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Chronic renal disease is more prevalent in patients with hemolytic uremic syndrome who had a positive history of diarrhea

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Many uncontrolled studies and a subsequent meta-analysis suggest that hemolytic uremic syndrome (HUS) with a positive history for diarrhea is associated with a significant increase in chronic renal disease. Two recent controlled studies that followed children with this type of HUS after *Escherichia coli* O157:H7 outbreaks, and where the controls were selected from a group exposed in the outbreak, gave conflicting results. To clarify this apparent difference, we retrospectively compared a cohort of 30 children with sporadic diarrhea-positive HUS with 30 healthy controls who had no history of bloody diarrhea or HUS and who had similar age and gender. Significantly more children with previous HUS than the controls had albuminuria over a median follow-up of 6.2 years. Of these albuminuric patients, one-third had macroalbuminuria compared with none of the controls. Following HUS, children were three times more prone to hypertension and prehypertension, although the difference was not statistically significant. Glomerular filtration rates, estimated by cystatin C, were significantly lower by 30 ml/min/1.73 m^2. Thus, children with sporadic HUS with positive history of diarrhea compared with unexposed controls had a higher prevalence of chronic renal disease; results consistent with the meta-analysis. Prospective studies with appropriate controls are needed to completely resolve this issue.

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KEYWORDS: albuminuria; diarrhea-positive; *E. coli* O157:H7; GFR; HUS; renal sequelae

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Diarrhea-positive hemolytic uremic syndrome (D+ HUS) is a major cause of acute kidney injury in children.[1,2] Similar to other acute renal injuries, a HUS episode has the potential to decrease nephron endowment initially with subsequent increase in proteinuria, hypertension, and impaired glomerular filtration rate (GFR).[3] A clear understanding of the risk of these chronic renal sequelae is important to guide the long-term follow-up after a HUS episode.

Chronic renal sequelae after D+ HUS have been the subject of considerable debate. Some studies have reported minimal renal sequelae after D+ HUS.[4–6] However, most studies have found an increase in proteinuria, hypertension, and impaired GFR after D+ HUS.[7–20] A meta-analysis based on these studies estimated chronic renal abnormalities (hypertension, overt proteinuria, GFR <80 ml/min/1.73 m^2) in a quarter of children after an average follow-up of 4 years.[21] A major limitation of the studies in the meta-analysis is a lack of matched controls. This is an important consideration as inclusion of a control arm will reduce misestimating the risk of renal sequelae by controlling for unrelated asymptomatic proteinuria, prehypertension, and hypertension, which increase with the increase in obesity in otherwise healthy children.[22–24]

To highlight this point further, a recent prospective study on D+ HUS after an *E. coli* O157:H7 outbreak with a matched control arm did not find a higher prevalence of overt proteinuria, hypertension, and chronic kidney disease in children after D+ HUS.[25] The results of this study suggested the possibility of an inflated estimate of chronic renal sequelae in the meta-analysis because the uncontrolled design of the included studies.[21] As the controls in this controlled study were exposed to *E. coli* O157:H7 during the outbreak, it is also possible that unrecognized subclinical renal injury in controls from the exposure of shiga toxin[26–28] or by the inclusion of subjects with undetected incomplete HUS in the control group[29,30] could have masked the difference in renal abnormalities in the HUS patients versus their controls. Also, a further masking in the difference is possible due to the attenuation of acute renal injury in

D+ HUS during an outbreak, by an early diagnosis, and by prompt fluid therapy.[31]

On the basis of these assumptions, we hypothesized that renal outcome after an outbreak of D+ HUS assessed with exposed controls could be less severe than that after sporadic D+ HUS with unexposed controls. To test our hypothesis, we assessed the long-term renal sequelae of children, who had previous sporadic D+ HUS, and compared their outcomes with age and gender-matched healthy controls who had not knowingly been exposed to an *E. coli* O157:H7 outbreak.

RESULTS

Screening for the study cohort

In all, 52 subjects with previous D+ HUS satisfying our inclusion criteria were identified from the records. A total of 22 could not be enrolled (15 not tracked, 1 moved out of province, and 6 did not consent). In those not enrolled, median age at the time of HUS episode was 3.4 years (range 7 months–16 years), and 20 (91%) of them aged below 12 years had HUS. In all, 15 (58%) were females; 18 (81%) had an *E. coli* documentation on stool culture, and 11 (50%) required dialysis initially. Finally, 30 subjects constituted the HUS group and were compared with 30 age and gender-matched healthy controls.

Table 1 | Characteristics of patients with previous HUS and the matched controls

	HUS patients (*n*=30)	Controls (*n*=30)	*P*-value
Median age; range (year)	9.0; 3, 26	9.0; 3, 26	NS
Age distribution (years)			
<12	19 (63.3%)	19 (63.3%)	
12–18	5 (16.6%)	5 (16.6%)	
>18	6 (20.0%)	6 (20.0%)	
Female (%)	16 (53.3)	16 (53.3)	NS
Family history of kidney disease (%)	3 (10.0)	1 (3.3)	0.61
Previous dialysis (%)	16 (53.3)	—	
BMI *z*-score[a]	0.87 ± 1.71	0.83 ± 1.79	0.93
BMI percentile[a]	66.84 ± 30.61	64.07 ± 36.08	0.77
BMI	22.09 ± 1.72	24.36 ± 4.37	0.28
Underweight (%)	1 (3.3)	3 (10)	0.90
Overweight (%)	4 (13.3)	3 (10)	0.54
Obese (%)	7 (23.3)	9 (30)	0.29

Abbreviations: BMI, body mass index; HUS, hemolytic uremic syndrome; NS, not significant.
[a]*z*-scores and percentiles of BMI are reported for those aged below 18 years. For those who are 18 years and older, absolute values of BMI are stated.
Data are expressed as mean ± s.d., unless stated otherwise.
For <18 years, underweight: <5th percentile; normal weight: 5th–85th percentiles; overweight: 85th–95th percentiles; and obese: ⩾95th percentile.[43] For ⩾18 years, underweight: <18.5; normal: 18.5–24.9; overweight: 25–29.9; and obese ⩾30.

Demographics of HUS patients and controls

The HUS patients and controls were matched for age (median 9 years; range 3–26) and gender (female 53%) as per the study design. These two groups were comparable with regard to body mass index (BMI) *z*-scores ($P=0.93$), and the proportion of underweight ($P=0.90$), overweight ($P=0.54$), and obese ($P=0.29$) subjects (Table 1).

In the HUS group, median age at the time of HUS episode was 2.5 years (range 3 months–15.3 years). In all, 29 (97%) had HUS below 12 years of age and 16 (53%) required a short-term dialysis initially. None of them required dialysis after the initial recovery. A total of 24 (80%) patients were stool culture or verotoxin-positive. Median follow-up was 6.2 years (range 3.3–20.1).

Chronic renal sequelae in the HUS patients and controls

The HUS subjects and controls were compared for albuminuria, systolic and diastolic blood pressure (BP) *z*-scores, and estimated GFR (eGFR) by cystatin C and creatinine formulae (Table 2). The HUS subjects had a markedly higher urine albumin to creatinine ratio (median and 95% confidence interval, 2.70 (3.42, 17.25) versus 0.60 (0.72, 1.60), $P=0.001$) (Figure 1). In all, 40% of the HUS patients showed albuminuria in contrast to only 3% of controls (relative risk 12; $P=0.001$). One-third of HUS subjects with albuminuria had macroalbuminuria, whereas none of the control subjects had macroalbuminuria. The HUS group had a lower cystatin C eGFR (30 ml/min/1.73 m^2; $P=0.001$) and creatinine eGFR (20 ml/min/1.73 m^2; $P=0.02$) than controls. There was a trend towards higher BP after HUS (three times more hypertension and prehypertension, and a higher systolic and diastolic BP in HUS subjects), although the difference did not reach statistical significance. This may be because of five HUS subjects on angiotensin converting enzyme inhibitors (three for albuminuria and two for hypertension) versus none in controls (Table 2).

Comparison of the HUS patients with different follow-up durations

The HUS patients with shorter follow-up (<5 years) were compared with those with a follow-up of ⩾5 years. Age at the time of HUS and initial dialysis requirement were similar in the two groups (Table 3). Both groups did not differ in their urine albumin to creatinine ratio, BP *z*-scores, eGFR, and subjects with albuminuria, hypertension, prehypertension, and angiotensin converting enzyme inhibitor therapy.

Comparison of the enrolled HUS subjects with those not enrolled

We analyzed the impact of non-enrollment on our results. Previous studies have shown that a randomly missed follow-up, rather than a systematic missing pattern, does not impact the applicability of results.[32–34] Absence of consent, despite awareness with regard to the study, in only 6/22 non-enrolled HUS subjects did not suggest a systematic pattern.[32] A random pattern of the missing data was also confirmed by two suggested techniques: first, by establishing similar characteristics of those enrolled and not enrolled (age ($P=0.20$), gender distribution ($P=0.30$), *E. coli* documentation ($P=0.40$), and dialysis requirement ($P=0.50$)), and second, by predictive logistic regression by creating a dummy dependent variable (enrolled = 1, non-enrolled = 0).[33,34] We

Table 2 | Renal parameters in patients with previous HUS and the matched controls

	HUS patients (*n*=30)	Controls (*n*=30)	*P*-value
Systolic BP *z*-score; median (95%CI)[a]	−0.25 (−0.52, 0.36)	−0.07 (−1.22, 0.46)	0.65
Systolic BP percentile[a]	48.31 ± 32.0	46.18 ± 24.05	0.82
Systolic BP (mm Hg)	120.85 ± 11.96	112.66 ± 9.13	0.21
Diastolic BP *z*-score; median (95%CI)[a]	0.31 (−0.03, 0.65)	−0.57 (−1.66, 0.51)	0.09
Diastolic BP percentile[a]	58.44 ± 24.98	48.43 ± 26.31	0.17
Diastolic BP (mm Hg)	73.66 ± 9.72	64.16 ± 7.33	0.08
Hypertension and prehypertension (%)	9 (30)	3 (10)	0.10
Hypertension (%)	2 (6.6)	0	
Prehypertension (%)	7 (23.3)	3 (10)	
Albumin/creatinine (mg/mmol); median (95%CI)	2.70 (3.42, 17.25)	0.60 (0.72, 1.60)	0.001
Albuminuria (%)	12 (40)	1 (3.3)	0.001
Microalbuminuria (%)	8 (26.6)	1 (3.3)	
Macroalbuminuria (%)	4 (13.3)	0	
Medications for blood pressure or albuminuria (%)[b]	5 (16.6)	0	
Creatinine-based eGFR	117.84 ± 33.08	140.88 ± 41.45	0.02
Cystatin C (mg/l)	0.76 ± 0.20	0.62 ± 0.09	0.002
Cystatin C-based eGFR	131.60 ± 32.17	159.67 ± 28.32	0.001

Abbreviations: BP, blood pressure; CI, confidence interval; eGFR, estimated GFR (ml/min/1.73 m^2); HUS, hemolytic uremic syndrome.
To convert urine albumin to creatinine ratio from mg/mmol to mg/g, multiply by 8.84; to convert GFR from ml/min/1.73 m^2 to ml/s/1.73 m^2, multiply by 0.01667.
[a]*z*-scores and percentiles of BP are reported for those aged below 18 years. For 18 years and older subjects, absolute values of BP are stated. For <18 years, BP levels were classified according to the fourth report (prehypertension: 90th and 95th percentile or in adolescents BP ⩾120/80 even if BP <90th percentile; hypertension: BP >95th percentile or antihypertensive medication already initiated for a diagnosed hypertension).[51] For ⩾18 years, BP was categorized according to the seventh JNC report (prehypertension: 120–139/80–89; hypertension: ⩾140/90).[52]
[b]Enalapril, for albuminuria in three and for hypertension in two subjects.
Data are expressed as mean ± s.d., unless stated otherwise.
Microalbuminuria was defined as urine albumin/creatinine ratio of 3.4 mg/mmol (30 mg/g) to 33.9 mg/mmol (300 mg/g), and macroalbuminuria as urine albumin/creatinine ratio >33.9 mg/mmol (300 mg/g).
Creatinine-based GFR was estimated by the Schwartz formula[48] in children and adolescents aged below 18 years, and by the modified diet in renal disease (MDRD) equation[49] in 18 years or older subjects.
Cystatin C-based estimated GFR was estimated by the Filler formula.[39]

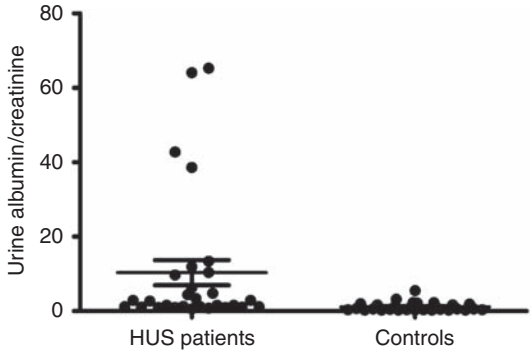

Figure 1 | Urine microalbumin to creatinine ratio (mg/mmol) in HUS patients (subjects with hemolytic uremic syndrome in the past) and controls (matched controls).

did additional analysis by extrapolating the proportion of albuminuria (3%), prehypertension, and hypertension (10%) from the enrolled control group ($n=30$) to non-enrolled HUS subjects ($n=22$) and to additional 22 controls. The difference in the extrapolated groups ($n=52$, each) still showed significant difference in albuminuria ($P=0.003$); however, the difference in prehypertension and hypertension did not reach statistical significance ($P=0.17$), which was similar to the results of enrolled HUS subjects and controls ($P=0.10$). To assess a sample-size effect, we did similar extrapolation based on albuminuria (40%), prehypertension, and hypertension (30%) in the enrolled HUS group ($n=30$) to those not enrolled ($n=22$), and by extrapolating corresponding proportions (3 and 10%) from the enrolled controls ($n=30$) to additional 22 controls. In these extrapolated groups ($n=52$, each), albuminuria was expectedly significantly different ($P<0.001$); however, the difference in prehypertension and hypertension also reached statistical significance ($P=0.01$).

DISCUSSION

Two controlled studies following children after *E. coli* outbreaks[25,35] failed to confirm the opinion, on the basis of previous uncontrolled studies and a large meta-analysis,[7–20] that D+ HUS induces a major increase in long-term renal sequelae. Concern about these conflicting observations led us to investigate the patients with sporadic D+ HUS, with healthy controls not knowingly exposed to an *E. coli* O157:H7 outbreak.

In the initial controlled study, Ogborn *et al.*[35] compared 18 children who had D+ HUS in an *E. coli* O157:H7 outbreak with 18 exposed controls. After a 4-year follow-up, both HUS and control groups remained comparable with respect to systolic and diastolic BP, proteinuria, and creatinine-based eGFR but HUS survivors had a higher prevalence of hematuria. This study suggested that *E. coli* HUS, in the setting of an outbreak, does not increase chronic renal sequelae. The interpretation of this study was limited by not reporting BP *z*-scores or percentiles, to account for the

Table 3 | Renal parameters in patients with previous HUS with a follow-up <5 years and ⩾5 years

	Follow-up <5 years (*n*=12)	Follow-up ⩾5 years (*n*=18)	*P*-value
Median age at the time of HUS; range (year)	2.75; 0.70, 15.30	2.25; 0.30, 11.50	0.58
Female (%)	4 (33%)	12 (66%)	0.07
Dialysis (%)	6 (50%)	10 (55%)	0.70
Systolic BP *z*-score[a] median (95%CI)	−0.25 (−0.75, 0.67)	−0.35 (−0.76, 0.52)	0.86
Systolic BP percentile[a]	47.08 ± 33.34	45.55 ± 32.07	0.91
Systolic BP (mm Hg)	—	120.85 ± 10.14	
Diastolic BP *z*-score[a] median (95%CI)	0.30 (−0.20, 0.89)	0.19 (−0.23, 0.79)	0.84
Diastolic BP percentile[a]	34.08 ± 32.92	18.75 ± 29.96	0.24
Diastolic BP (mm Hg)	—	73.66 ± 8.46	
Hypertension and prehypertension (%)	4 (34%)	5 (28%)	0.18
Hypertension (%)	2 (17%)	0	
Prehypertension (%)	2 (17%)	5 (28%)	
Albumin/creatinine (mg/mmol) median (95%CI)	2.90 (−0.30, 20.44)	1.60 (0.50, 20.50)	0.95
Albuminuria (%)	5 (42%)	7 (39%)	0.86
Microalbuminuria (%)	3 (25%)	5 (28%)	
Macroalbuminuria (%)	2 (17%)	2 (11%)	
Medications for blood pressure or albuminuria[b] (%)	3 (25%)	2 (11%)	0.30
Creatinine-based eGFR	125.69 ± 25.07	113.04 ± 36.98	0.32
Cystatin C (mg/l)	0.76 ± 0.27	0.76 ± 0.14	0.95
Cystatin C-based eGFR	137.06 ± 43.07	129.04 ± 23.08	0.47

Abbreviations: BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate (ml/min/1.73 m^2); HUS, hemolytic uremic syndrome.
[a]*z*-scores and percentiles of BP are reported for those aged below 18 years. For 18 years and older subjects, absolute values of BP are stated. For <18 years, BP levels were classified according to the fourth report (prehypertension: 90th and 95th percentile or in adolescents BP⩾120/80 even if BP <90th percentile; hypertension: BP >95th percentile or antihypertensive medication already initiated for a diagnosed hypertension).[51] For ⩾18 years, BP was categorized according to the seventh JNC report (prehypertension: 120–139/80–89; hypertension: ⩾140/90).[52]
[b]Enalapril, for albuminuria in three and for hypertension in two subjects.
Data are expressed as mean ± s.d., unless stated otherwise.
Microalbuminuria was defined as urine albumin/creatinine ratio of 3.4 mg/mmol (30 mg/g) to 33.9 mg/mmol (300 mg/g), and macroalbuminuria as urine albumin/creatinine ratio >33.9 mg/mmol (300 mg/g).
Creatinine-based GFR was estimated by the Schwartz formula[48] in children and adolescents aged below 18 years, and by the modified diet in renal disease (MDRD) equation[49] in 18 years or older subjects.
Cystatin C-based estimated GFR was estimated by the Filler formula.
To convert urine albumin to creatinine ratio from mg/mmol to mg/g, multiply by 8.84; to convert GFR from ml/min/1.73 m^2 to ml/s/1.73 m^2, multiply by 0.01667.

age dependency of BP. Also proteinuria quantification was performed by dipstick only. The controlled study by Garg *et al.*[25] reported 5-year follow-up of 19 children with D+ HUS in an *E. coli* outbreak. This study found a comparable BP and macroalbuminuria in the HUS group and controls; however, more children with HUS had microalbuminuria, and cystatin C eGFR was 10 ml/min/1.73 m^2 lower in the HUS group. This study had the methodological strengths of an adequate statistical power, a control arm, urinary albumin quantification, and GFR estimation by cystatin C. Both studies were similar in their selection of controls from the outbreak-exposed population, which may introduce a potential contamination from subclinical renal damage[27,28] or by the inclusion of cases with incomplete HUS[29,30] in the control arm. In relative terms, Garg *et al.*[25] had a lower risk of contamination as their controls were asymptomatic, whereas the controls in the study by Ogborn *et al.*[35] had gastroenteritis.

When compared with these two controlled studies,[25,35] our HUS group showed more renal sequelae than controls. However, these renal sequelae were still lower than that estimated by meta-analysis on the basis of previous uncontrolled studies.[7–21] Subjects with albuminuria were double in our HUS group than in study by Garg *et al.*[25] Macroalbuminuria was present in one-third of our HUS subjects with albuminuria as compared with none from the Garg's study.[25] When compared with respective controls, cystatin C eGFR was three times lower and the prevalence of hypertension and prehypertension was three times higher in our HUS subjects than that observed by Garg *et al.*[25] Angiotensin converting enzyme inhibitor prescription was five times more in our HUS subjects than in the study by Garg *et al.*[25]

Despite the similarity of controlled designs, a higher prevalence of chronic renal sequelae in our analysis as compared with other two controlled studies made us look at the possible reasons.[25,35] Similar distribution of overweight and obese subjects in our HUS patients and controls accounted for a possible confounding from obesity-associated albuminuria, prehypertension, and hypertension.[23,24] The *z*-scores of weight and height[35] and also BMI[25] were comparable between the HUS and control groups in other controlled studies. *E. coli* documentation in 80% of our HUS subjects was similar to 84% confirmed cases reported by Garg *et al.*[25] Recent evidence suggests a strain-specific variation in *E. coli* O157:H7 virulence,[36] which may affect the severity of acute renal injury. In the absence of strain recognition in our study and other studies,[25,35] we cannot comment on a possible impact of *E. coli* virulence on the results. This issue needs consideration in future studies. Although our median follow-up of 6.2 years was somewhat longer than 4- and 5-year follow-up in other studies,[25,35] our

HUS subjects with 5-year or longer follow-up did not have a higher albuminuria, BP *z*-scores, and eGFR than those with shorter follow-up. Other studies have also observed proteinuria and decreased GFR within the first few years in the majority of D+ HUS survivors who manifest chronic renal sequelae.[4,12,37]

It is also important to understand that D+ HUS is not a uniform disease and a severe acute renal injury predicts higher long-term renal sequelae.[10,12] We argue that sporadic setting versus outbreak exposure can affect the severity of HUS. Also, the assessment of chronic renal sequelae may vary if the control arm is exposed to an outbreak. Our hypothesis is based on the previous work by Ake *et al.*[31] reporting the attenuation of renal injury by an early recognition of *E. coli* O157:H7 infections and parenteral volume expansion before the development of HUS. An outbreak setting offers the advantage of prompt recognition of new cases and lesser delay in the delivery of medical care. A water-borne origin of *E. coli* in some outbreaks,[25,35] rather than a food source, could also dilute the inoculum resulting in a lesser shiga toxin exposure and potentially milder initial renal injury. Conversely, sporadic D+ HUS may have a longer time lag in the diagnosis and a larger inoculum in many cases due to food-borne contamination, which may result in greater initial renal injury. Our data supported this hypothesis as our HUS group had greater acute renal injury suggested by a higher initial dialysis need (53%) than in other two outbreak studies (22[35] and 37%[25]). Our reasoning of a higher probability of undiagnosed renal injury in controls selected from an outbreak-exposed population is based on *in vivo* evidence of subclinical renal injury from the exposure to *E. coli* O157:H7 shiga toxin,[26–28] and clinical studies reporting incomplete HUS not requiring medical attention.[29,30] Our data supported the risk of renal injury in exposed controls, as the exposed controls enrolled by Garg *et al.*[25] had about one and a half times higher urine microalbumin to creatinine ratio and BP percentiles, 5% controls with hypertension (none in our controls), and 30% lower cystatin C eGFR than our unexposed controls. The risk of long-term renal sequelae simply from *E. coli* exposure even without HUS was suggested by a higher prevalence of hypertension and reduced GFR in 1985 adults followed for 4 years after a self-limited *E. coli* O157:H7 gastroenteritis.[38]

Extrapolating these findings about exposure, the lack of difference in chronic renal sequelae between the HUS patients and controls in the study by Ogborn *et al.*[35] could be due to milder acute renal injury in their HUS patients because of an outbreak setting, and greater undetected renal injury in the exposed controls as they had diarrhea. Despite an outbreak setting, no diarrhea in the controls of Garg *et al.*[21,24,25,38] suggests a lower risk of subclinical renal injury in the exposed controls, and therefore, a trend towards higher renal sequelae than in the study by Ogborn *et al.*[35] In our study, the combination of a sporadic setting (more chronic sequelae from greater acute renal injury) and lack of exposure to an outbreak (absence of subclinical renal injury in the controls) can explain the higher chronic renal sequelae than that reported by Ogborn *et al.*[35] and Garg *et al.*[21,24,25,38] Our lower chronic renal sequelae, than in previous uncontrolled studies, and the meta-analysis[7–21] could be because of the inclusion of a control arm that accounted for unrelated proteinuria, prehypertension, and hypertension in the general population.[22–24]

Selection of HUS patients from a hospital database exposes our study to referral bias. However, referral bias may not be significant given that we remained the sole referral center for our designated geographical area, and the prevalent practice warranted a referral to the pediatric nephrology service for HUS. Our screening of database for HUS rather than for renal failure secondary to HUS lowered the bias towards including more cases with severe renal injury. Similar to missed follow-up in other studies that was around 30% in study by Garg *et al.*,[25] we could not enroll 42% of initially identified HUS subjects, which can be partly because of our study design and a longer follow-up. Missed follow-up may induce a wellness or sickness bias. However, the analysis of our missed follow-up suggested a random rather than a systematic pattern, which has been shown to maintain the validity of observations.[32] Further, extrapolation based on controls to non-enrolled HUS subjects still showed a significant difference in albuminuria between the HUS group and controls. Similar extrapolation for prehypertension and hypertension replicated the trend of higher prehypertension and hypertension without reaching the statistical significance seen in enrolled HUS subjects. Although our enrolled sample provided adequate statistical power to detect a clinically meaningful difference in renal sequelae between the HUS subjects and controls, extrapolation analysis based on renal sequelae in the HUS group to non-enrolled HUS subjects suggested the possibility of picking a bigger difference in renal sequelae with a larger study sample. A treatment bias seems unlikely, as the policy of supportive care for D+ HUS remained consistent throughout the period.

The strengths of our study are the inclusion of a matched healthy control arm, urine albumin quantification, eGFR estimation by cystatin C, and accounting for age and gender-dependency of body mass and BP by calculating the respective percentiles and *z*-scores. Estimation of chronic renal sequelae after D+ HUS with matched unexposed control arm is reported for the first time in our analysis. This is an important issue considering a high prevalence of asymptomatic unrelated derangement of renal parameters in pediatric population, which increase with obesity epidemic,[22–24] and possibility of asymptomatic renal impairment in controls from the exposure of shiga toxin in outbreaks.[19,27,29,30,38] Despite the fact that microalbuminuria may be the first marker of renal injury, albumin quantification after HUS has been done in only a few studies.[9,25] Although nuclear GFR would have provided a better estimate of kidney functions, its application in healthy controls was not practical. Cystatin C is believed to be a better marker of GFR estimation than serum creatinine.[39–41]

In summary, our retrospective cohort-matched analysis of sporadic D+ HUS survivors using healthy controls reveals a high risk of developing chronic renal sequelae in the follow-up. This observation is different from that in controlled studies after *E. coli* O157:H7 outbreaks, with symptomatic and asymptomatic exposed controls.[25,35] Prospective studies with appropriate controls are needed to completely resolve this issue.

MATERIALS AND METHODS

Study setting and participants

This retrospective cohort-matched study was approved by the University of Western Ontario Research Ethics Board, with written informed consents obtained from the parent/guardian or participants, in cases older than 18 years, before study enrollment. Electronic medical records were screened from the period 1988 to 2005 for children admitted with the diagnosis of HUS. The findings of HUS were verified. An established HUS episode with a documented preceding diarrhea was labeled as D+ HUS. Presence of non-bloody diarrhea was not an exclusion criterion.[42] Exclusion criteria were a D+ HUS from an outbreak, a diagnosis of atypical HUS or use of plasma therapy (suspicion of thrombotic thrombocytopenic purpura). Details on demographic data and dialysis need were extracted. Letters were sent to the identified patients explaining the rationale of the study, with phone call follow-up to expand on the provided information and to answer related questions. Those not located by letter or phone call were tracked by calling the family doctor or the family member recorded in their hospital record. Those enrolled in the study underwent a standardized BP check-up, weight and height recording, and blood and urine testing.

Healthy controls were age and gender-matched for enrolled HUS subjects, selected from subjects requiring blood work not expected to affect study parameters e.g. pre-surgery screening for minor elective surgery. Healthy status of controls was carefully ensured by excluding any chronic disease including diabetes and hypertension, no previous history of bloody diarrhea and/or HUS, or acute renal issue and a recent acute illness. The controls underwent BP, weight, height recording and blood testing according to the same protocol as for the HUS patients.

Measurements of BMI, renal function, and BP

Weight and height were recorded on a calibrated digital scale and stadiometer (Holton, Crymatch, UK). BMI was calculated as height/weight squared (kg/m^2). In children and adolescents aged below 18 years, BMI *z*-scores and BMI percentile were calculated using the 2000 Centers for Disease Control and Prevention growth charts (underweight: $<$5th percentile; normal weight: 5th–85th percentiles; overweight: 85th–95th percentiles; obese: $\geqslant$95th percentile).[43] In 18-year and older subjects, body weight was categorized according to absolute BMI (underweight: $<$18.5; normal:18.5–24.9; overweight: 25–29.9; obese $\geqslant$30).

Urine albumin level was measured using the Image Beckman Coulter immunoassay (Beckman Coulter, Fullerton, CA, USA). Microalbuminuria was defined as a urine albumin/creatinine ratio of 30–300 mg/g (3.4–33.9 mg/mmol).[22,44–46] In subjects aged below 18 years, creatinine-based eGFR was estimated by using the Schwartz formula;[47] $\mathrm{GFR} = k \times (\text{height (cm)}/\text{serum creatinine (}\mu\text{mol/l)})$. Recalibrated k constants for the formula were used for a better estimate: for males aged 2–13 years and all females, k was 0.523; and for males aged 13–18 years, k was 0.564.[48] In 18 years or older subjects, creatinine-based eGFR was estimated by the modified diet in renal disease equation;[49] $\mathrm{GFR}\ (\mathrm{ml/min/1.73\,m^2}) = 186 \times (S_{cr})^{-1.154} \times (\mathrm{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African–American})$ (conventional units). In addition, eGFR was assessed by serum cystatin C level;[39] GFR: $\log(\mathrm{GFR}) = 1.962 + (1.123 \times \log(1/\text{cystatin C}))$. The cystatin C equation is independent of age, sex, and body mass, and is considered to be more reliable than creatinine-based eGFR.[39,41,50] In the present study, cystatin C was measured in the same laboratory at which this equation was derived.[39]

Casual BP was measured in supine position after 10 min rest. The cuff covered two-third length of the upper arm, with the bladder covering the arm circumference. An average of three BP measurements, recorded at 2-min intervals, with the standard oscillometric method (Dynamap; Critikon, Tampa, FL, USA) was used for the analysis. For children and adolescents, BP measurements were converted to *z*-scores and percentiles on the basis of age, sex, and height,[51] and BP levels were classified according to the fourth report (prehypertension: 90th–95th percentile or in adolescents BP $\geqslant$120/80 even if BP $<$90th percentile; hypertension: BP $>$95th percentile or antihypertensive medication already initiated).[51] In 18-year or older subjects, BP was categorized according to the seventh JNC report (prehypertension: 120–139/80–89; hypertension: $\geqslant$140/90).[52]

Statistical analysis

Continuous data were tested for normal distribution using the Shapiro–Wilks test. Categorical data were compared using Fisher exact test. Normally distributed data were analyzed using parametric methods (mean, s.d., and *t*-test). When data were not distributed normally, non-parametric methods (median, range, 95% confidence interval, and Wilcoxon rank–sum test) were used. Two-tailed *P*-value <0.05 was considered significant. On the basis of variance estimate, our data had 80% statistical power to detect a difference of 9 mg/mmol in urine albumin to creatinine ratio, 20 ml/min/1.73 m^2 for cystatin C eGFR, 9 mm Hg for systolic BP, and 7 mm Hg for diastolic BP. We used SPSS version 17 (SPSS, Chicago, IL, USA) for the statistical analysis.

DISCLOSURE

All the authors declared no competing interests.

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