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Clinical Characteristics and Circulating Collagen and Laminin Metabolites in Insulin-Dependent Diabetic Children with Joint and Skin Manifestations

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ABSTRACT. One hundred seventy-four children and adolescents with insulin-dependent diabetes mellitus were examined for joint contractures and skin manifestations in their hands. Joint contractures were found in 52 (29.8%) and skin manifestations in 29 (16.6%) patients. To eliminate the possible confounding effects of age and duration of diabetes on the variables to be studied, patients younger than 7 y and with a duration of diabetes shorter than 3 y were excluded from the subsequent analyses. Of the remaining 108 children, those with joint contractures had lower serum concentrations of the 7-S domain of type IV collagen and the P1 fragment of laminin than the other patients ($p = 0.033$) but higher mean glycated Hb levels ($p = 0.048$). A clear association was noted between the occurrence of joint contractures and skin changes ($p = 0.007$). Background retinopathy was found in six patients (5.6%), three of whom had stage II joint contractures ($p = 0.064$). The children with skin changes and those with combined joint and skin manifestations more often had insulin-dependent diabetes mellitus in their first-degree relatives ($p = 0.038$ and $p = 0.043$, respectively). No difference in relative height was found between the groups. No association could be seen between disease susceptibility antigens in the HLA-D locus and joint or skin manifestations. The lower levels of circulating collagen and laminin metabolites in the diabetic children with joint contractures suggest that these patients are characterized by a reduced turnover of basement membranes in tissues. In addition, our data suggest that the development of joint contractures is associated with impaired metabolic control but not necessarily with growth retardation. (*Pediatr Res* 33: 501–505, 1993)

Abbreviations

IDDM, insulin-dependent diabetes mellitus
HbA_{1C}, hemoglobin A_{1C}
IBA, insulin-binding antibody
PIIINP, aminoterminal propeptide of type III procollagen
CI, confidence interval

There have been a number of reports over the last 15 y indicating that joint contractures and skin thickening, mainly affecting the small joints of the hands, are common manifestations of IDDM in childhood and adolescence (1–10). The prevalence of joint contractures has varied from 8 to 50% (11), and skin manifestations have been reported to occur in 34 to 51% of children with IDDM (5, 7, 12) often in association with joint contractures (3, 8, 12).

In many reports, the presence of joint contractures in diabetic patients has shown a positive correlation with the duration of the disease (1, 3, 4, 6–8, 13) and the actual age of the patients (1, 2, 4, 7, 10), which appears to be the most important variable in the expression of diabetic joint contractures (11). The association of joint contractures with growth retardation has remained controversial (1, 6, 7, 10, 14). No correlation has been found between joint contractures and poor metabolic control in earlier reports (1, 3, 4, 6, 10), but recently a positive association between elevated blood HbA1 levels and the presence of joint contractures has been described among adolescents and adults with IDDM (15). Furthermore, an increased risk of microvascular complications, such as retinopathy, has been observed in the presence of joint contractures (3, 8, 9, 12, 13, 15).

The etiology of the joint and skin manifestations is unknown. The collagen content of capillary basement membranes is increased in patients with IDDM (7, 16, 17), and it has been suggested that alterations in collagen structure, possibly related to glycation, may result in more stable forms (7, 18).

The aim of the present work was to determine the prevalence of joint and skin manifestations in an unselected series of diabetic children and adolescents and to identify factors associated with these changes, especially the possible relationship between serum concentrations of laminin and collagen metabolites and joint or skin manifestations.

PATIENTS AND METHODS

Patients. The series included 174 diabetic children and adolescents (102 boys) who visited the Diabetes Clinic at the Department of Pediatrics, University of Oulu, Oulu, Finland, in the course of 1 y. The mean age of the patients was 12.0 (4.1 SD) y (range 3.1 to 21.9 y), and the mean duration of diabetes 5.2 (3.6 SD) y (range 0.1 to 16.5 y). Twenty-four patients (13.9%) had one or more first-degree relatives with IDDM.

When analyzing the relationship between joint and skin manifestations and other factors, patients younger than 7 y and with a duration of diabetes shorter than 3 y were excluded to eliminate the possible confounding effects of age and duration of the disease. The mean age of the remaining 108 patients was 13.6

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(3.0 SD) y (range 7.2 to 21.9 y), and the mean duration of diabetes 7.2 (3.1 SD) y (range 3.0 to 16.5 y).

Serum concentrations of laminin P1 and collagen metabolites were measured in 84 subjects who did not differ in their clinical characteristics from the larger group of 108 subjects.

Clinical methods. All patients were examined for joint and skin manifestations by the same observer (R.V.). The presence of joint contractures in the fingers was evaluated by the method described by Grgic *et al.* (1). The patient was asked to place his right hand on the tabletop palm down with fingers fanned, and the examiner determined the contact between the fingers and the plane surface by looking at table level. The entire palmar surface of the fingers should normally make contact. The patients were classified as having stage I contractures if they were unable to make contact with some portion of one finger and as having stage II contractures if they could not make contact with two or more fingers. Another method for demonstrating joint mobility in the fingers was to ask the patient to place his hands together in a praying position and to assess the ability to oppose the full palmar surfaces of the fingers (4). Again, the patients were classified as having stage I contractures if they were unable to make contact with some portion of one pair of fingers and as having stage II deformations if flexion contractures were seen in two or more pairs. Contractures were confirmed by the examiner's attempt to passively extend the fingers. If there was any discrepancy between these two methods, the patient was classified according to the milder finding. Skin changes in the hands were evaluated by palpation and inspection (1, 5). Skin thickening and induration were most readily discernible in terms of an inability of the examiner to grasp a fold over the dorsal surface of the proximal phalanges of the fingers.

The growth of the patients was monitored by means of height and weight measurements on every visit to the Diabetes Clinic. The height was measured with the same Harpenden stadiometer. The results were expressed in terms of relative height (SD score) and relative weight (%) by reference to Finnish growth charts (19).

A retinal examination through dilated pupils was performed by an ophthalmologist in a darkened room to detect early signs of microvascular complications of diabetes. The finding of one or more microaneurysms or red dot lesions was considered to indicate background retinopathy. No one was observed to have proliferative retinopathy.

Biochemical methods. Metabolic control of diabetes was assessed by the level of blood HbA_{1C}, which was determined by ion-change chromatography using a method modified from that of Trivelli *et al.* (20) with a reference range of 4.1 to 6.3% in nondiabetic children.

Random serum C-peptide concentrations were measured with an RIA using antiserum M 1230 (Novo Research Institute, Bagsvaerd, Denmark), as previously described (21). The detection limit was 0.02 nmol/L. IBA were assayed by a modification of the method described by Welborn *et al.* (22), using dextran-coated charcoal instead of ethanol precipitation to separate free and bound insulin. HLA-Dw typing was carried out with local homozygous typing cells (23).

Serum concentrations of the 7-S domain of type IV collagen and the laminin P1 fragment were measured using specific RIA with reference intervals for adults of 4.1 to 9.7 μg/L and 15 to 37 μg/L, respectively (24). These reference values have been found to be independent of age in a study including children younger than 4 y (25) as well as among adults (24). Serum levels of PIIINP were quantified with a rapid equilibrium RIA (26). The reference intervals for PIIINP were 3.6 to 12.2 μg/L in 7- to 10-y-old children, 3.1 to 17.2 μg/L in 11- to 13-y-olds and 2.0 to 21.5 μg/L in 14- to 17-y-olds. These measurements were performed on all available samples at the time of clinical examination. In addition, a second sample was obtained from a subgroup of 30 subjects after a mean interval of 9 mo (range 3 to 20 mo) to analyze the consistency of serum laminin and collagen metabolite levels.

Statistical methods. The data were evaluated statistically by means of cross-tabulation and χ^2 statistics, t test in the case of normally distributed variables, and the Mann-Whitney U test in the case of an unequal distribution. Linear regression analysis was used to compare serum laminin and collagen metabolite levels at different time points.

RESULTS

Joint contractures were observed in 52 diabetic children and adolescents (29.8%) in this cross-sectional survey (95% CI, 23.1 to 36.7%), and 18 subjects (10.3%) (95% CI, 5.8 to 14.9%) were classified as having stage II contractures. Skin manifestations were seen in 29 patients (16.6%) (95% CI, 11.1 to 22.2%).

In the series including 108 subjects (age 7 y or more; duration of diabetes 3 y or more) the prevalences for joint contractures and skin manifestations were 40.7% and 24.1%, respectively (Table 1). The mean HbA_{1C} level was higher in those with joint contractures than in the others. There were no significant differences in sex distribution, age at diagnosis, circulating levels of IBA, or daily insulin dose between the two groups. The relative height and weight were of the same magnitude in both groups, and no significant difference was found in relative height between those with stage II contractures ($n = 17$) and other patients. Background retinopathy was found in six patients (5.6%) (95% CI, 1.2 to 9.9%), three of whom had stage II joint contractures. The association between more severe joint manifestations and background retinopathy was not significant ($p = 0.064$).

The patients with skin manifestations more often had first-degree relatives with IDDM than those without skin manifestations (Table 2). Similarly, six (35.3%) patients out of 17 having both joint and skin manifestations (95% CI, 12.6 to 58.0%) had IDDM in their first-degree relatives, compared with 11 (12.2%) out of the remaining 90 patients (95% CI, 5.5 to 19.0%; $p = 0.043$). We did not find any significant differences in HbA_{1C} level or relative height between those with skin manifestations and those without. Joint contractures were observed in 17 patients (65.4%) with skin manifestations as compared with 27 (32.9%) without skin changes ($p = 0.007$). Disease susceptibility antigens were detected in the HLA-D locus as often in those with joint or skin manifestations as in the other diabetic children.

Serum concentrations of 7-S collagen and laminin P1 were lower in the patients with joint contractures than in those without contractures (Table 3). No difference was found in the serum levels of 7-S collagen, PIIINP, and laminin P1 between the skin manifestation group and the other patients (Table 4). The consistencies of serum laminin P1, 7-S collagen, and PIIINP concentrations over a period of 9 mo (range 3 to 20 mo) were good ($r = 0.79$ for serum laminin P1, $r = 0.76$ for 7-S collagen, and $r = 0.82$ for PIIINP concentrations; $p < 0.001$ for all three analyses).

DISCUSSION

The prevalence of joint contractures in diabetic children found in this study, 29.8%, is similar to figures reported previously (1, 3, 6, 11). Thick, tight, waxy skin was often seen in association with these joint contractures, although the prevalence of the skin manifestations (16.6%) was lower than in previous reports (5, 7, 12). This may be because evaluation of the skin is subjective and accordingly prone to considerable interobserver variation. In the series of 108 subjects, the prevalences for both joint contractures and skin manifestations were higher because older subjects with longer duration of IDDM were included in this subgroup.

Other authors have failed to find any association between metabolic control and joint contractures in diabetic children (1, 3, 4, 6, 10). Our observations suggest, however, that impaired metabolic control may be related to the development of joint and skin manifestations, inasmuch as the mean level of HbA_{1C}

Table 1. *Clinical and laboratory data for 108 diabetic children and adolescents with and without joint contractures**

	Joint contractures		Significance of difference
	+ ($n = 44$)	− ($n = 64$)	
Sex (male/female)	23/21	38/26	NS
Age (y)	14.1 (13.4–14.8)	13.3 (12.4–14.1)	NS
Age at diagnosis (y)	6.5 (5.6–7.4)	6.4 (5.5–7.3)	NS
Duration of diabetes (y)	7.6 (6.7–8.5)	6.9 (6.1–7.7)	NS
IDDM in first-degree relatives, prevalence (%)	20.9 (8.8–33.1)	12.5 (4.4–20.6)	NS
Relative height (SD-score)	−0.5 (−0.8–−0.3)	−0.6 (−0.8–−0.4)	NS
Relative weight (%)	104.5 (101.4–107.5)	102.3 (99.8–104.8)	NS
Daily insulin dose (U/kg)	0.69 (0.66–0.72)	0.69 (0.65–0.73)	NS
Detectable serum C-peptide, prevalence (%)	11.6 (2.1–21.1)	13.1 (4.8–21.4)	NS
HbA_{1c} (%)	12.3 (11.5–13.1)	11.2 (10.6–11.9)	$p = 0.048$
IBA (%)	5.0 (1.7–8.2)	11.2 (6.6–15.8)	NS
Dw3, prevalence (%)	18.2 (6.8–29.6)	15.6 (6.7–24.5)	NS
Dw4, prevalence (%)	47.7 (33.0–62.5)	40.6 (28.6–52.7)	NS
Dw3/Dw4, prevalence (%)	11.4 (2.0–20.7)	15.6 (6.7–24.5)	NS
Skin changes, prevalence (%)	38.6 (24.2–53.0)	14.1 (5.5–22.6)	$p = 0.007$
Retinopathy, prevalence (%)	7.1 (0.0–14.9)	4.8 (0.0–10.0)	NS

* Values are means or prevalences (95% CI). Subjects were age 7 y or more with a duration of diabetes of 3 y or more.

Table 2. *Clinical and laboratory data for 108 diabetic children and adolescents with and without skin manifestations**

	Skin manifestation		Significance of difference
	+ ($n = 26$)	− ($n = 82$)	
Sex (male/female)	16/10	45/37	NS
Age (y)	13.9 (13.1–14.8)	13.5 (12.8–14.2)	NS
Age at diagnosis (y)	6.7 (5.7–7.6)	6.4 (5.6–7.1)	NS
Duration of diabetes (y)	7.3 (6.1–8.4)	7.1 (6.4–7.8)	NS
IDDM in first-degree relatives, prevalence (%)	30.8 (13.0–48.5)	11.1 (4.3–18.0)	$p = 0.038$
Relative height (SD score)	−0.6 (−1.1–−0.2)	−0.6 (−0.8–−0.4)	NS
Relative weight (%)	102.7 (98.9–106.6)	103.3 (101.1–105.6)	NS
Daily insulin dose (U/kg)	0.71 (0.66–0.76)	0.68 (0.65–0.71)	NS
Detectable serum C-peptide, prevalence (%)	12.0 (0.0–24.7)	12.7 (5.3–20.0)	NS
HbA_{1c} (%)	12.1 (11.1–13.0)	11.6 (10.9–12.2)	NS
IBA (%)	7.2 (0.5–13.9)	9.6 (5.8–13.4)	NS
Dw3, prevalence (%)	23.1 (6.9–39.3)	13.4 (6.0–20.8)	NS
Dw4, prevalence (%)	34.6 (16.3–52.9)	46.3 (35.5–57.1)	NS
Dw3/Dw4, prevalence (%)	19.2 (4.1–34.4)	12.2 (5.1–19.3)	NS
Joint contractures, prevalence (%)	65.4 (47.1–83.7)	32.9 (22.8–43.1)	$p = 0.007$
Retinopathy, prevalence (%)	4.0 (0.0–11.7)	6.3 (0.9–11.6)	NS

* Values are means or prevalences (95% CI). Subjects were age 7 y or more with a duration of diabetes of 3 y or more.

Table 3. *Serum concentrations of laminin P1 and collagen metabolites for diabetic children and adolescents with and without joint contractures**

	Joint contractures		Significance of difference
	+ ($n = 34$)	− ($n = 50$)	
Serum laminin P1 (μg/L)	28.6 (25.8–31.4)	35.9 (30.7–41.2)	$p = 0.033$
Serum 7-S collagen (μg/L)	5.5 (5.1–5.9)	6.2 (5.8–6.5)	$p = 0.033$
Serum PIIINP (μg/L)	7.1 (6.2–7.9)	7.8 (7.2–8.5)	NS

* Values are means (95% CI). Subjects were age 7 y or more with a duration of diabetes of 3 y or more.

Table 4. *Serum concentrations of laminin P1 and collagen metabolites for diabetic children and adolescents with and without skin manifestations**

	Skin manifestations		Significance of difference
	+ ($n = 22$)	− ($n = 62$)	
Serum laminin P1 (μg/L)	31.9 (25.4–38.4)	33.3 (29.3–37.2)	NS
Serum 7-S collagen (μg/L)	5.9 (5.4–6.4)	5.9 (5.6–6.3)	NS
Serum PIIINP (μg/L)	7.3 (6.2–8.3)	7.6 (7.0–8.2)	NS

* Values are means (95% CI). Subjects were age 7 or more with a duration of diabetes of 3 y or more.

was higher in the presence of joint contractures even after exclusion of the confounding effects of age and duration of diabetes.

The initial investigations into this topic described growth retardation in association with severe joint contractures (1, 14). More recently, in a survey including 142 patients with diabetes, presumably diagnosed before puberty, and a duration of diabetes longer than 3 y, Rosenbloom *et al.* (10) found that subnormal statural growth was associated with both mild and severe joint contractures but was also present in diabetic patients without joint manifestations. No statistical evaluation was included, however, to verify the association between joint contractures and growth retardation. We could not find any association between the relative height of the diabetic patients and the presence of joint or skin manifestations, nor could Starkman and Brink (6) in their examinations of 100 diabetic children in which patients with and without joint contractures were matched for age and duration of diabetes.

Our results, which showed no relationship between the frequency of disease susceptibility antigens in the HLA-D locus and joint or skin manifestations in IDDM, are in agreement with previous findings (27, 28). The fact that a positive family history of IDDM was noted more often in the patients with concurrent skin and joint manifestations or isolated skin manifestations has not been reported previously and indicates that unidentified genetic factors may be involved in the development of diabetic joint contractures and skin changes.

The fact that we could not find any difference in the levels of insulin binding antibodies between the patients with and without joint contractures does not support previous suggestions that circulating insulin antibodies may contribute to the development of chronic complications of diabetes (29, 30). The possible role of immune complexes in the pathogenesis of diabetic joint and skin manifestations remains to be investigated.

Thickening of the capillary basement membranes is a characteristic lesion in long-term diabetes and is considered to play a role in the pathogenesis of diabetic microangiopathy (16, 31). The total amount of collagen in basement membranes is known to increase in diabetes (7, 16, 17), and decreased acid solubility, increased resistance to enzymatic degradation, and increased nonenzymatic glycation of skin and tendon collagen have been reported in patients with IDDM (7, 32–34). Accordingly, it has been suggested that these changes may lead to increased stability and accumulation of collagen in various tissues all over the body, which may contribute to the development of microangiopathic complications (7, 32).

Basement membranes contain both collagenous (type IV) and noncollagenous proteins. An aminoterminal crosslinking part of the type IV collagen molecule is known as the 7-S domain, whereas the main noncollagenous glycoprotein characterized in basement membranes is laminin, which comprises several antigenic determinants. Quantification of the circulating concentrations of these antigens can provide useful information on the metabolism of basement membranes. Serum concentrations of 7-S collagen and the laminin fragment P2 have been shown to increase in streptozotocin-induced diabetic rats, the former effect being inhibited by insulin treatment (35). Increased serum levels of the laminin fragment P1 have been found in adult patients with IDDM as compared with healthy persons, but no significant differences have been observed between type 1 diabetic patients with and without established microangiopathy (36). The serum concentration of PIIINP has been used as an indicator of the metabolism of interstitial collagens (37).

In the light of previous results, increased serum levels of laminin P1, 7-S collagen, and PIIINP could have been expected among the diabetic patients with joint and skin manifestations, but the results showed lower concentrations in the presence of joint contractures. Thus, we could not find any sign of accelerated collagen synthesis. In contrast, our observations do suggest a reduced turnover in basement membranes and interstitial tissues. This finding is in line with the hypothesis that the stability of collagens could be increased in the presence of joint and skin manifestations of diabetes.

The correlation between joint and skin manifestations and the degree of nonenzymatic glycation of skin collagen in diabetic patients has been investigated with conflicting results (7, 33, 34, 38), whereas no clear association has been found between the level of glycated Hb and these chronic complications of diabetes (7, 33). The present results showed a significant increase in HbA_{1C} in children with joint manifestations, and, accordingly, it seems logical that because metabolic control is impaired the nonenzymatic glycation of interstitial and basement membrane collagens could also be accelerated, leading to increased stability of collagen and its accumulation in various tissues.

Joint and skin manifestations are common complications of IDDM in childhood. The present findings suggest that the development of diabetic joint contractures is associated with lower serum concentrations of collagen and laminin metabolites. Our data also indicate that joint manifestations are associated with impaired metabolic control in diabetic children. The development of joint and skin manifestations seems to be unrelated to known genetic markers of IDDM because we could not find any association between the disease susceptibility antigens in the HLA-D locus and the presence of such manifestations. On the other hand, the overrepresentation of familial cases with IDDM among the patients with both joint and skin manifestations implies that genetic factors unrelated to the HLA system may play a role in the pathogenesis of these diabetic complications.

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