Childhood Nephrotic Syndrome in Cambodia: An Association with Gastrointestinal Parasites

Lawrence Copelovitch, MD, Orng Sam Ol, MD, Sarah Taraquinio, BA, and Ngoun Chanpheaktra, MD

Objectives To describe childhood nephrotic syndrome (NS) in Cambodia and to evaluate whether initial presentation or relapse is associated with gastrointestinal parasitic infection.

Study design We reviewed the records of 112 children with NS. A retrospective cross-sectional study compared 99 stool exams from 63 children with NS with 12,365 stool exams from 9,495 controls.

Results The male-to-female ratio was 1.7; the mean age of presentation was 8.95 years–44% were hypertensive, 44% had microscopic hematuria, 40% had eosinophilia, and 41% had acute renal failure; 92.7% were steroid sensitive, 12.7% were steroid dependent, and 8.9% were frequent relapers. Peritonitis and death were rare outcomes. *Giardia lamblia* (OR, 3.62; 95% CI, 2.0 to 6.1), *Strongyloides stercoralis* (OR, 3.59; 95% CI, 1.3 to 8.2), and Hookworm species (OR, 2.57; 95% CI, 1.0 to 5.5) were more likely to be isolated from the children with NS than the controls.

Conclusions The clinical course of childhood NS in Cambodia is similar to the developed world. Differences at presentation included older age and increased prevalence of microscopic hematuria, hypertension, eosinophilia, and acute renal failure. This study demonstrates an association between *G lamblia, S stercoralis,* and possibly Hookworm species and the onset of NS. (J Pediatr 2010;156:76-81)

Children's nephrotic syndrome (NS) is a worldwide disease whose incidence and prognosis vary greatly with geography. The estimated incidence of NS in white children from the United States and the United Kingdom is 2 to 7 per 100,000, with a prevalence of 15 per 100,000 under 16 years of age. There seems to be higher incidence in South Asian (Pakistani, Indian, and Bangladeshi) and Arab children. Additionally, long-term prognosis, treatment response, and renal pathology vary with ethnicity. Children with NS of African or Hispanic descent have worse outcomes, less responsiveness to treatment, and a higher incidence of focal segmental glomerulosclerosis as compared with whites and South Asians. Whether these disparities result from genetic differences, local environmental influences, or a complex interplay between the two is unclear.

There are few systematic clinical studies of children with NS from mainland Southeast Asia (Cambodia, Thailand, Vietnam, Laos, Burma, and Malaysia) and none from Cambodia. A retrospective review of 133 Thai children with idiopathic NS showed clinical features similar to children of European ancestry: 91% were steroid sensitive, more than half had at least 1 relapse, and 16.5% were frequent relapers. Conversely, a separate histopathologic study of 91 Thai children with NS found far higher numbers of mesangiproliferative glomerulonephritis (33%) and membranoproliferative glomerulonephritis (30.8%) compared with developed countries in East Asia, Europe, and North America. Another study from Thailand showed that more than 50% of children with steroid-resistant or difficult-to-treat NS had mesangial immunoglobulin M (IgM) deposits. In contrast, IgM nephropathy is found in only 20% to 25% of similar European patients with NS.

These results suggest that ethnic background and environmental conditions may play a role in the etiology and clinical picture of NS in Southeast Asia. Throughout the world, parasitic infections, including *Plasmodium malariae, Schistosoma mansoni, Leishmania donovani,* and *Strongyloides stercoralis* have been associated with NS. Altered T-helper cell cytokine expression, binding of autoantibodies to glomerular autoantigens, and immune complex formation have been proposed as mechanisms explaining causation. Because gastrointestinal parasitic infections are widely prevalent in Cambodia, this is an excellent etiologic candidate to study.

AHC Angkor Hospital for Children
ARF Acute renal failure
BP Blood pressure
GFR Glomerular filtration rate
Ig Immunoglobulin
IL Interleukin
NS Nephrotic syndrome
The Angkor Hospital for Children (AHC) patient database contains the demographic information, primary and secondary discharge diagnosis, and laboratory values of all the patients seen in the outpatient clinic and inpatient department between June 2001 and June 2008. Four hundred fifty patients had at least 1 primary or secondary diagnosis of NS, nephritic syndrome, or acute glomerulonephritis. Nineteen patient charts could not be found. The remaining 431 charts were reviewed in detail by the same pediatric nephrologists. One hundred thirty-one patients were incorporated into the database and did not have a relevant diagnosis. Of the remaining 300 patients, 112 had idiopathic NS, 169 had acute glomerulonephritis, 12 had nephritic/nephrotic features whose diagnosis could not be determined, 5 had systemic lupus nephritis, 1 had Henoch-Schonlein nephritis, and 1 had HIV nephropathy. None of the 112 patients with idiopathic NS had gross hematuria. The institutional review board of AHC approved the study.

Nineteen patient charts could not be found. The remaining 300 charts were reviewed in detail by the same pediatric nephrologists. One hundred thirty-one patients were incorporated into the database and did not have a relevant diagnosis. Of the remaining 300 patients, 112 had idiopathic NS, 169 had acute glomerulonephritis, 12 had nephritic/nephrotic features whose diagnosis could not be determined, 5 had systemic lupus nephritis, 1 had Henoch-Schonlein nephritis, and 1 had HIV nephropathy. None of the 112 patients with idiopathic NS had gross hematuria. The institutional review board of AHC approved the study.

Methods

The medical records of the 112 children with NS were reviewed for sex, age, BP, antihypertensive medications, urinalysis, serum creatinine, serum albumin, and eosinophil count. Sex and age data were available for all 112 patients. At the first NS encounter 109 children had a BP, 110 had a urinalysis, 107 had a serum creatinine, 110 had a serum albumin, and 104 had a complete blood count documented in the medical record. The mean estimated GFR was 106 mL/min/1.73 m², with a standard deviation of 48.3 mL/min/1.73 m². Two children under the age of 2 years were not included in the ARF analysis because their GFRs were <90 mL/min/1.73 m² (72 and 66 mL/min/1.73 m²) but may have been appropriate for age. The clinical data at presentation are summarized in Table I. The mean absolute eosinophil count of the 42 children with eosinophilia was 2200 cells/μL, with a range of 600 to 8900 cells/μL.

Response to and duration of prednisone treatment, the number of relapses, episodes of peritonitis, and death were documented. Of the 112 children with NS, 96 had sufficient

Results

The medical records of the 112 children with NS were reviewed for sex, age, BP, antihypertensive medications, urinalysis, serum creatinine, serum albumin, and eosinophil count. Sex and age data were available for all 112 patients. At the first NS encounter 109 children had a BP, 110 had a urinalysis, 107 had a serum creatinine, 110 had a serum albumin, and 104 had a complete blood count documented in the medical record. The mean estimated GFR was 106 mL/min/1.73 m², with a standard deviation of 48.3 mL/min/1.73 m². Two children under the age of 2 years were not included in the ARF analysis because their GFRs were <90 mL/min/1.73 m² (72 and 66 mL/min/1.73 m²) but may have been appropriate for age. The clinical data at presentation are summarized in Table I. The mean absolute eosinophil count of the 42 children with eosinophilia was 2200 cells/μL, with a range of 600 to 8900 cells/μL.

Response to and duration of prednisone treatment, the number of relapses, episodes of peritonitis, and death were documented. Of the 112 children with NS, 96 had sufficient
follow-up time to establish steroid sensitivity or resistance. The rate of steroid dependence was recorded in 63 steroid-sensitive patients who were followed at least 2 weeks after prednisone treatment was discontinued. The number of frequent relapers was evaluated in the 45 steroid-sensitive patients who were followed for at least 6 months. The clinical course data are summarized in Table II. Six patients had no relapses and were followed for a mean of 19.4 months (range, 9.9 to 35.6 months). Seven patients were steroid resistant, and the records were reviewed in detail. Two had development of chronic renal failure, 2 had persistent proteinuria with normal renal function, and 3 patients achieved remission, 1 after a course or oral cyclophosphamide and 2 after prolonged treatment with corticosteroids. The mean follow-up time for the steroid-resistant patients was 15.1 months (range, 3.7 to 32.8 months). One patient died of apparent septicemia, although no organism was identified.

The stool exam results from the patients with NS and controls are summarized in Table III. All 40 positive exams from the nephrotic group were positive for 1 or more of the following: Giardia lamblia, S trematodes, Hookworm species, or Ascaris lumbricoides. Of the positive stool exams, 1463 of 1668 (87.8%) from the NS cohort were positive for at least 1 parasite other than other than G lamblia, S stercoralis, or Hookworm species were performed (Table IV). G lamblia alone (OR, 3.62; 95% CI, 2.0 to 6.1; \( P < .0001 \)) and S stercoralis alone (OR, 3.59; 95% CI, 1.3 to 8.2; \( P < .01 \)) were more likely to be found in the stools of NS children as compared with the controls. Hookworm was also more likely (OR, 2.57; 95% CI, 1.0 to 5.5; \( P < .05 \)) to be found in patients with NS; however, the confidence interval included 1.0, raising concerns regarding limited sample size from which to draw conclusions. In the subgroup analysis, none of the G lamblia alone, S stercoralis alone, and Hookworm species alone stool exams from the NS cohort demonstrated multiple parasites. By including exams with multiple organisms in the control group, we lessened the likelihood of biasing the data toward an erroneous association.

Of the 63 patients with stool exams, 35 had at least 1 positive exam and 28 had all negative stool exams. Comparison of the 2 groups showed no statistically significant difference in the rates of steroid sensitivity, steroid dependence, frequent relapers, chronic renal failure, or death (data not shown). To evaluate whether the patients with stool exams sampled were representative of all children with NS, we compared the 63 patients who underwent stool examination with the 48 who did not (data not shown). There was no statistically significant difference in male-to-female ratio, hypertension, hematuria, serum creatinine, ARF, duration of follow-up, steroid sensitivity, steroid dependence, or the rate of frequent relapers. Age (8.25 years vs 9.85 years, \( P .02 \)) and serum albumin (1.7 g/dL vs 1.4 g/dL, \( P < .005 \)) were significantly different among the 2 groups. We hypothesize that younger patients who presented with edema were more likely to get stool exams as part of their initial evaluation. The clinical relevance of the significantly different albumin levels is unclear.

### Table I. Clinical data at presentation

<table>
<thead>
<tr>
<th>Sex (M:F)</th>
<th>Age</th>
<th>Hypertension</th>
<th>Hematuria</th>
<th>Albumin</th>
<th>ARF</th>
<th>Eosinophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7:1 (71:41)</td>
<td>8.95 y</td>
<td>44%</td>
<td>43.6%</td>
<td>1.6 g/dL</td>
<td>41%</td>
<td>40.4%</td>
</tr>
<tr>
<td>Range: 8 mo to 15.75 y</td>
<td>(48/109)</td>
<td>(48/110)</td>
<td>(43/105)</td>
<td>(42/104)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table II. Clinical course

<table>
<thead>
<tr>
<th>Steroid-sensitive</th>
<th>Steroid-dependent</th>
<th>Frequent relaper</th>
<th>Intermediate relaper</th>
<th>Peritonitis</th>
<th>ARF</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>92.7% (89/96)</td>
<td>12.7% (8/63)</td>
<td>8.9% (4/45)</td>
<td>75.6% (34/45)</td>
<td>3.6% (4/112)</td>
<td>0.9% (1/112)</td>
<td></td>
</tr>
</tbody>
</table>

The original descriptions of steroid-sensitive or minimal change NS in Western children show a male-to-female ratio of 2.3 to 3.3:1 and a mean onset at 3 years of age. At presentation, there was microscopic hematuria in 11% to 13%, ARF in 19% to 26%, and hypertension in 9% to 28% of the patients. Our findings are distinct: Although the male-to-female ratio was similar at 1.7:1, the age at presentation (8.95 years), incidence of hypertension (44%), hematuria (44%), and ARF (41%) were significantly different. Whether children in Cambodia have NS at a later age, have a different underlying pathology, or present later in the course of illness as a result of socioeconomic barriers is unclear. Given that the vast majority of our patients had rapidly reversible ARF, it is probable that a greater percentage of our patients presented with intravascular volume depletion. However, it is also possible that different pathophysiologic mechanisms might be involved because a biopsy study of children with NS from rural Northeastern Thailand (bordering Cambodia) found that 77% of children presented after the age 5 years. In addition, a large number had mesangioproliferative glomerulonephritis (33%) or membranoproliferative glomerulonephritis (30.8%). It is unclear if the higher incidences of microscopic hematuria, hypertension, and ARF that we observed are related to these findings. One possible reason for the higher incidences of hypertension and ARF may be that we had more inclusive definitions. The original studies defined hypertension as a diastolic blood pressure >90 mm Hg or 2 standard deviations above the mean and ARF as a BUN...
Table III. Stool examination results

<table>
<thead>
<tr>
<th></th>
<th>NS (n = 99)</th>
<th>Control (n = 12365)</th>
<th>OR</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of parasites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris*</td>
<td>40 (40.4%)</td>
<td>1668 (13.5%)</td>
<td>4.35</td>
<td>2.8-6.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Giardia, Strongyloides, Hookworm, or Ascaris</td>
<td>40 (40.4%)</td>
<td>1463 (11.8%)</td>
<td>5.05</td>
<td>3.3-7.7</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Giardia*</td>
<td>22 (22.2%)</td>
<td>777 (6.3%)</td>
<td>4.26</td>
<td>2.5-7.0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Strongyloides*</td>
<td>12 (12.1%)</td>
<td>314 (2.5%)</td>
<td>5.29</td>
<td>2.6-9.8</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Hookworm*</td>
<td>11 (11.1%)</td>
<td>455 (3.7%)</td>
<td>3.27</td>
<td>1.6-6.2</td>
<td>.0012</td>
</tr>
<tr>
<td>Ascaris*</td>
<td>2 (2%)</td>
<td>58 (0.47%)</td>
<td>4.38</td>
<td>0.51-16.5</td>
<td>.0822</td>
</tr>
<tr>
<td>+ others</td>
<td>0</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Parasite alone or in combination with other parasites.

Table IV. Stool examination subgroup analysis

<table>
<thead>
<tr>
<th></th>
<th>NS (n = 99)</th>
<th>Control (n = 12365)</th>
<th>OR</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia*</td>
<td>18 (18.2%)</td>
<td>716 (5.8%)</td>
<td>3.62</td>
<td>2.0-6.1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Giardia alone</td>
<td>18</td>
<td>689</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giardia plus others</td>
<td>0</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strongyloides*</td>
<td>6 (6.1%)</td>
<td>218 (1.8%)</td>
<td>3.59</td>
<td>1.3-8.2</td>
<td>0.0088</td>
</tr>
<tr>
<td>Strongyloides alone</td>
<td>6</td>
<td>207</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strongyloides plus others</td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hookworm*</td>
<td>7 (7.1%)</td>
<td>356 (2.9%)</td>
<td>2.57</td>
<td>1.0-5.5</td>
<td>0.0254</td>
</tr>
<tr>
<td>Hookworm alone</td>
<td>7</td>
<td>341</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hookworm plus others</td>
<td>0</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giardia and Strongyloides</td>
<td>3 (3.0%)</td>
<td>32 (0.32%)</td>
<td>12.04</td>
<td>2.3-39.5</td>
<td>0.0026</td>
</tr>
<tr>
<td>Giardia and Hookworm</td>
<td>1 (1.0%)</td>
<td>35 (0.33%)</td>
<td>3.59</td>
<td>0.1-21.8</td>
<td>0.2499</td>
</tr>
<tr>
<td>Hookworm and Strongyloides</td>
<td>3 (3.0%)</td>
<td>70 (0.57%)</td>
<td>5.49</td>
<td>1.1-17.2</td>
<td>0.0202</td>
</tr>
</tbody>
</table>

*Parasite alone or in combination with any parasite other than Giardia, Hookworm, or Strongyloides.

>20 to 40 mg/dL.24,25 Our use of the NIH guidelines for BP norms23 and calculated GFRs based on the Schwartz formula probably resulted in more patients being included.

More than 90% of Western patients with minimal change in NS respond to prednisone.26 About 20% to 30% never have a relapse,24,27,28 and 40% become either steroid dependent or frequent relapers.28 Our follow-up data are similar: 92.7% of our patients were steroid sensitive, 13.3% had no relapses, 12.7% were steroid dependent, and 8.9% were frequent relapers.

Cambodia has a high prevalence of gastrointestinal parasitic infections in rural and urban communities. Reports on the prevalence of gastrointestinal parasitic infections in Cambodian children vary because of differing time periods, varied regions, accessibility to government-provided deworming medications, and the type of stool test. In our control group, 6.3%, 2.5%, 0.47%, and 3.7% of children had stool exams positive for G lamblia, S stercoralis, A lumbricoides, and Hookworm species, respectively, by direct microscopy. The G lamblia results are consistent with reported rates in the literature, which range from 2.9% to 4.2%.29-32 Reported prevalences of S stercoralis stool infections range from 2.7% to 20.2%.30,33,34 Exemplifying the methodological discrepancies, 1 study30 reported positive exam rates of 2.7% using the sodium–acetic acid–formalin fixation and 20.2% with the modified Baermann technique. Both methodologies are plausible mechanisms. Interestingly, 65.8%,30-35 respectively. This probably represents a high variance in the evaluation of parasitic infection status or a different environmental background. It is also conceivable that patients with NS were more likely to be admitted, have more stool exams performed, and therefore have a higher positivity rate. This seems unlikely because 63 patients with NS with at least 1 exam culture had an average of 1.6 stool cultures tested per patient, and the control group had a similar average of 1.3 stool exams per patient. Finally, it is possible that the immunosuppressive effects of NS results in an increased susceptibility to parasitic infections.

If the association between gastrointestinal parasitic infection and NS is causative, several possible pathogenic mechanisms can be invoked. Eosinophilia with altered T-helper 2 immune response, including the production of interleukin (IL)-4, IL-5, IL-13, and IgE have been implicated.41 In addition, the binding of autoantibodies to glomerular autoantigens, immune complex formation, or localized intestinal mucosal damage allowing intraluminal toxins to pass into circulation are plausible mechanisms. Interestingly, 40.4% of our patients had eosinophilia. The association between peripheral eosinophilia and atopy is well established. In 1959, Hardwicke et al12 first described an association between NS and pollen hypersensitivity. There are many reports of NS association with Gastrointestinal Parasites.
in association with allergic reactions to food and inhaled allergens. Furthermore, the incidence of atopy, serum IgE levels, and absolute eosinophil count are higher in patients with idiopathic NS, and oligoantigenic diets may help induce remission.

Neoplastic, inflammatory, and infectious eosinophilic diseases have been associated with nephrotic syndrome: Kimura disease, angiolymphoid hyperplasia, eosinophilic gastroenteritis, and *S. stercoralis*. Kimura disease, a benign angiolympathoma proliferation with eosinophilic infiltration that occurs most frequently in male East Asians, is associated with proteinuria in 12% of patients and NS in 7%. The renal pathology includes minimal change disease, mesangioproliferative glomerulonephritis, IgM nephropathy, focal segmental glomerulosclerosis, and membranous nephropathy.

NS in Cambodian children represents a small but significant burden to the healthcare system. Between June 2001 and June 2006, 0.53% (127/23 916) of all admissions to AHC were for NS. Our results indicate that the overall prognosis and response to treatment of childhood NS in Cambodia is similar to that of children of European descent. Whether older age at presentation, increased incidences of hematuria, eosinophilia, ARF, and hypertension results from different underlying pathophysiologic processes is unclear. This study demonstrates an association between *G. lamblia*, *S. stercoralis*, and possibly Hookworm species with NS. Whether gastrointestinal parasites are nonspecific triggers, causative agents, or a consequence of the NS itself requires further investigations. Regardless, we recommend empiric treatment with appropriate anti-parasitic agents to all children who present with NS in tropical or developing countries. The possibility that Giardiasis is unrecognized in NS patients in developed countries should be considered.

The authors thank Srey Sopeha, Sun Sopheap, Phok Raphy, Deth Sophay, and Sun Sophay for their assistance with medical record retrieval. We also send our gratitude to Michelle Denburg, MD, for her assistance with the statistical analysis and Bernard Kaplan, MBBCh, and Mary Leonard, MD, MSCE, for their helpful critique.
Childhood Nephrotic Syndrome in Cambodia: An Association with Gastrointestinal Parasites


44. Howanieth Z, Lube G. Idiopathic glomerulopathy, treated with steroids for five years, found to be allergic reaction to pork. Lancet 1985;2:450.


