LOW MOLECULAR WEIGHT PROTEINURIA IN DIABETIC CHILDREN – A MARKER OF EARLY DIABETIC NEPHROPATHY?

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Summary

Twenty-four hour urine specimens of 67 diabetic children aged 1–17 years without any renal manifestations were examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The excretion of high molecular weight, i.e. glomerular proteins was compared to that of low molecular weight, i.e. tubular proteins corresponding to more or less than 68,000 daltons. The glomerulotubular protein ratio (GTPR) obtained was significantly lower in diabetic patients compared with 30 healthy children of the same age and showed a linear decrease with longer duration of diabetes.

Introduction

About one-third of young insulin dependent diabetics develop diabetic nephropathy leading finally to terminal renal failure. The characteristic histological lesion, glomerulosclerosis, is usually accompanied by proteinuria starting 10–15 years after the first manifestation of diabetes. Proteinuria rapidly increases during the following years concomitant with the progressive damage of the glomerular capillaries. Conventional clinical parameters such as measurement of total proteinuria have failed to recognise renal involvement in diabetes before advanced morphological lesions become evident. Measurement of microalbuminuria determined overnight and after exercise seems to be a better indicator of impending kidney dysfunction [1,2]. Exercise-induced microalbuminuria increased significantly in diabetic children even with a duration of diabetes of only more than five years [3]. In recent years SDS-PAGE has proven to be a sensitive method of characterising proteinuria according to the molecular size of proteins excreted. This enables SDS-PAGE to differentiate glomerular, i.e. high molecular weight (HMW) proteinuria and tubular, i.e. low molecular weight (LMW) proteinuria. It has been applied to adult patients at different stages of diabetes, who often showed an unselective glomerular proteinuria. We have used SDS-PAGE in a group of paediatric patients without clinical manifestations of renal disease, in order to recognise disturbed handling of protein excretion at a subclinical stage.
Patients and methods

Sixty-seven children aged 1–17 years with type I diabetes known for one month to 12 years were examined. All patients were treated by insulin and diet on an ambulatory basis. No patient was known to have renal disease as demonstrated by sterile urine with normal cell counts. Urinary protein excretion was negative to Albustix and sulfosalicylic acid. Serum creatinine and blood pressure were within normal limits for age. In 34 patients blood Haemoglobin A1c were determined. All patients were in good metabolic control. Thirty healthy children aged 2–15 years served as controls.

Twenty-four hour urine samples were collected after addition of 0.05% sodium azide. Protein concentration was measured by a modified Lowry technique. Urine samples were concentrated and dialysed at 4°C against 20% polyethyleneglycol using flexible ‘Visking’ tubes (Serva, Heidelberg). SDS-PAGE was performed on slab gel units (Hoelzel, Munich) using the Laemmli buffer system [4]. The acrylamide concentrations used were %T=10, %C=2.7 [5]. The gels were stained with Coomassie Blue G 250. For densitometry and integration of the protein peaks Desaga Quick Scan and Desaga Quick Quant units were used (Desaga, Heidelberg). All chemical reagents were obtained from Serva (Heidelberg) and Merck (Darmstadt). GTPR was calculated by dividing the total excretion of HMW proteins with molecular weight (MW) above 68,000 daltons by that of LMW proteins with MW below 68,000 daltons. The albumin peak was excluded from the calculation.

Results

In no patient total proteinuria exceeded 100mg/24hr/m² body surface area. Glomerulo-tubular protein ratio (GTPR) values in diabetics and healthy children are compared in Figure 1. In the diabetic group GTPR ranged between 0.2 and

![Figure 1. GTPR-values (GTPR=glomerulo-tubular ratio) of 30 healthy and 67 diabetic children](image-url)
1.9 (mean 0.8, median 0.8). In the control group the range was between 0.7 and 5.0 (mean 2.0, median 1.3). This difference was significant with a confidence interval of 99 per cent in the Wilcoxon test. About 35 per cent of the diabetic patients had GTPR values lower than 0.7 which was the lowest value seen in the control group. The LMW proteinuria and the corresponding GTPR values in the diabetics did not correlate with age of patients, urine volume per 24 hours, total proteinuria and blood Haemoglobin A1c, but the GTPR values decreased with longer duration of diabetes (coefficient of correlation 0.32, p<0.01, ‘t’ test), as shown in Figure 2.

![Figure 2](image_url)

**Discussion**

There is a general belief that proteinuria in manifest diabetic nephropathy is of glomerular, i.e. of HMW, origin, but some recent data indicate a very early involvement of tubular protein handling in diabetics. In a study of 200 adult diabetics Boesken et al, using SDS-PAGE, found that 50–60 per cent of patients had an unselective glomerular type of proteinuria and 10 per cent a tubular proteinuria, whereas the rest had a normal pattern [6]. In another study using SDS-PAGE three out of 10 young adult patients showed a predominant LMW proteinuria [7]. Persistent LMW proteinuria was also described in diabetic rats investigated during 12 months after infusion of streptozotocin [8]. Michels et al found a surprising discrepancy between reduced anionic dextran sulphate clearances and increased excretion of proteins, especially of albumin in diabetic rats. They suggested that the marked increase of albumin excretion was the result of decreased tubular reabsorption rather than of increased glomerular filtration [9].

Our data clearly express that during the first years after manifestation of juvenile diabetes in the absence of any pathological rise of total proteinuria LMW proteins are excreted at higher rates than in healthy controls. These findings are in good concordance to recent reports of LMW proteinuria in diabetic children with hyperglycaemic ketoacidosis [10]. At early stages of insulin-treated diabetes glomerular filtration rate is increased by about 10–25
per cent. Therefore, it is possible that an increased filtered load of LMW proteins is responsible for the LMW proteinuria observed. Even if the limited capacity of the tubular system to handle a LMW overflow proteinuria is considered it seems unlikely that an increased delivery of these proteins at the rates described above could cause the degree of excretion observed. Moreover proteinuria induced by increased glomerular filtration rate could not explain the reduction in GTPR values with the duration of diabetes. For this reason we would suggest that the low molecular weight proteinuria in diabetic children is the consequence of beginning alteration of renal protein handling by the tubular epithelium due to, for example, early changes in the peritubular vasculature.

References
1 Mogensen CE, Christensen CK. N Engl J Med 1984; 311: 89
2 Viberti GC, Jarrett RJ, McCartney M, Keen H. Diabetologia 1978; 14: 293

Open Discussion

MIGONE (Chairman) Have you considered the possibility of a relationship between low molecular weight proteinuria and the level of serum $\beta_2$ microglobulin which has been shown to be increased in diabetic patients?

WARTHA The problem is there are very few studies on children. There is one from Belgium reporting that $\beta_2$ microglobulin is increased in diabetic children and the excretion of $\beta_2$ microglobulin then decreases with a decrease in GFR in older patients. At this time serum $\beta_2$ microglobulin seems to increase.

MIGONE What happens to the microproteinuria in your patients with decreasing glomerular filtration rate?

WARTHA I think that tubular proteinuria in diabetic patients is still manifest in adults, but you won’t detect it because it will be masked by the increased glomerular proteins that are excreted in consequence of the progressive damage of the glomerular filter. Our patients were only followed in childhood.