Microalbuminuria three years after recovery from *Escherichia coli* O157 hemolytic uremic syndrome due to municipal water contamination

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**Background.** Knowledge of the long-term renal prognosis of diarrhea associated hemolytic uremic syndrome (HUS) is important for patient counseling and follow-up. However, estimates of long-term risk are highly variable, with previous studies not using a healthy control group.

**Methods.** A municipal water system in the small rural town of Walkerton, Ontario, became contaminated with *Escherichia coli* O157:H7 in 2000. A cohort of 19 children who recovered from HUS was randomly age- and sex-matched to 38 children with no symptoms at the time of the outbreak. Both groups had detailed renal function testing 3 years after the outbreak, including a random urine albumin to creatinine, glomerular filtration rate estimated by Schwartz formula, and automated and manual blood pressure measurements.

**Results.** There were no baseline differences between the groups with respect to age (mean 4.8 years, range 1 to 15), sex, or birth weight (mean 3.4 kg). In follow-up there were no differences between the groups in body surface area (mean 1.0 m²), or in the methods by which renal function was assessed. Compared to the group with no symptoms, patients with HUS demonstrated more microalbuminuria (32% vs. 5%, relative risk 4.8 (95% CI 1.1 to 22.0)), a nonsignificant trend toward lower GFR (124 vs. 134 mL/min per 1.73m²), and no difference in blood pressure.

**Conclusion.** Children may demonstrate microalbuminuria 3 years after recovering from HUS. Longer follow-up is needed to determine if this finding has clinical relevance and utility.

**Key words:** cohort study, *Escherichia coli* O157, hemolytic-uremic syndrome, hypertension, proteinuria, chronic kidney disease.

Received for publication August 11, 2004
And in revised form September 28, 2004
Accepted for publication October 14, 2004

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of the outbreak, heavy rainfall contributed to the surface transport of livestock fecal contaminants into inadequately chlorinated drinking water supplied from a drilled well. This outbreak produced an estimated 2300 cases of acute gastrointestinal illness, over 750 emergency room visits, 65 hospital admissions, and 28 pediatric cases of confirmed HUS, 1 of which was fatal [3]. The Walkerton Health Study provides a unique opportunity to characterize the association between HUS and long-term renal sequelae, and guides the need for long-term screening of individuals exposed to E. coli O157:H7. There are also issues of financial compensation for those affected by the environmental disaster [4]. Research questions of this report were: (1) compared to healthy controls, are pediatric HUS survivors at higher risk of renal sequelae (proteinuria, hypertension, reduced GFR) 3 years after the initial infection?; (2) what is the history of identified sequelae at 3 years—are findings progressing, regressing, or remaining constant from the time of initial recovery from HUS?

METHODS

Participants and study design

In May 2000, municipal water in Walkerton became contaminated with E. coli O157:H7. Phage type 14 was isolated [5], and the majority of specimens tested for verotoxin genotype were found to be verotoxin 2. Beginning in February 2002, all surviving individuals who either lived in the Walkerton area or who had consumed municipal water, irrespective of whether they developed an acute illness, were invited to participate in the Walkerton Health Study. The London Health Sciences Center ethics review board approved the study. Participants underwent an annual standardized interview, physical assessment, and laboratory testing by trained staff unaware of the acute exposure status of the participant. A review of family physician and hospital charts before, during, and after the outbreak was conducted with participant consent.

Case definition for HUS

In the absence of another medical explanation, children with all of the following 3 criteria were defined as having HUS: (1) a hemoglobin ≤100 g/L with evidence of destruction of erythrocytes on blood smear; (2) platelets ≤150 × 10⁹/L; and (3) a serum creatinine ≥95th percentile for age and sex (for example: age <7 creatinine ≥62 µmol/L; age 7 to 11 ≥76 µmol/L, girls age 12 to 16 ≥89 µmol/L, boys age 12 to 16 ≥109 µmol/L) [6]. In addition, during the outbreak, strained health care resources led to limited laboratory testing in some patients. Five children with elevated serum creatinine approached but did not exceed the 95th percentile for age and sex. These children otherwise met all diagnostic criteria (marked hemolytic anemia, thrombocytopenia, and red blood cell casts on urine microscopy), and were characterized as having HUS by 2 independent physicians.

Selection of controls

Each participant with HUS was matched to 2 children who were healthy at the time of the outbreak. These control participants were randomly selected from a group of 352 healthy eligible children who participated in the Walkerton Health Study. Cases were frequency matched to controls in 8 unique stratum, defined by age and sex.

Renal function

Urine albumin to creatinine ratios were calculated on a random specimen using clean catch techniques and sterile containers [7]. Urine albumin was initially assessed at the London Health Sciences Center Laboratory with the IMMULITE 2000 Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA), using a solid-phase competitive chemiluminescent enzyme immunoassay. During the study, testing was moved to the Walkerton laboratory, which used an IMAGE Beckman Coulter immunoassay (Beckman Coulter, Fullerton, CA, USA). There was excellent reliability on 100 duplicate samples sent to both labs for urine albumin to creatinine ratio measurements (Pearson correlation R² = 0.98). Serum and urine creatinine were measured by the modified kinetic method of Jaffe using a Vitros 950 Auto Analyzer (Johnson & Johnson, Skillman, NJ, USA), with an interassay coefficient of variation less than 4%. For children, the laboratory serum creatinine reference range of upper normal for this analyzer was 70 µmol/L for ages 1 to 7, and 90 µmol/L for ages 8 to 12. The reference normal range was 59 to 117 µmol/L for adult males, and 51 to 95 µmol/L for adult females. Walkerton serum creatinine was reading on average 3 µmol/L higher compared to the Cleveland Clinic (the primary lab for the Modification of Diet in Renal Disease Study) based on 139 duplicate samples sent to each lab in 2002.

Urinary albumin to creatinine ratios are challenging to interpret in children because normal levels are not well established [8]. To facilitate comparisons, in addition to using a healthy control group within the study, we defined microalbuminuria with similar cut-off points used in the Third National Health and Nutrition Examination Survey (NHANES III) [9, 10]. In NHANES III, 5000 healthy participants ages 6 to 19 years were assessed for microalbuminuria, defined by a random urine albumin to creatinine ratio between 3.4 and 33.9 mg/mmol (30 to 300 mg/g).

GFR was estimated by the Schwartz formula [11]. In addition, a 24-hour urine was collected for protein. Blood pressure was assessed in-center by protocol using an appropriate cuff size for measured arm circumference. Staff unaware of the acute exposure status of the participant
were trained to perform 3 blood pressure measurements (2 automated by Dynmap and 1 manual). To reduce variability, the first blood pressure measurement was ignored, and second automated and third manual measurements were averaged.

**Analysis**

We specified a priori 3 primary continuous renal measures to be compared between the groups—1 representative of proteinuria (random urine albumin to creatinine ratio), clearance (Schwartz calculated GFR), and blood pressure (mean arterial blood pressure). To account for multiple comparisons in these primary outcomes, we used a Bonferroni correction with a two-tailed \( P \) value < 0.017, considered significant when interpreting positive results. Supplementary analyses supporting the primary outcomes considered a two-tailed \( P < 0.05 \) as significant. To account for the initial matching and small cell sizes, the group differences on outcomes were assessed using a mixed model with exposure as the fixed effect and subjects defined by stratum. Supplementary techniques to confirm the robustness of the primary analyses included stratified ordinary least squares regression (OLS) and nonparametric comparisons. Relative risks for microalbuminuria were calculated as described elsewhere [12], as stratified logistic regression demonstrated no effect modification by stratum. Box plots and trends over time were graphed in Microsoft Excel.

**RESULTS**

**Study enrollment**

During the outbreak, there were 28 children with HUS. One child had proteinuria which predated the outbreak, and 1 died at the time of the outbreak. Of the remaining 26 children, 1 declined study participation, 1 was unable to be located, 3 declined complete follow-up measurements, and 2 were from out of the area and were unable to be contacted. These 7 potentially eligible nonparticipants were similar to participants in their baseline characteristics and disease severity—their average age at the time of the outbreak was 4, 4 were male, 2 developed oliguria or anuria, and 1 required hemodialysis for 5 days. The remaining 19 participating children with HUS were matched to 38 children with no symptoms at the time of the outbreak.

**Baseline characteristics**

All participants were Caucasian, and ranged in age from 1 to 15 years at the time of the outbreak. As shown in Table 1, there were no significant differences between the groups in baseline characteristics at the time of the outbreak. Similarly, as shown in Table 2, there were no significant differences between the groups in relevant factors related to renal function testing during follow-up.

Of the 19 children with HUS, all were known to be previously healthy without medical conditions (such as diabetes, hypertension, renal disease, recurrent infections, or failure to thrive) predating the outbreak. At the time of acute illness, 16 (84%) had stools positive for *E. coli* O157:H7 bacteria or verotoxin. The average lowest hemoglobin was 61 g/L (SD 14, range 45 to 95), the average lowest platelet count was 56 × 10^9/L (SD 38, range 12 to 150), and the average highest serum creatinine was 246 \( \mu \)mol/L (SD 248, range 42 to 861). None developed convulsions, coma, stroke, or severe colitis requiring surgery. Seven anuric children were treated with peritoneal dialysis for an average of 14 days (range 10 to 19). One child was treated with antihypertensive therapy acutely. Three children were treated with antihypertensive therapy [an angiotensin converting enzyme inhibitor (ACE-I)] for variable amounts of time in the 3 years after HUS (Fig. 3). All the 19 children made an excellent recovery in the months following the acute infection—none required long-term dialysis, and all had a serum creatinine that returned to the lab defined normal range.

**Renal function 3 years after HUS**

Renal function tests 3 years after the outbreak are presented in Table 3. Compared to controls, children who

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**Table 1. Baseline characteristics of children at the time of a municipal water contamination with *E. coli* O157:H7 in May 2000**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Children with HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>Age years</td>
<td>4.8 ± 3.4</td>
<td>4.7 ± 3.8</td>
</tr>
<tr>
<td>Female</td>
<td>53%</td>
<td>53%</td>
</tr>
<tr>
<td>Birth weight kg</td>
<td>3.4 ± 0.7</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>Family history of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney failure</td>
<td>2.6%</td>
<td>0%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.6%</td>
<td>0%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13.2%</td>
<td>5.3%</td>
</tr>
</tbody>
</table>

Complete data were collected on all participants, with the exception of birth weight, which was recorded in 44% of medical records. Children who were asymptomatic at the time of the outbreak (controls) were compared to those children who developed hemolytic uremic syndrome (HUS). Mean ± standard deviation.

**Table 2. Follow-up characteristics 3 years after a municipal water contamination with *E. coli* O157:H7**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Children with HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight kg</td>
<td>29.1 ± 14.4</td>
<td>31.0 ± 16.2</td>
</tr>
<tr>
<td>Height cm</td>
<td>128 ± 18</td>
<td>127 ± 20</td>
</tr>
<tr>
<td>Body mass index kg/m^2</td>
<td>16.9 ± 2.8</td>
<td>17.9 ± 3.4</td>
</tr>
<tr>
<td>Body surface area m^2</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Time from outbreak to clinic visit years</td>
<td>3.0 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>24 hour urine volume mL</td>
<td>549 (239)</td>
<td>567 (274)</td>
</tr>
</tbody>
</table>

Children who were asymptomatic at the time of the outbreak (controls) were compared to those children who recovered from hemolytic uremic syndrome. Mean ± standard deviation.
Table 3. Renal function testing 3 years after a municipal water contamination with E. coli O157:H7

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Children with HUS</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteinuria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random urine albumin to creatinine ratio mg/mmol</td>
<td>1.1 ± 1.0</td>
<td>3.0 ± 2.9</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Random urine albumin mg/L</td>
<td>11.0 ± 12.5</td>
<td>32.1 ± 44.9</td>
<td>P = 0.003</td>
</tr>
<tr>
<td>24 hr proteinuria mg/day</td>
<td>31 ± 4</td>
<td>31 ± 3</td>
<td>P = 0.94</td>
</tr>
<tr>
<td>24 hr proteinuria mg/m²/hour</td>
<td>1.3 ± 1.7</td>
<td>1.2 ± 1.1</td>
<td>P = 0.88</td>
</tr>
<tr>
<td><strong>Clearance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular filtration rate mL/min per 1.73m²/a</td>
<td>137 ± 19</td>
<td>127 ± 18</td>
<td>P = 0.06</td>
</tr>
<tr>
<td>Serum creatinine μmol/L</td>
<td>40 ± 10</td>
<td>49 ± 10</td>
<td>P = 0.16</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure mm Hg</td>
<td>75 ± 7</td>
<td>74 ± 7</td>
<td>P = 0.70</td>
</tr>
<tr>
<td>Systolic blood pressure mm Hg</td>
<td>100 ± 8</td>
<td>100 ± 8</td>
<td>P = 0.98</td>
</tr>
<tr>
<td>Diastolic blood pressure mm Hg</td>
<td>63 ± 7</td>
<td>62 ± 8</td>
<td>P = 0.60</td>
</tr>
</tbody>
</table>

Children who were asymptomatic at the time of the outbreak (controls) were compared to those children who recovered from hemolytic uremic syndrome (HUS). Mean ± standard deviation. To convert albumin to creatinine ratio from mg/mmol to mg/g, multiply by 8.84.

*GFR as estimated by Schwartz formula.

Fig. 1. Random urine albumin to creatinine ratios 3 years after a municipal water contamination with E. coli O157:H7. Thirty-two percent of children who recovered from hemolytic uremic syndrome (HUS) demonstrated microalbuminuria (6 of 19) compared to 5% of controls (2 of 38, P = 0.01). The relative risk of microalbuminuria after HUS was 4.8 (95% CI 1.1 to 22.0). No child in either group had overt proteinuria, as defined by a random urine albumin to creatinine ratio > 33.9 mg/mmol (300 mg/g).

As shown in Table 3, at 3 years there were no significant differences in blood pressure or GFR between HUS survivors and healthy controls. Five percent of both HUS children (1/19) and controls (2/38) had a systolic or diastolic blood pressure > 95th percentile for age and sex. The difference in Schwartz-estimated GFR between the groups approached but did not achieve statistical significance (Fig. 2; P = 0.06).

In addition to testing at 3 years, most of children who developed HUS had urine albumin to creatinine testing 1 and 2 years after the acute illness (descriptively presented in Fig. 3). In the 6 children with microalbuminuria 3 years after HUS, the urine albumin to creatinine ratio was decreasing with time in 3 children, increasing with time in 2 children, and stable in 1 child (Fig. 3). Descriptively, those with stable or increasing urine albumin to creatinine ratios did not appreciably differ in their illness and treatment characteristics from those with decreasing levels, other than in the use of ACE-I.

In those with HUS, none of the following factors during the acute illness were statistically associated with increased urine albumin to creatinine ratio or lower GFR 3 years after the outbreak: higher white blood count (≥ 11.0 vs. < 11.0 × 10⁹/L), lower hemoglobin (≤ 107.5 g/L vs. > 107.5 g/L), lower platelets (≤ 74.5 vs. > 74.5 × 10⁹/L), higher serum creatinine (≥ 141 vs. < 141 μmol/L), elevated lactose dehydrogenase (≥ 1910 vs. < 1910 IU/L), or the presence of fever, anuria, or the need for acute dialysis.

**DISCUSSION**

Despite its importance, the long-term renal prognosis of D+ HUS remains controversial. Here, 3 years after HUS recovery, one third of children demonstrated...
microalbuminuria. HUS survivors were comparable in GFR and blood pressure to healthy children, and none had overt proteinuria.

The outcome of these children at 3 years is better than expected. Our systematic review summarizing all long-term studies suggested that an average of 4 years following D+ HUS (follow-up range 1 to 22 years), a quarter of children develop renal abnormalities (GFR less than 80 mL/min per 1.73m², hypertension, or overt proteinuria) [2]. Similarly, when follow-up studies of shorter duration are considered, appreciable renal sequelae in survivors have been described in most [13–15], but not all [16] studies. Reasons for differences between our results and other studies are likely multifactorial, and may relate to differences in the severity of acute disease or the use of ACE-I therapy in some children during follow-up. Furthermore, due to limited statistical power in our study, we cannot exclude a 10 mL/min per 1.73m² reduction in GFR in HUS compared to control participants, if in truth such a difference did exist. Despite these encouraging short-term results, the clinical relevance and prognostic importance of isolated microalbuminuria in our children remains uncertain. Similar to other renal insults, it has been suggested that D+ HUS can lead to a critical reduction in nephron number, with unsustainable remnant single-nephron hyperfiltration and progressive renal disease [17]. Microalbuminuria may be the first marker of such hyperfiltration, and its importance in the natural progression of other renal diseases, such as diabetic nephropathy, is well described. In 2 children who recovered from HUS, the microalbuminuria level appeared to increase with time. Thus, longer follow-up is needed to clarify the clinical significance and utility of this early finding. In addition, whether those who contracted E. coli O157:H7 gastroenteritis in the absence of HUS could also demonstrate hypertension, proteinuria, or chronic kidney disease is an untested research question for future consideration.

The Walkerton outbreak has had important legal and financial remuneration implications for those affected. Communicating the nuances and uncertain prognostic significance of microalbuminuria to the population, legal experts, and government representatives has proven challenging.

Our study used the strongest feasible epidemiologic design, a cohort analytic study, to answer the research question posed. Unlike other studies where the primary etiology of HUS was not always certain [18–24], all participants in this study were clearly delineated to have HUS due to E. coli O157:H7. The use of a healthy internal control group to compare renal outcomes such as microalbuminuria is particularly important, given the uncertain prevalence of such findings in the pediatric population. Finally, the majority of children who developed HUS from the outbreak were participants in this study.

Despite these merits, limitations of the present research should be appreciated. Random urine specimens were used here for reasons of feasibility. However, using first morning urine samples may have reduced measurement variability due to postural changes. While the homogenous exposure of this study is a strength, these long-term results may not be generalizable to other strains of toxigenic E. coli or contact sources other than water. Finally, the control group used in this study may have ingested contaminated municipal water during the
outbreak, but otherwise remained healthy without diarrhea. It is conceivable that asymptomatic \textit{E. coli} O157:H7 exposure results in acute silent renal disease and long-term microalbuminuria. However, this would bias toward demonstrating no difference between the groups, a finding that was not present in this study at least for microalbuminuria. Furthermore, we believe the control group used in this study represents an appropriate norm, given their levels of microalbuminuria were equal or less than healthy children used in population reference standards [25].

In this study, we conclude D+ HUS caused microalbuminuria 3 years after recovery. However, the majority of children had no renal testing before an unpredictable event like an outbreak. Thus, it could be proposed that a group of children with microalbuminuria predating the outbreak may have been more susceptible to \textit{E. coli} O157:H7 infection and HUS than controls, which accounts for the microalbuminuria observed at 3 years. While theoretically possible, we believe this to be implausible for a number of reasons. The HUS subjects were children, and children are unlikely to have substantial undetected health conditions associated with microalbuminuria. In addition, the baseline characteristics of HUS participants predating the outbreak were similar to healthy controls.

CONCLUSION

Children may demonstrate microalbuminuria 3 years after recovering from HUS. Longer follow-up of these participants will clarify the natural history and importance of this early finding.

ACKNOWLEDGMENTS

Supported by Ontario Ministry of Health and the Kidney Foundation of Canada. Dr. Garg was supported by a Canadian Institutes of Health Research (CIHR) Clinician Scientist Award. Other Walkerton Health Study investigators include Dr. S. Collins, Dr. G. Garofeannu, Dr. J. Howard, Dr. J. Mahon, Dr. J. Marshall, Dr. L. Moirl, Dr. J. Pope, Dr. J. Ray, and Dr. P. Rosas-Arellano. We thank Dr. A. Bagga and Dr. C. White for their commendable patient care during the outbreak. This research was first presented in abstract form at the Annual Meeting of the Canadian Society of Nephrology in Toronto, May 2004.

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REFERENCES


Fig. 3. Spot urine albumin to creatinine ratios in each of the 19 children who survived HUS, separated into decreasing, similar, and increasing trends over time. Microalbuminuria was defined by a urine albumin to creatinine ratio between 3.4 and 33.9 mg/mmol. In the 6 children with microalbuminuria 3 years after HUS, the urine albumin to creatinine ratio was decreasing with time in 3 children, increasing with time in 2 children, and stable in 1 child. Children who were treated with an ACE-I during follow-up are highlighted with a star (*).


