Archives of Disease in Childhood, 1989, **64,** 984–991

Original articles

Early markers of the renal complications of insulin dependent diabetes mellitus

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SUMMARY We investigated the associations between albuminuria, metabolic control, glomerular filtration, blood pressure, and platelet function in children with insulin dependent diabetes mellitus. The geometric mean (95% tolerance levels) albumin excretion (expressed as the geometric mean albumin to creatinine ratio on two overnight urine collections (UA/UC)), in 60 diabetic children was 0·72 (0·80–6·9) mg/mmol, significantly greater than in 45 normal children (geometric mean 0·41(0·14–1·17)). Mean (SD) glomerular filtration rate, measured by ^{51}Cr edetic acid clearance during constant infusion, was significantly greater in diabetic children (129 (20) ml/min/1·73 m^2) compared with normal controls (109 (13)). Mean (SD) renal length for height standard deviation score was +0·25 (1·1); systolic blood pressure standard deviation score was 0·15 (0·65), and diastolic blood pressure was 0·51 (0·82). Spontaneous platelet aggregation, expressed as percentage fall in platelet count in stirred whole blood after 2 minutes was 17·8 (9·2)% in the diabetic compared with 12·3 (7·9)% in normal children. UA/UC correlated with renal length and of the children with UA/UC above the normal range, 70% also had a glomerular filtration rate above the normal range. There was a weak correlation between UA/UC and glycated haemoglobin (HbA_{1c}). All children with spontaneous platelet aggregation above normal had had diabetes for more than seven years. These cross sectional data define some of the early markers and inter-relationships that may be important in the development of nephropathy.

The risk of developing nephropathy is highest in those individuals with insulin dependent diabetes mellitus (IDDM) in whom the disease starts in childhood,[1 2] but it is difficult to identify accurately the susceptible individuals in the early years. The only marker that has been shown in prospective studies of adult diabetic individuals to predict nephropathy is the presence of microalbuminura.[3–6] This predates the onset of 'macroproteinuria', decline in glomerular filtration rate, and onset of hypertension.[7] The level of urine albumin excretion, however, which best predicts nephropathy remains unclear as studies have varied in their methods of urine collection and attention to variability, which is high in both normal and diabetic individuals.[8–10] Microalbuminuria, defined as urine albumin excretion above the 95th centile for normal children has been reported to be raised in 12–20% of diabetic children.[11–14]

Other possible early markers of nephropathy in diabetes include an increase in glomerular filtration rate, kidney size, and blood pressure. A high glomerular filtration rate has been reported in 20–30% of diabetic children under conditions of standard insulin treatment,[14–16] and although poorly studied in childhood, increased renal size has been described in adults with IDDM, both at diagnosis and during the early years of the disease.[17–19] Hypertension is an accepted feature of diabetic nephropathy after the appearance of microalbuminuria and its treatment has been shown to slow the decline of renal function.[20 21] In children with IDDM, blood pressure data are conflicting, but where increases have been shown, they have been small.[22–24] A problem in most of these studies is the choice of controls. Some studies have used the siblings of the diabetic children,[22] whereas others have related the results to normal published data.[13] At present there are no such data for British children and it is necessary to use either continental[25] or American data.[26]

Abnormal platelet function has been implicated in

the pathogenesis of diabetic, nephropathy,[27 28] and increased platelet aggregation has been reported in children with IDDM in the early stages of the disease.[29]

Weak correlations between some of these markers have been reported in previous studies,[11 13 30] but they have not been systematically described together in a population of children with IDDM. We have therefore examined the associations between them and urine albumin excretion in order (a) to determine whether a single population of diabetic children share these possible risk factors and (b) to examine the role of other compounding factors such as age, puberty status, metabolic control, and duration of disease.

Subjects and methods

DIABETIC SUBJECTS

Sixty children and adolescents attending the diabetic clinics at the Hospitals for Sick Children, London, agreed to participate in the study. Their mean (SD) age was 13·1 (3·5) years and the mean duration of IDDM was 7·0 (4·3) years; 16 (27%) of the children were prepubertal, 17 (28%) in early puberty (stages 2 and 3), and 27 (45%) were in late puberty (stages 4 and 5); there were 27 boys and 33 girls. Two of the 60 diabetic children were on anticonvulsant drugs (carbamazepine and sodium valproate). The remainder were receiving no other drugs apart from insulin. None of the diabetic children or controls had taken aspirin during the preceding two weeks and none of the children had evidence of urinary infection at the time of study.

Complete studies were undertaken on all 60 children with the exception of glomerular filtration rate measurement; only 44 children agreed to this procedure.

CONTROLS

The urine albumin excretion was measured in 45 normal schoolchildren of mean age 13·4 (2·7) years; nine (19%) were prepubertal, 14 (32%) in early and 22 (49%) in late puberty; there were 23 boys and 22 girls.

Platelet studies were undertaken in 26 normal children of mean age 13·1 (4·9) years before minor surgery; blood samples were taken at the same time as the routine check for haemaglobin concentration.

It was not possible to obtain control data for glomerular filtration rate measurements in normal children. As glomerular filtration rate corrected for surface area reaches adult values by the age of 2 years,[31] however, it was considered justifiable to use adults as controls. Glomerular filtration rate was measured in 15 normal young adults, mean age 24·1 (16·5) years who were recruited from hospital staff and friends; there were seven men and eight women.

All of the studies described had the approval of the ethical committee at the Hospital for Sick Children, Great Ormond Street, London.

METHODS

Urine albumin excretion

We measured urine albumin excretion on overnight urine samples because the variability is less than in 24 hour or daytime collections[9 32] and they are more practical to collect at home. The duration of overnight collections was similar in normal and diabetic children.

Urine albumin concentration was measured by double antibody radioimmunoassay (Diagnostic Products Corporation) with a sensitivity of 0·5 mg/l, an intra-assay coefficient of variation of 4%, and interassay coefficient of variation of 5%. Urine creatinine concentration was measured by the Jaffé reaction and urine albumin excretion was expressed as the geometric mean urine albumin creatinine concentration ratio (UA/UC) in mg/mmol of two consecutive overnight urine samples. We have previously shown that overnight UA/UC correlated well with albumin clearance and urine albumin excretion rate.[8]

Glomerular filtration rate

Glomerular filtration rate was measured using a constant infusion of 51-chromium ethylene-diamine-tetra-acetic acid (^{51}Cr edetic acid) under conditions of water diuresis. We used this technique because the unicompartmental plasma clearance method usually used for measuring glomerular filtration rate in children overestimates normal and high glomerular filtration rate and is therefore inappropriate for investigating hyperfiltration states.[33 34] All measurements were performed in the morning. Diabetic children had their normal morning insulin and all subjects were instructed to omit tea, coffee, meat, and milk for their breakfast meal; two to three hours later a diuresis was established with an oral water load of 20 ml/kg body weight. The mean (SD) urine flow rate in the diabetic children was 11·0 (3·5) ml/min and in the controls was 12·6 (4·0) ml/min. A priming dose of ^{51}Cr edetic acid (0·025 MBq/kg) was administered, followed by a constant infusion of $2{\cdot}3\times10^{-4}$ MBq/kg/minute. After 45 minutes for equilibration, three or four carefully timed, spontaneously voided urine samples were collected at 20–30 minute intervals. A blood sample was drawn from a peripheral vein at the midpoint of each collection period. The mean of three or four clearances (UV/P) was then calculated and corrected to 1·73 m^2 surface area using the Dubois

formula. Estimates of glomerular filtration rate were rejected if one or more individual clearance differed from the mean by more than 15%. Acceptable estimates of glomerular filtration rate were obtained in 41 of the 44 children studied.

Renal size

Renal length was measured by ultrasonography with the child in the prone position and related to normal data for right and left kidney lengths plotted against body height.[35] Data on the diabetic children was expressed as the mean of the right and left kidney length standard deviation scores, compared with these normal published sonographic growth charts.

Blood pressure

Blood pressure was measured in the right arm with the child seated, using a random zero sphygmomanometer with the appropriate sized cuff and taking the fifth sound as the diastolic blood pressure. All measurements were taken by the same observer after the glomerular filtration rate measurement was completed. Results were expressed as a standard deviation score related to the 1987 Task Force Data for blood pressure in normal children.[26]

Platelet aggregation

Platelet aggregation was measured in whole blood using the Ultraflo 100 Whole Blood Platelet Counter. A total of 5 ml blood was taken into a polypropylene syringe using a 19 gauge needle and immediately transferred into a polystyrene tube containing 0·5 ml 3·8% trisodium citrate dihydrate. A 0·5 ml aliquot was then stirred in a plastic tube at 1000 rpm at 37°C. At 1, 2, 3, 4, and 8 minutes after stirring had commenced, 20 µl aliquots were taken and immediately fixed by mixing with 40 µl of formalin-edetic acid solution to prevent further aggregation or disaggregation before counting. Aggregation was expressed as the percentage fall in platelet count at 2 minutes compared with the count in an aliquot taken immediately before stirring.[36] All experiments were performed between one and three hours after venepuncture and a control was included in every platelet aggregation experiment.

Glycated haemoglobin

Glycated haemoglobin (HbA_{1c}) was measured by an electrophoretic method, the normal range being 5·0–8·0%.

STATISTICAL METHODS

Computation and statistical analysis was performed using the Statistical Analysis System (SAS Institute Inc).

UA/UC in both normal and diabetic children was best described by a log normal distribution and therefore results were log transformed before analysis and expressed as the geometric mean and 95% tolerance limits (antilog −2 SD to +2 SD).[8] Comparisons of variables between normal and diabetic children and diabetic children with high versus normal UA/UC were made by use of unpaired (two tailed) Student's *t* tests. Associations between variables were examined using linear regression by the least squares method, Pearson's correlation coefficients, χ^2 analysis, and multiple regression.

Results

PRIMARY DATA

Mean (SD) HbA_{1c} concentration was 11·8 (2·3)% and correlated with increasing age ($r=0·44$, $p<0·001$).

The geometric mean (95% tolerance limit) for UA/UC in the diabetic children was 0·72 (0·08–6·9) mg/mmol, significantly greater than that of the normal children, 0·41 (0·14–1·17, $p<0·001$) and 15 (25%) of diabetic children had UA/UC values above the normal range (fig 1). The characteristics of these 15 diabetic children with high UA/UC are shown in table 1.

Mean (SD) glomerular filtration rate in the diabetic children (41 studied) was 129 (20) ml/min/

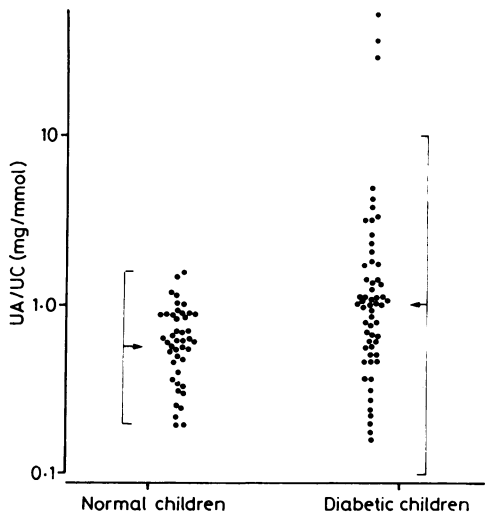

Fig 1 *Geometric mean and 95% tolerance limits for UA/UC in mg/mmol plotted on a log scale in normal and diabetic children.*

Table 1 *Comparison of diabetic children with high compared with normal UA/UC. Results expressed as mean (SEM)*

Variable	*High UA/UC (n=15) (>1·17 mg/mmol)*	*Normal UA/UC (n=45) (≤1·17 mg/mmol)*	*p Value (t test)*
Sex (M/F)	5/10	22/23	
Age (years)	14·5 (0·73)	12·7 (0·53)	<0·1
No at puberty stage:			
1	3	13	
2 and 3	2	14	
4 and 5	10	18	
Disease duration (years)	8·10 (0·92)	6·80 (0·65)	<0·5
Glycated haemoglobin (%)	13·40 (0·53)	11·20 (0·31)	<0·001
Glomerular filtration rate (ml/min/1·73 m^2)	140·10 (3·36)	124·80 (3·65)	<0·01
Renal size standard deviation score	1·16 (0·28)	−0·03 (0·15)	<0·001
Systolic blood pressure standard deviation score	0·03 (0·06)	0·19 (0·10)	<0·2
Diastolic blood pressure standard deviation score	0·59 (0·23)	0·48 (0·12)	>0·5
Platelet aggregation at 2 minutes	18·80 (2·45)	17·40 (1·36)	>0·5

1·73 m^2 surface area, significantly greater than the 109 (13) ($p<0·001$) observed in controls, and 16/41 (39%) had values above this normal range (fig 2).

The mean (SD) systolic blood pressure standard deviation score in the diabetic children did not differ significantly from zero (0·15 (0·65), $0·1>p>0·05$), whereas the diastolic standard deviation score was significantly raised with reference to the normal published data (0·51 (0·82), $p<0·001$).

Renal size was also normal in the diabetic children with a mean renal length for child height standard deviation score of +0·25 (1·1) ($0·1>p>0·05$).

Platelet aggregation, expressed as the percentage fall in platelet count was significantly greater in the diabetic children when compared with normal children at 2 minutes ($p<0·01$). Significant differences were also observed at 1, 3, and 4 minutes but not at 8 minutes after stirring (table 2). The 2 minute value was used for subsequent analysis.

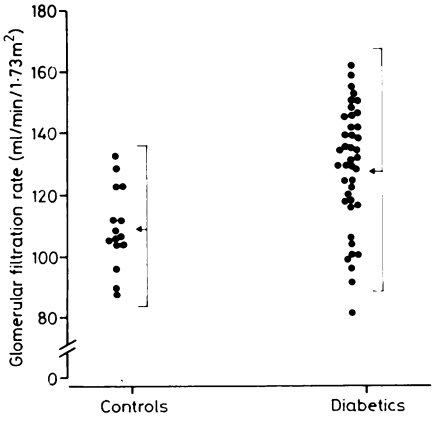

Fig 2 *Geometric mean and range (2 SD) for glomerular filtration rate in ml/min/1·73 m^2 surface area for diabetic children and controls.*

Table 2 *Platelet aggregation expressed as percentage fall in platelet count in stirred whole blood in the diabetic children compared with normal children*

Time (minutes)	*Diabetic children Mean (SEM)*	*Normal children Mean (SEM)*	*p Value (t test)*
1	10·4 (0·9)	6·1 (1·0)	<0·05
2	17·8 (1·2)	12·3 (1·5)	<0·01
3	27·5 (1·4)	22·2 (2·1)	<0·01
4	36·5 (1·5)	31·4 (2·2)	<0·01
8	49·0 (1·4)	45·0 (2·5)	<0·5

INTER-RELATIONSHIPS

UA/UC correlated with age ($r=0·35$, $p<0·005$) in the diabetic but not in the normal children ($r=0·14$). The association in the diabetic children was not attributed to disease duration, as this did not correlate significantly with UA/UC ($r=0·08$). Of the diabetic children who had UA/UC above the normal range, five were prepubertal or in early puberty and 10 were in late puberty ($\chi^2=3·2$, $0·05>p>0·1$). In the normal children, the geometric mean (range) UA/UC was similar in those in Tanner pubertal stages 1, 2, and 3, 0·41 (0·2–1·0), to those in pubertal stages 4 and 5, 0·41 (0·3–1·3). There was no significant relationship between UA/UC and age at onset of diabetes.

UA/UC correlated weakly with HbA_{1c} ($r=0·36$, $p<0·01$). As both UA/UC and HbA_{1c} correlated with age, multiple regression was performed to hold this confounding variable constant. The association between UA/UC and HbA_{1c} was still significant ($p<0·05$). There were no significant associations

between HbA_{1c} and the other parameters measured.

Examining the association between UA/UC and glomerular filtration rate it was observed that, although there was no significant linear correlation ($r=0{\cdot}29$, $p<0{\cdot}07$), seven of the 10 diabetic children who had UA/UC above the normal range also had glomerular filtration rate above the normal range (fig 3) ($p<0{\cdot}001$, χ^2).

There was a significant correlation between UA/UC and renal size ($r=0{\cdot}45$, $p<0{\cdot}001$) and although mean renal size in the diabetic children was not significantly increased, those with UA/UC above normal more often had kidneys above normal mean length for height ($p<0{\cdot}002$, χ^2) (fig 4). The correlation between UA/UC and renal size was still significant ($r=0{\cdot}45$) if only those children in whom glomerular filtration rate had been measured were included ($n=41$). There was also a significant correlation between glomerular filtration rate and renal size ($r=0{\cdot}56$, $p<0{\cdot}001$) (fig 5).

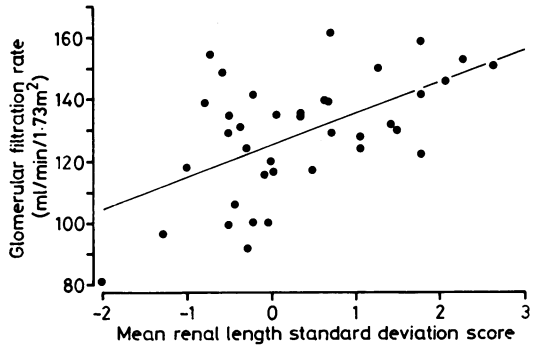

Fig 5 *Association between glomerular filtration rate and renal length for height standard deviation score.*

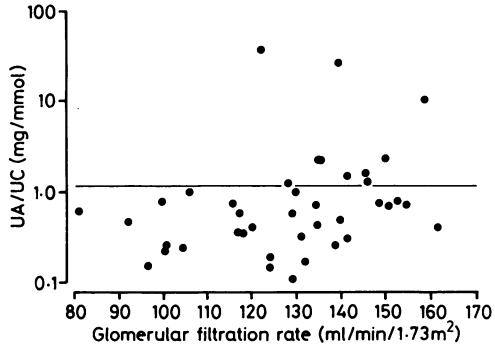

Fig 3 *Association between UA/UC in mg/mmol (log scale) and glomerular filtration rate in 41 diabetic children. The upper 95% tolerance limit for UA/UC in the controls is indicated.*

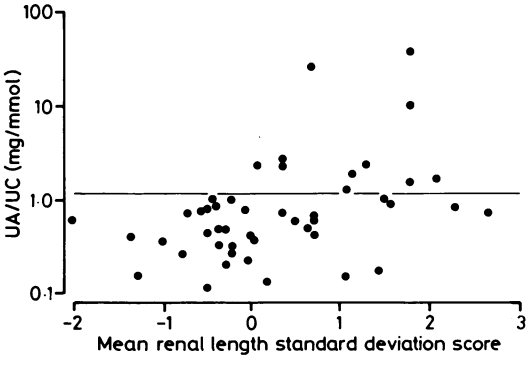

Fig 4 *Association between UA/UC in mg/mmol (log scale) and renal size standard deviation score. The upper 95% tolerance limit for UA/UC for the controls is indicated.*

Neither systolic nor diastolic blood pressure correlated significantly with any of the above markers.

There was no significant association between platelet aggregation and any of the parameters of renal function measured. However, there was a significant correlation with disease duration ($r=0{\cdot}35$, $p<0{\cdot}01$). Eight of the 25 (32%) children who had had diabetes for more than seven years (the mean disease duration for the group) had platelet aggregation at 2 minutes above the normal range, whereas no child who had had the disease for less than seven years had abnormal platelet aggregation ($p<0{\cdot}01$, χ^2). If age, which correlated with disease duration, is held constant using multiple regression analysis, the association between platelet aggregation and disease duration remains significant ($p<0{\cdot}01$). Furthermore, there was no significant correlation between platelet aggregation in the controls.

Discussion

The prevalence of high UA/UC observed in the diabetic children in this study was similar to that in previous studies.[11–14] UA/UC increased with age and progression through puberty and was not correlated with disease duration. Rowe *et al* also observed an association between UA/UC and age, after controlling for disease duration.[12] In both his study and that of Mathiesen *et al*,[11] raised urinary excretion of albumin was more prevalent in children

over the age of 14 or 15. At variance with our results, Davies *et al* reported a small effect of age on the urinary albumin excretion rate but not UA/UC in normal children.[13] Although he observed no significant association between urinary albumin excretion rate and age in diabetic children, however, those with a urinary albumin excretion rate above the normal range were significantly older than those with a normal rate.[13] These studies have used urinary albumin excretion rate to express results of albumin excretion whereas results were expressed as UA/UC in this study. In 47 of the 60 diabetic children where accurate timed urine collections had been made, however, urinary albumin excretion rate values were also available and in these children an association between the rate and age was also found ($r=0{\cdot}33$, $p<0{\cdot}02$).

Rowe *et al* suggested that puberty might be an important factor in the development of nephropathy. He divided the children into two groups according to age (less than or more than 12 years), however, and not according to pubertal stage. In our study, 66% of children with increased UA/UC were in late puberty irrespective of duration of diabetes. Although suggestive of an effect of puberty on the level of UA/UC, the effect of age was greater and it is impossible to separate these two confounding variables.

UA/UC tended to increase with an increasing concentration of HbA_{1c} even allowing for the effect of increasing age on HbA_{1c}. The degree of correlation between UA/UC and HbA_{1c} observed in this study was similar to that reported by Rowe *et al*[12] and by Davies *et al*,[13] but this is in contrast with other studies where no associations between urinary albumin excretion and degree of metabolic control were observed.[14 37]

We found that 39% of diabetic children had a raised glomerular filtration rate compared with normal controls. The glomerular filtration rate values in this study were very similar to previous reports,[13–16] although we have found that the clearance of ^{51}Cr edetic acid gives estimates of glomerular filtration rate which are about 6% lower than that of inulin.[38] Although adult control subjects were used in this study, their mean glomerular filtration rate was very similar to that of Berg and Thalme; using an inulin clearance technique, they observed a mean value of 114 ml/min/1·73 m^2 in group of 13 normal children and adults.[15]

There was no association observed between glomerular filtration rate and age or disease duration in this study. This is in accordance with both Dalquist *et al* who observed no difference in glomerular filtration rate between children with disease duration of less than five, five to 10, or more than 10 years,[14] and Davies *et al* who observed no correlation between either age or disease duration.[13] Berg and Thalme observed an increase in glomerular filtration rate up to a five year disease duration and an inverse relationship thereafter, despite the absence of albuminuria. However, this was assessed by Albustix and not by more sensitive techniques.[1]

When examining the association between UA/UC and glomerular filtration rate, significance was found with a χ^2 test but not with linear regression, which suggests a threshold effect rather than a linear relationship. This is in contrast to the findings of Davies *et al* who observed no difference in glomerular filtration rate between those with normal and those with increased urinary excretion of albumin,[13] and there have been few other studies of these two parameters in diabetic children. Of the three children with raised UA/UC but glomerular filtration rate in the normal range, one had persistent microalbuminuria and marginally raised blood pressure. Her glomerular filtration rate may have been declining with increasing UA/UC.[4] Most of the children with raised UA/UC had values above normal but below the 'microalbuminuric' range, however, the consensus for which is >20 μg/min/1·73 m^2, and which has been shown to predict nephropathy.[3–7] We have reported the association between UA/UC and urine albumin excretion rate in normal and diabetic children.[8] The 95th centile for UA/UC in the normal children on two overnight urines was equivalent to an albumin excretion rate on two overnight urines of 8·2 μg/min/1·73 m^2, very similar to that reported by Davies *et al*.[39] In this study, UA/UC above this level was associated with abnormally high glomerular filtration rate and larger than average kidney size.

We found no significant increase in renal size in diabetic children compared with a population of normal children. These findings are surprising, and contrast with previous reports of increased kidney size in adults with IDDM.[17–19] We did, however, find a significant correlation between glomerular filtration rate and kidney size as has been reported previously in both normal and diabetic individuals,[19] and our data also showed an association between UA/UC and renal size.

Hypertension is known to increase the rate of deterioration in renal function in diabetic nephropathy. Although some studies have shown minor increases in blood pressure in diabetic children,[22] most studies of diabetic children without microalbuminuria have found them to be normotensive.[23] In this study, we found no significant differences in systolic blood pressure standard deviation score but did find an increase in diastolic blood pressure standard deviation socre compared with normal

children from the 1987 American Task Force Data.[26] These results are in agreement with Davies *et al,* who used the French normal data from which to calculate standard deviation scores.[13] A criticism of the Task Force Data has been that blood pressure during childhood may relate better to height than to age.[24] In our study, however, we related the raw blood pressure data not only to age, but also to height, weight, and surface area using a multiple regression model. We found that blood pressure related best to age (data not shown), possibly because half of these diabetic children were in late adolescence and therefore more comparable with an adult population. Contrary to some[11 30] but not other[13] studies, we observed no correlation between blood pressure and either UA/UC or glomerular filtration rate.

We found an increase in platelet aggregation in stirred whole blood in this group of diabetic children compared with normal children. The role of platelets in the pathogenesis of diabetic nephropathy is unclear. Abnormal platelet function has been reported in other renal diseases including glomerulosclerosis[40] and platelet activation results in the release of cationic substances including platelet factor 4 (PF4) and mitogens such as platelet derived growth factor. Both could be involved in the pathogenesis of diabetic nephropathy, PF4 by binding to glomerular polyanions thereby increasing the permeability of glomerular capillaries to anionic albumin and platelet derived growth factor by causing mesangial cell proliferation.[41] Despite these theoretical reasons for linking abnormal platelet aggregation to the early increase in urine albumin excretion seen in these young diabetics, however, we were unable to show any association in this cross sectional study. In addition, the association found between platelet aggregation and disease duration would tend to support the argument that abnormalities in platelet function are secondary to microvascular damage rather than integral to any pathogenetic mechanisms in the development of nephropathy.

Combining these observations on early markers of renal function in children and adolescents with IDDM, we conclude that urinary albumin excretion that is above normal but not necessarily in the predictive microalbuminuric range for the development of nephropathy, is associated with a higher glomerular filtration rate and larger than average kidney size, but there is no association with blood pressure. Longitudinal follow up of these early markers is required to study their variability and to delineate their possible pathogenetic and predictive roles in the development of nephropathy in IDDM.

We thank Dr D Grant and Dr M Savage for permitting the diabetic children at the Hospitals for Sick Children to take part in the study, and Professor M Preece for permission to obtain urine samples from the children of Holyrood School, Chard, Somerset. We thank Ms J Jones for help with collecting urine samples and Dr Y Kotecha for help with obtaining blood samples for normal controls. We thank Mr W Wilson for measuring urinary creatinines and his help with measuring glomerular filtration rate.

The project was supported by grants from Boehringer Ingelheim and the Kidney Research Aid Fund.

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Accepted 2 December 1988