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M. Janner
S. Eberhard Knill
P. Diem
K. A. Zuppinger
P. E. Mullis

Persistent microalbuminuria in adolescents with Type I (insulin-dependent) diabetes mellitus is associated to early rather than late puberty

Results of a prospective longitudinal study

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M. Janner · S. Eberhard Knill
K. A. Zuppinger · P. E. Mullis (✉)
Department of Paediatrics,
Paediatric Endocrinology,
University of Berne, Inselspital,
CH-3010 Berne, Switzerland

P. Diem
Department of Medicine, Diabetes Station,
University of Berne, Inselspital,
CH-3010 Berne, Switzerland

Abstract Microalbuminuria is generally accepted to be highly predictive of overt diabetic nephropathy which is the leading cause of end-stage renal failure and, consequently, of death in patients with type 1 (insulin-dependent) diabetes mellitus (IDDM). Its early identification and therapy are exceedingly important. We studied prospectively the occurrence of microalbuminuria (MA) in relation to puberty and its pubertal stages in 164 children and adolescent patients (83 girls and 81 boys) with IDDM. Analysing 100 healthy subjects, normal values for albumin excretion (range: 0–10.1 μg/min/1.73 m^2) according to sex and the different pubertal stages were defined. No significant difference between the groups were noted and, therefore, 20 μg/min per 1.73 m^2 (3 SD above the mean) was generally defined as cut-off for MA. Of the patients with IDDM studied, 20% (20 females and 12 males) developed persistent MA (22.1–448.2 μg/min/1.73 m^2) during the study period of 8 years. The first manifestation of persistent MA was in 69% (13 females and 9 males) during stages of early and midpuberty; and in 28% (6 females and 3 males) at a late pubertal stage or at the end of puberty. The only child who developed MA before the onset of puberty (range: 23.5–157.4 μg/min/1.73 m^2) was found to have dystopic kidney. Therefore, all patients with IDDM should be screened for MA regardless of diabetes duration, sex and level of diabetes control beginning at the very first stage of puberty and neither earlier nor after puberty as suggested by the American Diabetes Association.

Key words Insulin-dependent diabetes mellitus · Childhood Microalbuminuria · Puberty Diabetic nephropathy

Abbreviations *ADA* American Diabetes Association · *AER* albumin excretion rate · *CV* coefficient of variation · *IDDM* type I (insulin dependent) diabetes mellitus *MA* microalbuminuria (20–200 μg/min/1.73 m^2)

Introduction

Approximately 40% of patients with type 1 (insulin-dependent) diabetes mellitus (IDDM) develop diabetic nephropathy with proteinuria, hypertension and reduced glomerular filtration rate leading to end-stage renal disease [2]. Mogensen et al. [19] have classified the progression of diabetic nephropathy from its onset (stages I and II), where only physiological and histological abnormalities are present, to stage IV, when nephropathy becomes clinically overt. Stage III is described as "incipient" diabetic nephropathy; its only manifestation is the present microalbuminuria (MA). In addition, MA is generally accepted to be highly predictive of overt diabetic nephropathy [18]. Several studies without exactly focusing on puberty and the stages itself have suggested that prepubertal diabetic subjects are less prone to microvascular compli-

cations than postpubertal subjects with equal duration of diabetes and the same level of control [7, 15, 20, 23, 24]. These findings have raised the possibility that hormonal changes during puberty may modify the development of diabetic microangiopathy causing both retinopathy and nephropathy.

Therefore, the aim of the present prospective study was first to determine the exact occurrence and prevalence of persistent MA in children and adolescents with IDDM, second to define the relation of the persistent MA to the different pubertal stages, and third to evaluate the current concept suggested by the American Diabetes Association (ADA) that screening for MA should begin only after puberty [1].

Subjects and methods

Patients

A total of 164 patients (83 girls and 81 boys) with IDDM regularly treated at the University Children's Hospital in Bern were enrolled into the study and followed through puberty. Before and during puberty there were no drop outs. After the age of 18 years, the diabetic patients were referred to the Adult Diabetic Clinic at the Inselspital in Bern where these patients were followed until the end of the study period. However, 7 patients (4 girls, 3 boys) left Bern and, therefore, no data were available after puberty; consequently, we do not know whether they developed MA or not.

At the beginning of the study, the children studied were prepubertal, the median age was 10.2 years (range: 5.0–13.5) and the range of the duration of diabetes 1–12 years. The treatment consisted of an age-adapted diabetes regimen and injections of a combination of short (Actrapid; Novo-Nordisk, Copenhagen, Denmark) and intermediateacting (Insulatard; Novo-Nordisk) insulin. At 3 monthly intervals, mean blood pressure, body mass index and pubertal stages according Tanner [26] were recorded; in addition, glycated haemoglobin, fructosamine and creatinine clearance were measured. After puberty some patients were seen in 6 monthly intervals. Macroproteinuria was exluded by negative results of an Albymtest (Boehringer, Mannheim, Germany; sensitivity 150 mg protein/l). Metabolic control was usually assessed by urinary glucose and acetone estimations three to four times a day (Ketodiabur 5000; Boehringer Mannheim), and by frequent home blood glucose monitoring (Haemoglucotest, Boehringer). There was no history of nephrological disorders; and in patients with MA both abdominal ultrasound and IgA measurement were performed to exclude urinary tract malformations and IgA-nephritis respectively.

Control-subject

As a reference for sex and their different pubertal stages a cohort of 100 children (10 boys and 10 girls for each pubertal stage) were studied. Urinary albumin excretion (AER) was measured during 3 consecutive nights. The exact procedure of 12 h overnight urine collection was carefully instructed.

Methods

The prospective study lasted for 8 years. It was approved by the ethical committee and informed parental consent was obtained.

Regular measurement of AER at 3 monthly intervals became a routine for our patients with IDDM. During the night preceding the visit to our outpatient clinic, urine was collected quantiatively over a 12 h period in a plastic bottle without preservatives. AER was determined by a radioimmunoassay (Pharmacia AB, Uppsala, Sweden) with sensitivity of 0.4 mg/l and an interassay coefficient of variation of 7.2% at 3.2 mg/l. AER of 20–200 µg/min per 1.73 m^2 was considered as MA (see Result section). It is recognized that albuminuria may initially be intermittent, or might be related to remarkable physical exercise or illness [8, 18–20]. Therefore, persistent MA was considered when three out of four consecutive AER levels were pathologically high and remained at that increased level [18]. For the purpose of the current study, we have designated the onset of persistent MA as the date on which the first pathological value for AER was measured.

In addition, a morning mid-stream urine specimen was obtained from each patient. Macroproteinuria was ruled out by Albymtest and urinary tract infection was excluded by Uricult.

Plasma creatinine levels determined by the Jaffé reaction using a Greiner G 400 analyser and creatinine clearance was estimated from the plasma creatinine concentration and body height measured by a Harpenden stadiometer [25]. Stable haemoglobin A_1 (HbA_1) was measured chromatographically by a thermostabilized microcolumn method (Boehringer Mannheim) after aldimine removal. The normal range was 5.8%–8% (mean ± 1 SD; 6.5 ± 0.69) with an interassay coefficient of variation (CV) of 4.5%, 3.4% and 2.6% at HbA_1 of 7%, 10% and 16% respectively. HbA_1 intra-assay CV was 2.6% at 9.8%. The value was corrected for the presence of HbF as determined by high-pressure liquid chromatography (Kyoto Daichi). Fructosamine was determined using a commercial kit (F. Hoffmann, LaRoche, Basel, Switzerland). This colourimetric test is based on the ability of ketoamines to reduce nitroblue tetrazolium in alkaline medium (0.25 mmol/l NBT in 0.1 mol/l sodium carbonate buffer, pH 10.35 at 25° C). The intra-assay CV was 4% at 2.0 mmol/l and 4.5% at 3.1 mmol/l, interassay CV 3.8% at 2.4 mmol/l and 2.3% at 4.9 mmol/l. Normal values of fructosamine (> 5 years of age) are 1.5–2.4 mmol/l.

Blood pressure was measured in the supine position on the right upper arm using the largest cuff possible [21]. The diastolic pressure was noted at disappearance of the Korotkoff sounds (phase 5) and the mean arterial pressure was calculated as the diastolic pressure plus one-third of the difference between systolic and diastolic pressure.

Statistics

For statistical analysis, the paired and unpaired Student's *t* tests were used for parametric data and the Wilcoxon and Spearman rank test for non-parametric data. All hypothesis tests were two-tailed and were calculated at the 0.05 level of significance.

Results

Reference values

The sex and pubertal stage specific reference values for 12 h overnight AER were determined in 100 healthy subjects during three nights in a row and are shown in Table 1. Significant differences were noted neither between male and female nor the groups of different pubertal stages. Since the level of the AER did not exceed 10 µg/min per 1.73 m^2, a level of 20–200 µg/min per 1.73 m^2 was taken to represent MA. This value is 3 SD above the mean AER and is in agreement with literature data as cut-off for MA [18, 19].

Table 1 Normal values of albumin excretion in 12 h overnight urine collections for each pubertal stage in 100 healthy controls

n	Sex	Pubertal stage	Albuminuria (μg/min/1.73 m^2) Range (median)
10	F	B1	0 – 5.8 (2.5)
10	M	G1	0 – 4.9 (4.3)
10	F	B2	0.4– 4.9 (3.3)
10	M	G2 (Testes 3 ml)	0 – 5.2 (2.9)
10	F	B3	0.2– 5.8 (3.5)
10	M	G3 (Testes 6–10 ml)	0.2– 4.4 (2.2)
10	F	B4	0.5– 7.3 (3.2)
10	M	G4 (Testes 12–18 ml)	0 – 5.1 (3.8)
10	F	Adult	1.0–10.1 (3.9)
10	M	Adult	1.2– 9.5 (4.2)

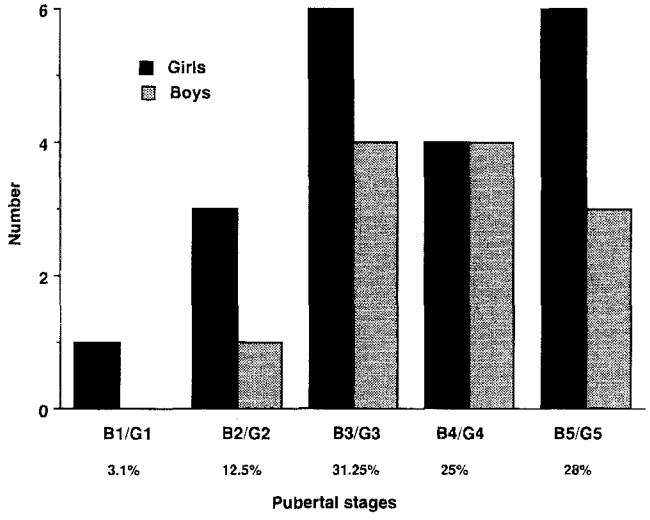

Fig. 1 First manifestation of persistent MA in 32 patients with IDDM and its relation to the pubertal stage

Albumin excretion rates in patients with IDDM

The AER values ranged from 0.7–448.2 μg/min per 1.73 m^2 (mean: 15.7). One hundred and thirty-two patients, 80% from a total of 164, 76% of the females (*n*: 63; total 83) and 85% of the males (*n*: 69, total 81) had normal AER values (0.5–18.9; mean: 6.2 μg/min/1.73 m^2).

Thirty-two patients (20%; 20 females and 12 males) developed persistent MA during the study (22.1–448.2; mean: 78.7 μg/min/1.73 m^2). The mean observation time of persistent MA was 4.6 years (1.2–7.8). Urinary tract infection and/or other specific causes increased AER such as IgA-nephritis were excluded [3]. An AER greater than 200 μg/min per 1.73 m^2 (approximately 300 mg/24 h) measured in two out of three timed urine collections within a 3-month period, was defined as macroalbuminuria and considered indicative of overt diabetic nephropathy [19]. This was the case in one male patient. The macroalbuminuria developed from MA over 4 years.

Onset of MA and its relationship to pubertal stages

Of the 164 patients studied, 20% (20 females and 12 males) developed MA during the study period. The first manifestation of MA was in 69% (13 females and 9 males) during stages of early and mid-puberty; and in 28% (6 females and 3 males) at a late pubertal stage or at the end of puberty. Figure 1 presents the occurrence of MA according to the different pubertal stages for boys and girls. There was no positive correlation between the occurrence of MA and age, sex, duration of diabetes before puberty and indices of diabetes control. Only one child (9-year-old girl) developed MA (range: 23.5–157.4 μg/min/1.73 m^2) before the onset of puberty and, interestingly, a dystopic kidney was diagnosed.

Table 2 Comparison of mean values (± SD) of glycated haemoglobin (HbA_1), fructosamine, creatinine clearance (creat. cl.) and mean blood pressure (BP) in two subgroups with and without MA derived from the 6 months before and after occurrence of MA and/or identical pubertal stage. The two groups were matched for sex, age, duration of diabetes, and duration of diabetes up to the pubertal stages at which in one group the first manifestation of diabetes occurred (*NS* non significant)

	Patients with MA			Patients without MA inclusive pubertal stage matched with MA group[a]		
	Before MA (*n* = 32)		After MA (*n* = 32)	Before pubertal stage matched with MA group (*n* = 32)		After pubertal stage matched with MA group (*n* = 32)
	•	NS	*	•	P = 0.07	*
HbA1 (%)[b]	9.4 (± 1.3)	NS	9.8 (± 1.4)	9.1 (± 1.1)	NS	9.0 (± 1.2)
Fructosamine (m*M*)[b]	3.4 (± 0.4)	NS	3.5 (± 0.3)	3.2 (± 0.5)	NS	3.1 (± 0.5)
Creat. cl. (ml/min/1.73 m^2)[b]	109.7 (± 11.5)	NS	109.6 (± 11.9)	109.0 (± 6.1)	NS	110.2 (± 9.8)
Mean BP (mmHg)[b]	86.8 (± 11.3)	NS	89.3 (± 9.2)	84.6 (± 5.1)	NS	85.9 (± 6.8)

[a] Matching: i.e. At the age of 12 years, a girl developing MA at the pubertal stage B2 after 5 year duration of diabetes was matched with a 12-year-old girl presenting B2 after a duration of diabetes of 5 years but without MA
[b] Data: mean ± 1 SD

Diabetes control and AER relationship

The patients with persistent MA were compared with a group of diabetic patients matched for sex, age, pubertal stages and total duration of diabetes. The data are shown in Table 2. There was no significant difference between mean values of indices of diabetes control such as glycated haemoglobin and fructosamine, creatinine clearance, body mass index, and blood pressure from at least three measurements derived from the 6-month-period prior to and after the first manifestation of MA in patients with MA and the matched patients without MA (Table 2). It should be emphasized that a slight but not statistically significant increase of HbA_1, fructosamine and mean blood pressure was found in the patients with MA after the development of MA in comparison to the values from the preceding 6-month period which was not seen in the matched control patient group without MA. In addition, the HbA_1 values derived from the 6-month period after the occurrence of MA were compared with the corresponding values of the matched control patients. The *P* value was 0.07 and, therefore, the level of significance was not attained (Table 2) possibly caused by the tight criteria of matching which included pubertal stages in relation to the duration of diabetes. (i.e: At the age of 12 years, a girl developing MA at the pubertal stage B2 after a 5-year duration of diabetes was matched with a 12-year-old girl presenting B2 after a duration of diabetes of 5 years but without MA).

Discussion

This study analysed prospectively the occurrence of MA in a group of 164 children and adolescents with IDDM over 8 years. Data from previous reports suggested, using age-related criteria only, that puberty is a critical period for the development of MA and, thus, routine screening for MA was also recommended in paediatric diabetes care [7, 20]. Since renal disease is the most important correlate of mortality in IDDM, early identification and therapy is exceedingly important. Therefore, the aim of this study was to define the extent of MA by pubertal stage and to evaluate the generally accepted guidelines of the ADA explicitly suggesting MA screening should begin after 5 years total duration of diabetes in adults, or just after puberty in children [1].

In 100 healthy children and adolescents, normal values for AER were defined according to sex and different pubertal stages. No significant difference were noted between sex and pubertal stages and, therefore, 20 μg/min per 1.73 m^2 (3 SD above the mean) was defined as cut-off for MA. Because of its skewed distribution, AER was examined compared with body surface area. Since AER is known to vary from day to day, sometimes quite significantly [22], multiple determinations are important for confirmation of AER and/or persistent MA in controls as well as in patients, as was done in our study.

Most interestingly, in the 164 young and adolescent patients with IDDM studied, persistent MA was found in 20% (*n*: 32) after the study period of 8 years. MA developed in 69% (13 females and 9 males) and in 28% (6 females and 3 males) during stages of early and mid-puberty and at a late pubertal stage, respectively. These data underline the fact that screening for MA is to perform neither prior to the puberty nor after puberty as recommended by ADA [1] but that it will be most important to perform tedious overnight urine collection soon after the onset of puberty (Tanner stage 2) [20, 26]. Furthermore, there are commercially available very sensitive, inexpensive methods suitable for MA screening that use enzyme-linked immunoabsorbent assays with both high sensitivity and specificity.

As reported for retinopathy, development of MA before the onset of puberty is rare, indicating some influence of pubertal development and sexual maturation [4]. When persistent MA in IDDM occurs before the onset of puberty, another cause such as IgA-nephritis, urinary tract infection etc. has to be considered.

In contrast to adult patients with IDDM, MA in paediatric patients occurred independently of duration of diabetes emphasizing that years before puberty seem to count less than those after [4, 6, 17].

Since there is a widespread clinical impression that during the pubertal ages diabetic children have poorer metabolic control compared to before puberty, it can be speculated whether the relation between AER and puberty is only an effect of poor metabolic control. In our patients, however, we found no significant differences in HbA_1 values within the two groups of children with and without MA. In contrast, comparing glycaemic control in the 6-month period after the first manifestation of MA a tendency towards a significantly poorer control in children with MA was found (Table 2). Therefore, although our results do establish neither a causal nor a statistically significant relationship between long-term hyperglycaemia (HbA_1 and fructosamine as indices of diabetes control, Table 2) and nephropathy in the individuals with persistent MA, they may lend support that better diabetes control decreases the likelihood of developing diabetic nephropathy or delay its onset. If metabolic control is already acceptable consideration should be given to other therapies, such as low protein diets and/or angiotensin enzyme inhibitors [5, 10, 27]. These strategies appear to alter some of the renal haemodynamic factors believed to contribute to the albuminuria.

Cross-sectional studies of patients with IDDM and MA have shown elevated blood pressure compared with patient with normal AER [14, 29]. In addition, prospective studies have demonstrated that progression of MA is associated with an increase in blood pressure [9]. The close association between systemic blood pressure and MA is

supported by the fact that acute and long-term reduction of blood pressure in normotensive IDDM patients with MA causes normalization of AER [11, 13]. Which of these two variables precedes the other in the development of nephropathy is open to debate. In fact, in some studies elevated AER preceded an increase in systolic blood pressure [16], but other authors reported prior blood pressure elevation in patients with IDDM who subsequently developed MA as compared with patients who remained normo-albuminuric [12, 28]. In our patients who developed MA mean blood pressure did not change significantly, but a slight increase was evident. These data support the concept that the increase of the blood pressure might be secondary to the pathological processes in the kidney in IDDM patients developing persistent MA [16].

In conclusion, persistent MA does occur not only after puberty but also at its very first stage. It is a major risk factor for diabetic nephropathy and, consequently, routine screening is to be performed soon after the onset of puberty if primary prevention of end-stage renal disease is the final aim.

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