 degrees defined as abnormal [3, 5]. In this study we used the angle of the MP joints as an index of the stiffening of connective tissue, which possibly reflects the extent of the cross-linking of collagen [13–15].

Using this method, we noted that the angle of the MP joints was affected by age and sex. The cross-links of collagen are known to increase with ageing, and the increase is assumed to be accelerated by the formation of AGEs in diabetes or hyperglycaemia [16, 17, 23–25]. It has also been suggested that the cross-linking of collagen is more extensive in men than in women [26, 27]. Therefore, the angle of the MP joints as an index of the stiffening of connective tissue varies not only with pathological changes but also with physiological changes associated with age and sex. Previous studies [5, 7, 8, 10] using the method of Rosenbloom et al. [3] demonstrated a significant relationship between LJM and age but not between LJM and sex (Clark et al. [7] showed a slightly higher prevalence in men than in women).

Similar to the case of LJM, the angle of the MP joints demonstrated a decrease in association with diabetic retinopathy and nephropathy. The association of the angle and nephropathy was significant, and this fact was supported by the finding that the angle was significantly smaller in non-diabetic patients undergoing haemodialysis than in age- and sex-matched normal subjects. These findings might be inferred from the recent studies [16, 17] that

Table 3. Correlation coefficients among the angle of the metacarpophalangeal joints, clinical features and biochemical parameters

	Angle	Age	BMI	Duration	SBP	FPG	HbA _{1c}	TBAR	LDL chol	HDL chol	TG
Angle	1	0.24 ^b	0.08	−0.05	−0.12	0.02	0.06	−0.15 ^a	0.00	0.19 ^b	−0.20 ^b
Age		1	−0.02	0.29 ^b	0.23 ^b	−0.03	−0.05	0.13	0.12	−0.15 ^a	0.11
BMI			1	−0.17 ^a	0.19 ^b	0.01	0.02	0.15 ^a	0.22 ^b	−0.20 ^b	0.25 ^b
Duration of diabetes				1	0.15 ^a	0.11	0.13	0.00	0.00	−0.05	−0.11
SBP					1	0.04	0.05	0.07	0.21 ^b	−0.12	0.04
FPG						1	0.80 ^b	0.05	0.19 ^b	0.12	0.08
HbA _{1c}							1	0.06	0.19 ^b	0.07	0.04
Serum TBAR								1	0.24 ^b	−0.23 ^b	0.41 ^b
LDL chol									1	−0.06	0.18 ^a
HDL chol										1	−0.44 ^b
TG											1

^a $p < 0.05$, ^b $p < 0.01$; SBP, Systolic blood pressure; FPG, Fasting plasma glucose; TBAR, Thiobarbituric acid reactants; LDL chol, LDL cholesterol; HDL chol, HDL cholesterol; TG, Triglyceride

Table 4. Clinical characteristics and the angles of the metacarpophalangeal (MP) joints in normal subjects, non-diabetic and diabetic patients on haemodialysis

	Normal subjects	Non-diabetic patients	Diabetic patients on haemodialysis
<i>n</i>	14	14	10
Male/Female	7/7	7/7	5/5
Age (years)	57.6 ± 8.9	57.6 ± 9.1	57.3 ± 9.9
Duration of haemodialysis (years)	—	8.9 ± 5.0	3.8 ± 3.4 ^c
Fasting plasma glucose (mmol/l)	5.4 ± 0.5	5.5 ± 0.6	8.7 ± 3.4 ^{b,c}
Serum triglyceride (mmol/l)	1.1 ± 0.6	1.4 ± 0.6	1.5 ± 0.6
Serum TBAR (μmol/l)	2.8 ± 0.6	3.6 ± 0.8 ^a	3.3 ± 0.6
Angle of MP joints (degrees)	53.8 ± 11.2	42.4 ± 13.5 ^a	37.8 ± 18.4 ^a

^a $p < 0.05$, ^b $p < 0.01$ vs normal subjects; ^c $p < 0.01$ vs non-diabetic patients

Means ± SD are shown. TBAR, Thiobarbituric acid reactants

AGEs on collagen increase with renal functional impairment.

Alternatively, it is noteworthy that the angle of the MP joints was weakly but significantly correlated with serum TBAR as a measure of lipid peroxides in all diabetic patients, despite no correlation between the angle and fasting plasma glucose or HbA_{1c}. It was previously postulated that non-enzymatic cross-links of collagen increased with ageing through oxygen-mediated reactions [28]. Also the formation of AGEs is known to be stimulated by oxygen and catalysts of oxidation reactions [29]. Although it is not known to what extent AGEs contribute to the non-enzymatic cross-links of collagen that increase with age, oxidation reactions are thought to play an important role in the increase of the non-enzymatic cross-links in hyperglycaemia, with consideration of the recent concept of auto-oxidative glycation [30] or glycoxidation [31]. If so, it is convincing that the angle of the MP joints was correlated with serum TBAR which is thought to some extent to reflect oxidative stress, but not with HbA_{1c} which is an index of early non-enzymatic glycation.

In fact, we have observed that the increase of the cross-links of collagen induced by hyperglycaemia, which was measured by the thermal stability of tail tendon collagen, was partially inhibited by the supplementation of vitamin E as an antioxidant in experimental diabetic rats [32]. Oxidative stress is known to increase in patients with renal failure [33], which may in part explain the stiffening of connective tissue as well as the rise of AGEs in renal failure despite normal glucose levels. In addition, it has been demonstrated that oestrogens help to reduce the production of lipid peroxides [34], which may in part lead to the difference between the sexes in the stiffening of connective tissue as seen in the present study.

The angle of the MP joints was significantly correlated with dyslipidaemia (high triglyceride and low HDL cholesterol) as well as serum TBAR. Since serum TBAR was also significantly correlated with serum triglyceride, serum TBAR might depend on the amount of serum triglyceride but not on the extent of oxidative stress. However, it is possible that oxidative stress increases in patients with hypertriglyceridaemia [35]. Of particular interest is that the factors contributing to the stiffening of

connective tissue shown (male sex, ageing, serum lipid peroxide, high triglyceride and low HDL cholesterol) are some of the risk factors for coronary heart disease [36, 37], especially in the case of normocholesterolaemia. Thus, the stiffening of connective tissue and arteriosclerosis ('stiffening of artery') might have a common underlying pathogenesis.

In conclusion, in elderly diabetic patients it was demonstrated that the stiffening of connective tissue assessed by the passive extension angle of the MP joints was associated with diabetic nephropathy, serum TBAR and dyslipidaemia. Stiffening of connective tissue, which possibly reflects the cross-linking of collagen, seems to be more affected by oxidative stress than non-enzymatic glycation per se. Factors contributing to the stiffening of connective tissue resemble those which are associated with arteriosclerosis.

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