Macroalbuminuria and Renal Pathology in First Nation Youth with Type 2 Diabetes Mellitus

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Submitted 7 October 2008 and accepted 7 February 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.

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Objective: to determine the prevalence of macroalbuminuria and to describe the clinical and renal pathological changes associated with macroalbuminuria in a population of Canadian First Nation children and adolescents with type 2 diabetes.

Research Design and Methods: We conducted a retrospective chart review at a single, tertiary care pediatric diabetes centre and a case series was constructed. We collected data on micro (≥ 3mg/mmol creatinine [26.5 mg/g]) and macroalbuminuria (≥ 28 mg/mmol creatinine [247.5 mg/g]), eGFR, renal pathology, aggravating risk factors (poor glycemic control, obesity, hypertension, glomerular hyperfiltration, hypercholesterolemia, smoking, exposure to diabetes in utero) were extracted from the charts.

Results: We reviewed 90 charts of children and adolescents with type 2 diabetes. 53% had at least one random urine albumin: creatinine ratio (ACR) ≥ 3 mg/mmol (26.5 mg/g). 14/90 (16%) had persistent macroalbuminuria at, or within 8 years of diagnosis of diabetes. Of these 14 subjects, one had orthostatic albuminuria and 3 had spontaneous resolution of albuminuria. 10 had renal biopsies performed. 9/10 exhibited immune complex disease or glomerulosclerosis and none had classic diabetic nephropathy.

Conclusions: this study suggests that the diagnosis of renal disease in children with type 2 diabetes cannot be reliably determined by clinical and laboratory findings alone. Renal biopsy is necessary for accurate diagnosis of renal disease in children and adolescents with type 2 diabetes and macroalbuminuria. The additional burden of non-diabetic kidney disease may explain the high rate of progression to end-stage kidney failure in this population.
The increasing prevalence of type 2 diabetes in children and youth has been well recognized over the past two decades. The geographic area in central Canada including Manitoba and northwestern Ontario has the highest reported prevalence of type 2 diabetes in youth in Canada. The prevalence is 1% in First Nation (FN) children age 4-19 years in specific communities from this region (1). 95% of the youth with type 2 diabetes from this region have Canadian FN heritage.

The Hepatic Nuclear Factor 1 alpha (HNF 1- alpha) is a transcription factor expressed in many tissues including the liver, intestine, pancreatic beta cell and kidney. A private polymorphism of this gene (HNF 1-alpha G319S) is found in the Oji-Cree of Manitoba and northwestern Ontario. It has been associated with early onset diabetes in this population and demonstrates a genotype-phenotype relationship (2, 3).

Youth onset type 2 diabetes is associated with an increased incidence of end stage kidney disease (ESKD) and mortality in middle age in the Pima Indians of the southwestern US (4). ESKD has been reported before the age of 30 years in Canadian FN young adults who had type 2 diabetes diagnosed in adolescence (5). In this series, the cause of ESKD was attributed to diabetic nephropathy as proteinuria was detected after the onset of diabetes. Renal biopsy was not performed to confirm the diagnosis and/or exclude other causes of kidney disease. Several small studies have reported an increased frequency (27-40%) of microalbuminuria in youth with type 2 diabetes (6, 7). These studies assessed microalbuminuria at a single time point and did not describe evolution over time.

Primary, non-diabetic renal disease is frequent in the FN population. Canadian FN children without diabetes have an increased rate of both congenital and acquired primary renal disease (8). FN adults also have an increased risk ratio for non-diabetic ESKD (9). In both FN children and FN adults, the most common renal pathology is primary glomerulonephritis. Childhood obesity is also increasingly common in this population and independently predisposes to secondary focal glomerulosclerosis and renal failure in children and adults (10, 11).

Diabetes associated ESKD is 7 times more frequent in FN compared to non-FN people in Canada (12). There is also a 2-fold increase in premature mortality rate in FN adults with diabetes compared to those with diabetes from the general Canadian population (12). It is thus imperative to describe and understand the natural history and etiology of renal disease in FN youth with type 2 diabetes as the first step in the development of intervention and treatment strategies in this vulnerable population.

The frequency of macroalbuminuria has not previously been described in the pediatric population with type 2 diabetes, nor has the nature of the associated renal pathology. The objectives of this study were to determine the prevalence of macroalbuminuria and to characterize the clinical and renal pathological changes associated with macroalbuminuria in a population of FN children and adolescents with type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

Clinic charts of children and adolescents with type 2 diabetes followed at the Diabetes Education Resource for Children and Adolescents (DER-CA) at the Winnipeg Children’s Hospital, Manitoba, Canada were reviewed between September and October 2003 and a case series of those with macroalbuminuria constructed. Cases were identified through the clinical database maintained at the DER-CA. The DER-CA is associated with the only tertiary care pediatric
hospital in the province of Manitoba, Canada with a catchment population of 1.2 million people.

Height, weight, blood pressure, self-declared ethnicity, gender, age, self-reported smoking status, history of prenatal exposure to hyperglycemia and laboratory data (HbA1c, albumin, creatinine, urine albumin to creatinine ratio and/or timed urinary collections for albumin and protein, HNF-1 alpha G319S genotype) were extracted from the chart. BMI was calculated and presented as BMI z-score. Obesity was defined as ≥ 95th percentile for age and gender (13). Blood pressure percentiles were defined using the National Heart, Lung and Blood Institute blood pressure levels for age, gender and height percentile (14) and fasting cholesterol level defined according to the National Cholesterol Education Program data (acceptable <4.4 mmol/L). Glomerular filtration rate was estimated using the Schwartz formula and expressed as ml/min/1.73m².

The diagnosis of diabetes was made according to the criteria of the American Diabetes Association (15). The diagnosis of type 2 diabetes was based on clinical criteria including presence of obesity, acanthosis nigricans, family history of type 2 diabetes and family heritage from a high-risk ethnic group (16). When available, the absence of diabetes-associated autoantibodies was used to support the diagnosis of type 2 diabetes (17).

Microalbuminuria was defined as urine albumin/creatinine ratio (ACR) 3-28 mg/mmol (26.5 -247.5 mg/g) or quantitatively as 30-300 mg of albumin / 24 hours. Macroalbuminuria was defined as urine ACR >28 mg/mmol (247.5 mg/g) or quantitatively as >300 mg of albumin / 24 hours. Persistent micro or macroalbuminuria was defined as 2/3 positive samples over 6 months. These patients underwent split timed-urine collections and /or a minimum of three first morning urine collections to identify those with orthostatic proteinuria. The standard approach to treatment of albuminuria in our clinic is the use of an ACE inhibitor and investigations aimed at confirmation of etiology.

Renal biopsy was performed in patients with persistent non-orthostatic macroalbuminuria (> 28 mg/mmol [247.5 mg/g]). Standard renal histology techniques included light microscopy (H&E, PAS, PAMS, Trichrome, Sirius red), immunofluorescence studies (IgG, IgA, IgM, C1q, C3, properdin, fibrinogen, kappa and lambda) and electron microscopy. Renal biopsy samples were considered adequate if they contained a minimum of two cores with a minimum of 10 glomeruli. All biopsies were interpreted by a single pathologist (IWG) who is a specialist renal pathologist. For those undergoing renal biopsy, clinical and biochemical findings are reported at the time of biopsy or the most proximal clinic visit.

HbA1c was measured using the DCA 2000 immunoassay (normal range 4-6%, Bayer Diagnostics). Fasting total cholesterol was measured before April 2003 using the Hitachi 917 Clinical Chemistry Autoanalyzer (Roche) and on the Roche Modular Analytics Analyzer after 2003. Plasma and urine creatinine were measured by kinetic colorimetric assay using the Jaffe method. Urine albumin was measured by immunoturbidimetric assay using anti-excess reagent. Genotyping for the HNF-1 alpha G319S polymorphism was performed by the Molecular Genetic Laboratory, Health Sciences Centre, Winnipeg, Manitoba.

Results

All clinical records of children and adolescents with type 2 diabetes followed at the DER-CA during the study period were reviewed. All patients had random urine ACR done at diagnosis and annually unless abnormal. If elevated, a first morning ACR was performed at each clinical visit (typically
Macroalbuminuria in youth with type 2 diabetes

Of the 90 records reviewed, 88/90 (98%) were of self declared FN or Métis heritage. The male to female ratio was 1:1.2. The mean age of this population was 15.2 years with a range from 10 to 19 years. Mean duration of diabetes was 2.5 years (range 0.4 -5 years). Mean BMI z-score at diagnosis was 1.88 (range -1.17 – 2.95). Approximately 80% lived outside urban centers in rural or remote settings.

Of the 90 children and adolescents, 48 (53%) had at least one ACR> 3 mg/mmol (26.5 mg/g). 26/90 (29%) had an elevated ACR at diagnosis of diabetes. 14 of the 90 (16%) had persistent macroalbuminuria. All youth with persistent macroalbuminuria were of self declared FN descent.

Of the 14 with persistent macroalbuminuria, one was shown with further testing to have orthostatic albuminuria, 3 had resolution of the albuminuria over a six month period awaiting work up and continue to be monitored closely, and 10 have had a renal biopsy (3 female and 7 male).

Of those biopsied, the mean age at diagnosis of diabetes was 12 years (range 9.7 to 16.7 years). The duration of diabetes at the time of biopsy ranged from 4 months to 8 years. Four of these 10 youth had macroalbuminuria at diagnosis. 7/10 were on an ACE inhibitor at the time of biopsy (Cases #1, 2, 3, 4, 5,9 ,10). The remaining three were started on an ACE inhibitor following biopsy (Case# 5, 6, 7). These three had all been diagnosed with diabetes for less than 12 months. The mean HbA1c in the year preceding biopsy ranged from 5.3 to 12.5 %. In 6/10, systolic blood pressure was above the 95 % percentile for age and gender. Fasting total cholesterol was elevated in all 10 (Table 1). 4/10 were self reported smokers (2 girls, 2 boys). 6/10 were exposed in utero to diabetes, either from conception (pre-pregnancy type 2 diabetes) or during pregnancy (gestational).

The HNF 1-alpha G319S polymorphism haplotype was present in 4/7 patients in whom genotyping was available (Table 1). 7/10 were obese with unbalanced distribution among those with the HNF 1-alpha G319S polymorphism present (mean BMI z-score: 1.4) and those without (mean BMI z-score: 2.6).

Biopsy results indicate significant, non-diabetic renal pathology (Table 2). Two or three cores were obtained for each patient with 12-25 total glomeruli per sample. Histological changes typically associated with diabetes were infrequently noted, and were insufficient to make a definitive diagnosis of diabetic nephropathy in any of the cases. Diabetes-related lesions seen included focal, mild hyaline arteriolosclerosis (#10), focal and mild glomerular basement membrane thickening (#6, 10) and a single Bowman’s capsular drop (#6).

Half of the patients exhibited evidence of immune complex deposition that was either IgA (n=3) or “full-house” immune complex deposition (n=2). In the latter group, a diagnosis of systemic lupus was eventually confirmed in both. One of these (#1) had WHO Class V lupus with membranous nephropathy changes, the other (#5) had WHO class IIB mesangial lupus nephritis. In those with IgA deposition, there was minimal-to-mild mesangial proliferation despite significant albuminuria.

90% of biopsies had at least one glomerulus exhibiting segmental or global glomerulosclerosis, and 60% had more than one glomerulus involved. In 70%, there was focal segmental glomerulosclerosis, with 3 cases showing perihilar lesions, one case showing glomerular tip lesion, and 3 cases showing peripheral segmental lesions. In 80%, the glomeruli were diffusely enlarged (#1, 2, 3, 4, 5, 7, 8, 9).

Complete and accurate timed urine collections to calculate creatinine clearance are difficult to obtain reliably and thus the Schwartz estimate was used. To validate this
approach, a sub-cohort analysis was done using all available urine collections for the patients reported in this study (n=90). Only 9 urine collections were suitable and complete (a minimum of 8 mmol/day [0.13 mmol/kg/day] of creatinine excretion). The urinary creatinine clearance was calculated and correlated with the Schwartz eGFR, using k=0.7 for boys > 12 years old, and k=0.55 for the remainder. Schwartz eGFR showed good correlation (r=0.67, p=0.046) and overestimated GFR in pair-wise comparison by 12 ± 15% (data not shown). The mean body surface area in this sub-cohort was 2.1 ± 0.4, so as a conservative measure, the eGFR corrected to 1.73m² rather than the absolute clearance, was reported.

For the patients reported in this series, all but one patient (#9) had elevated eGFR suggesting hyperfiltration, and 40% exhibited hyperfiltration in excess of 200 ml/min/1.73m². The patient with “normal” eGFR (#9) and 38% segmental glomerulosclerosis at the time of biopsy had demonstrable hyperfiltration at initial diagnosis (eGFR=217 ml/min/1.73m²) and has continued to have declining glomerular filtration rate in follow-up (at age 16 years, eGFR=44 ml/min/1.73m²).

Three cases exhibited nephrotic range proteinuria (ACR > 200 mg/mmol [1768 mg/g]), including one patient whose biopsy showed membranous nephropathy changes. Two of these three (#1, 9) had hypoalbuminemia (serum albumin: 27, 32 mg/L respectively). Two cases (#5, #10) with persistent macroalbuminuria prior to biopsy, had ACR <28 mg/mmol (247.5 mg/g) at the time of biopsy. In these cases concomitant immune complex deposition was noted in association with mild mesangial proliferation. The remaining cases had glomerulosclerosis and/or immune complex disease of sufficient severity to account for the presence of macroalbuminuria.

CONCLUSIONS

Persistent macroalbuminuria was documented in 16% of this population of children and adolescents with type 2 diabetes at, or within 8 years of diagnosis. The biopsy results demonstrate that non-diabetic renal disease in the form of immune complex disease or glomerulosclerosis is the most common etiology of macroalbuminuria in this young population.

In adults with type 2 diabetes, renal biopsy frequently identifies non-diabetic pathology. In a multicentre study of adults with type 2 diabetes undergoing renal biopsy, 45% (177/393) were diagnosed with non-diabetic glomerular disease, either superimposed on diabetic nephropathy (17% of all biopsies) or more commonly without underlying diabetic disease (28%) (18). The most common disease superimposed on diabetic nephropathy was post-infectious glomerulonephritis, and the most common glomerular diseases without underlying diabetic changes were membranous glomerulonephritis, IgA nephropathy and minimal change disease or focal segmental glomerulosclerosis. This is the first report to our knowledge of a similar finding in the pediatric population. Our study confirms that renal pathology in children with type 2 diabetes cannot be reliably predicted by clinical and laboratory findings alone and that renal biopsy is necessary for accurate diagnosis of renal disease in such patients.

HNF-1-alpha is involved in the differentiation of the nephron (19). Other, dominant mutations of the HNF 1-alpha gene are characterized by reduced renal tubular re-absorption of glucose (20). While the effect of the private Oji-Cree polymorphism of the HNF 1- alpha gene on the development and function of the kidney is not known, the observation that the polymorphism is present in 4/7 in whom genotyping was available is intriguing. We speculate that this polymorphism may play a role in the
development of the renal pathology found in this population.

Obesity is a well-recognized risk factor for insulin resistance and the development of type 2 diabetes. Obesity is associated with glomerular hyperfiltration and the development of glomerulosclerosis and kidney failure independent of the presence of diabetes (10, 11). The understanding of biologic mechanisms that explain this association are not fully understood but appears to be mediated through both hemodynamic and hormonal effects including increased renal plasma flow, GFR, glomerular filtration pressure and filtration fraction, hyperinsulinemia and activation of the renin-angiotensin system (11).

Furthermore, medications such as angiotensin-converting enzyme inhibitors which may mitigate hyperfiltration injury are beneficial in this context (11, 21). In our series, all of the patients without the HNF 1-alpha G319S polymorphism had a BMI z-score >2. Though 8/10 patients had a body-surface area >1.73 m² (mean 2.1 m²), the eGFR corrected to 1.73 m² was reported instead of the absolute GFR. By this conservative estimate, all patients currently or previously had an eGFR >140 ml/min/1.73m² and 4/10 had eGFR >200 ml/min/1.73m², which is higher than is reported in adults with recent-onset type 2 diabetes (22). In those with obesity, this was associated histologically with glomerulomegaly and significant glomerulosclerosis. The contribution of obesity, diabetes or the combination of the two to the hyperfiltration seen cannot be differentiated. However, the presence of obesity and hyperfiltration likely contributes to the evolution of renal injury in this population, and is likely additive to the other effects that are typically associated with hyperglycemia.

In humans, nephrogenesis occurs between the 5 and 36 weeks of gestation and new nephron formation ceases after 36 weeks of gestation (23). In the rat model, exposure to hyperglycemia during gestation decreases nephron number in the offspring by 35%. Minor elevations in glucose are associated with abnormal metanephros development in the offspring of streptozotocin treated diabetic rats (24). A decrease in nephron number may predispose to renal pathology in later life due to a reduction in renal reserve. Both glomerulomegaly and glomerulosclerosis are associated with decreased nephron number likely resulting from the decreased filtration surface and subsequent glomerular damage resulting from hyperfiltration. In young adult Pima Indians with type 2 diabetes, exposure to diabetes in utero increases the odds of having an elevated ACR by almost 4 fold (25). 60% of the youth in our series were exposed to either gestational or pre-gestational type 2 diabetes. We postulate that this may have resulted in a decreased nephron mass, accelerating their presentation with macroalbuminuria. As rates of youth onset type 2 diabetes increase, the frequency of fetal exposure to a diabetic environment will also increase. This may have significant detrimental effects on the renal health of subsequent generations.

Multiple risk factors for aggravating renal disease were found in this cohort including obesity, glomerular hyperfiltration, hypertension, hyperlipidemia, smoking, poor glycemic control and fetal exposure to a diabetic environment. This multiplicity of risk factors may explain the progression to ESRD at a relatively young age that has been observed in this population (5). Aggressive treatment of modifiable risk factors is warranted to prevent progression of renal disease and to prevent early cardiovascular disease associated with both renal disease and these aggravating risk factors.

Limitations to this report include the inability to obtain adequate, timed urine samples in all patients. Despite attempts, this testing presents significant practical
challenges particularly in adolescents and in remote communities. In addition, the findings of this report may not be generalizable to other pediatric populations affected by type 2 diabetes.

Our study suggests that the diagnosis of renal disease in children with type 2 diabetes cannot be reliably determined by clinical and laboratory findings alone. Renal biopsy is necessary for accurate diagnosis in this population. This finding needs confirmation in other populations to ensure its generalizability. The incidence of type 2 diabetes in First Nation youth is increasing at an alarming rate. The additional burden of non-diabetic kidney disease may contribute to the high rate of progression to end-stage kidney failure in this population.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Dr. Sarah McMorran, MBBS for the initial chart review and Dr. Gurmeet Singh for critical review of the manuscript. The authors also are appreciative for the support and constructive comments from the Canadian Young Investigators Group. Part of this work was presented in abstract form at the American Diabetes Association 2003 (Sellers) and the American Society of Nephrology 2003 (Blydt-Hansen).

Disclosure: The authors have no conflicts of interest to declare.
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### Table 1. Patient Characteristics

| Gender | Age at Diagnosis (years) | Age at Biopsy (years) | BMI z-score | SBP >95th%ile | Mean HbA1c* (%) | Fasting Total Cholesterol (mmol/L) | Maternal diabetes during pregnancy | HNF-1α G319S polymorphism |
|--------|--------------------------|-----------------------|-------------|--------------|----------------|-----------------------------------|----------------------------------|---------------------------|
| 1      | female                   | 10.0                  | 17.8        | no           | 10.1           | 5.09                              | no                               | homozygote                |
| 2      | male                     | 12.3                  | 12.9        | yes          | 6.3            | 5.02                              | pre-pregnancy                   | N/A                       |
| 3      | male                     | 11.1                  | 15.1        | yes          | 11.2           | 8.26                              | no                               | heterozygote               |
| 4      | male                     | 13.9                  | 17.4        | no           | 8.5            | 4.41                              | no                               | wild type                 |
| 5      | female                   | 13.3                  | 15.6        | no           | 6.0            | 6.00                              | no                               | N/A                       |
| 6      | male                     | 10.3                  | 10.7        | no           | 8.5            | 5.26                              | gestational                     | heterozygote               |
| 7      | male                     | 16.7                  | 17.1        | yes          | 6.0            | 4.71                              | gestational                     | N/A                       |
| 8      | male                     | 11.3                  | 12.5        | yes          | 12.5           | 5.94                              | pre-pregnancy                   | wild type                 |
| 9      | male                     | 12.3                  | 13.5        | yes          | 5.3            | 5.0                               | gestational                     | wild type                 |
| 10     | female                   | 9.7                   | 17.7        | yes          | 11.0           | 6.44                              | gestational                     | heterozygote               |

*mean HbA1c in 12 months prior to biopsy

SBP = systolic blood pressure; N/A = not available
| eGFR ‡ | Urine ACR mg/mmol | FSGS | GGS | Mesangial Proliferation | Immunofluorescence | Other Pathological Findings |
|--------|-------------------|------|-----|------------------------|-------------------|---------------------------|
| 1      | 156               | >270*| -   | mild                   | mesangial & capillary loop IgG, IgA, IgM, C3, C1q | subepithelial & mesangial deposits, epimembranous spikes |
| 2      | 204               | 66   | 2/12| mild                   | mesangial IgA      | focal mild chronic tubulointerstitial damage- |
| 3      | 162               | 105  | 1/25| -                      | negative           | -                          |
| 4      | 144               | 61   | 3/9 | -                      | negative           | mild-moderate chronic tubulointerstitial damage |
| 5      | 415               | 17†  | 2/19| mild                   | mesangial & capillary loop IgG, IgA, IgM, C3, C1q | paramesangial deposits & mesangial sclerosis |
| 6      | 194               | 178  | 1/22| 1/22                   | negative           | mild GBM thickening, capsular drop |
| 7      | 215               | 117  | 1/22| -                      | negative           | -                          |
| 8      | 190               | 215  | 1/14| tip lesion             | mesangial IgA      | focal arteriolosclerosis |
| 9      | 118               | 290  | 5/13| -                      | negative           | mild-moderate chronic tubulointerstitial damage |
| 10     | 210               | 23†  | 1/18| segmental mild         | mesangial IgA      | focal arteriolosclerosis, mild GBM thickening |

* estimated based on proteinuria 3.6 g/day
† persistent macroalbuminuria documented in these patients, ACR most proximal to time of biopsy demonstrated microalbuminuria

eGFR = estimated glomerular filtration rate (ml/min/1.73m²) by calculation of size-adjusted creatinine clearance using the Schwartz formula.

Urine ACR = urine albumin/creatinine ratio. When ACR > 200 mg/mmol, the urine total protein/creatinine ratio is reported

FSGS = focal segmental glomerulosclerosis; GGS = global glomerulosclerosis