Screening for kidney injury in hypertensive obese children, the use of NGAL as biomarker

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Abstract

Background Obesity-related childhood hypertension is a growing problem. Although hypertension is known to cause kidney injury in obese adults, studies in obese children are scarce. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is a marker for acute kidney injury and also seems to be a promising marker for chronic kidney injury. The aim of the study was to investigate if kidney damage is present in overweight and obese hypertensive children and whether NGAL is a suitable marker to detect chronic kidney damage in children.

Methods Random urine samples were collected from 1) overweight children with hypertension (n=38); 2) overweight children with normal blood pressure (n=86) and 3) non-overweight children with normal blood pressure (n=56), in 2013–2015 in the Netherlands. In addition to anthropometric and blood pressure measurements, urine was analysed for NGAL and albumin/creatinine ratio.

Results The mean age of the children was 10.5±3.4 (range 5–17) years and 53% were boys. There were no differences in urinary NGAL/creatinine, urinary albumin/creatinine ratio, and presence of microalbuminuria between the three groups. Girls had significantly higher urine NGAL/creatinine levels than boys (15.54 ng/mg (6.32–27.03) versus 3.18 ng/mg (2.28–5.38), β 1.78, 95%CI 1.60–1.99).

Conclusion Overweight-induced hypertension could not be associated with kidney injury in this pre- and postpubertal paediatric cohort. Further study of kidney injury and of the predictive value of NGAL will require longitudinal follow-up of overweight and obese children.
Introduction

As a result of the overweight and obesity epidemic, childhood hypertension is a growing public health problem, with a prevalence of 4–14% in overweight and 11–33% in obese children (1-4). This is of great concern, since overweight as well as hypertension have the tendency to track from childhood into adulthood (5;6). Hypertension is known to cause kidney injury in obese adults (7;8), and is suggested as being one of the most important causes of end-stage renal disease in adult patients (9).

Several studies in adults suggest that since the beginning of the obesity epidemic, the incidence of kidney disease has also increased (10;11). Obesity itself, even without the co-existence of hypertension, is also associated with kidney injury, both in children and adults. Obesity is thought to lead to glomerular hyperperfusion and hyperfiltration, as a result of an increased cardiac output and afferent arteriolar vasodilation. Kidney damage in obesity manifests itself clinically with microalbuminuria, proteinuria and/or renal insufficiency (7;12;13). Studies pertaining to the presence of kidney injury in children who are overweight or obese as well as being hypertensive are scarce.

An early sign of kidney injury in hypertension is the presence of abnormal levels of urinary (micro)albumin, as a consequence of hypertension-induced glomerular damage (8;14). Microalbuminuria is a strong predictor of renal and cardiovascular morbidity and mortality in adults with hypertension (15;16). In 20% of children with 24-hour ambulatory blood pressure measurement (ABPM) confirmed hypertension, microalbuminuria was found (17).

In recent years, neutrophil gelatinase-associated lipocalin (NGAL) has been discovered as a biomarker for acute kidney injury, and also seems to be a
promising marker for chronic kidney injury, both in adults and children (14;18;19). NGAL is a small protein, belonging to the lipocalin family, which is released from renal proximal tubular cells after injury (18). Several studies investigated the use of urinary NGAL in predicting the onset of acute kidney damage, and found that urinary NGAL levels increase prior to the increase in serum creatinine levels, for example after cardiac surgery both in children and adults (20;21). NGAL has also been found to indicate kidney damage in chronic diseases such as glomerular diseases in adults (22), and is believed to be a marker for the severity and risk of progression of kidney injury in adults as well as in children (18;23). Although one study found a significant association in children between elevated systolic blood pressure and higher urinary NGAL concentrations, no association with BMI was found (14). Another study on the other hand did not find a difference in urinary NGAL concentration between obese children with and without hypertension and a normal weight control group (24).
More research is needed to evaluate the use of NGAL as a marker for chronic kidney injury in hypertensive obese children. The aim of the study was to investigate if kidney damage is detectable in obese hypertensive children and whether NGAL would be a suitable marker to detect chronic kidney damage in children.

Methods

Study design
In the period September 2013–June 2015, random urine samples were collected from children aged 5–17 years. Three groups of children were enrolled in this cross-sectional study: 1) overweight children with hypertension; 2) overweight children with normal blood pressure; and 3) non-overweight children with normal blood pressure. Overweight children with
and without hypertension were recruited at a paediatric outpatient obesity clinic or through their participation in our ongoing study “Implementation study of screening for hypertension in overweight in Child Health Care”. In addition, healthy normal weight children, the control group, were recruited at a general paediatric outpatient clinic – which they visited for reasons such as check-ups for concussion or pneumonia – and at schools. Children with conditions or use of medication known to affect the kidneys, for example urinary tract infection, were excluded from participation in the study.

Informed consent was obtained from at least one parent and all children above the age of 12 years. The study has been approved by the VU University Medical Ethical Committee.

**Anthropometry and blood pressure measurements**

Body weight was measured to the nearest 0.1 kilogram using a digital scale, with children barefoot and wearing only light clothing. Height was measured to the nearest 0.1 centimetre with a stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by the square of body height in meters and categorized according to the International Obesity Task Force (IOTF) (25).

Blood pressure was measured three consecutive times after 5 minutes rest using an electronic oscillometric blood pressure monitor, on the right arm. The appropriate size cuff was used, according to the National High Blood Pressure Education Programme (NHBPEP) Working Group on Children and Adolescents (26). The lowest of the three blood pressure values was compared with cut-off values provided by the NHBPEP Working Group. Hypertension was defined as blood pressure ≥95th percentile for age, gender and height (26).
Urine sample analyses
Random urine samples were collected. During the data collection period, urine was stored at -80 °C until analyses took place (21). The urine was analysed for NGAL and microalbumin, and both are expressed over creatinine. Microalbuminuria was defined as albumin/creatinine ratio of 30–300 mg/g (3–30 mg/mmol) (27).

Microalbumin and creatinine in urine were measured using an immunoturbidimetric and enzymatic colorimetric assay, respectively (Cobas 8000, Roche Diagnostics, Mannheim, Germany). Urinary NGAL was measured using a fully automated immunoassay (Architect, Abbott Diagnostics, Abbott Park, IL) with a lower limit of quantitation of 2 ng/mL and imprecision of <5.3% for all levels (21).

Statistical analyses
BMI and height standard deviation (SD) scores were calculated using the LMS method (25), with respectively World Health Organization (WHO) (28) and Centers for Disease Control (CDC) data (29). Blood pressure SD scores were calculated using the equations provided by the NHBPEP Working Group on Children and Adolescents (26). Differences in characteristics between overweight children with hypertension, overweight children with normal blood pressure, and non-overweight children with normal blood pressure were tested with Student’s t-test for continuous variables or with X² tests for categorical data. NGAL and microalbumin followed a non-normal distribution, and were log transformed. Linear regression analysis was used to determine the associations between NGAL/creatinine levels and microalbumin/creatinine levels with high blood pressure and BMI status, with adjustment for possible confounding variables (age and gender). A level of \( p < 0.05 \) was considered
significant. The statistical analyses were performed using SPSS software version 22.0 (SPSS Inc., Chicago, IL).

**Results**

A total of 180 children and adolescents were included in the study, of which 38 overweight children with hypertension, 86 overweight children with normal blood pressure, and 56 non-overweight children with normal blood pressure. Table 1 shows the demographic, anthropometric and blood pressure data of the three study groups.

There was no significant difference in mean albumin/creatinine ratio between overweight hypertensive children, overweight children with normal blood pressure and non-overweight children with normal blood pressure. Microalbuminuria was present in 13 children (7.2%), with no differences between the three study groups (Table 2). Regarding urinary NGAL/creatinine levels, there was no significant difference between hypertensive and normotensive children ($\beta$ 1.08, CI 0.91–1.28, $p$=0.37), but there was a significant difference between overweight and non-overweight children ($\beta$ 1.20, CI 1.04–1.40, $p$=0.01). However, after adjustment for gender, this difference disappeared ($\beta$ 1.06, CI 0.36–1.36, $p$=0.31). There was no significant association between urinary NGAL/creatinine levels and albumin/creatinine ratio ($p$=0.90).
Table 1 Patient characteristics according to study group; hypertensive overweight children, normotensive overweight children, and normotensive non-overweight children.

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive overweight children (n=38)</th>
<th>Normotensive overweight children (n=86)</th>
<th>Normotensive non-overweight children (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys (%)</td>
<td>15 (40)\textsuperscript{a}</td>
<td>43 (50)</td>
<td>38 (68)</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>9.6±3.8</td>
<td>10.8±3.4</td>
<td>10.5±3.1</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western</td>
<td>8 (21)\textsuperscript{b}</td>
<td>17 (20)\textsuperscript{c}</td>
<td>49 (88)</td>
</tr>
<tr>
<td>Non-Western</td>
<td>30 (79)\textsuperscript{b}</td>
<td>65 (76)\textsuperscript{c}</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>-</td>
<td>4 (4.7)</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7±2.8\textsuperscript{b}</td>
<td>27.2±5.1\textsuperscript{c}</td>
<td>16.9±2.0</td>
</tr>
<tr>
<td>BMI-SDS</td>
<td>2.6±0.7\textsuperscript{b}</td>
<td>2.6±0.9\textsuperscript{c}</td>
<td>0.8±9.2</td>
</tr>
<tr>
<td>Overweight</td>
<td>9 (24)</td>
<td>24 (28)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>12 (32)</td>
<td>34 (40)</td>
<td></td>
</tr>
<tr>
<td>Morbidly obese</td>
<td>14 (37)</td>
<td>28 (32)</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean systolic BP-SDS</td>
<td>1.99±1.47\textsuperscript{b,d}</td>
<td>0.43±0.94\textsuperscript{c}</td>
<td>0.15±0.85</td>
</tr>
<tr>
<td>Mean diastolic BP-SDS</td>
<td>1.22±0.75\textsuperscript{b,d}</td>
<td>0.30±0.62\textsuperscript{c}</td>
<td>0.17±0.61</td>
</tr>
</tbody>
</table>

Values are shown in N (%) or in mean ± SDS. \textsuperscript{a} significant difference between hypertensive overweight and normotensive non-overweight children (p <0.02), \textsuperscript{b} significant difference between hypertensive overweight and normotensive non-overweight children (p <0.001), \textsuperscript{c} significant difference between normotensive overweight and non-overweight children (p <0.001), \textsuperscript{d} significant difference between hypertensive overweight and normotensive overweight children (p <0.001). Ethnicity: country of birth of the mother, unless the mother was born in the Netherlands, then country of birth of the father. Western: countries in Europe (Turkey excluded), North-America, or Oceania. Non-Western: countries in Africa, Latin-America, Asia, or Turkey. Weight is categorised according to the definition of International Obesity Task Force (25). BP= blood pressure.
### Table 2 Urinary microalbumin and NGAL/creatinine excretion in children.

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive overweight children (n=38)</th>
<th>Normotensive overweight children (n=86)</th>
<th>Normotensive non-overweight children (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR (mg/g)</td>
<td>6.45 (3.62 – 10.51)</td>
<td>5.22 (3.45 – 9.11)</td>
<td>4.77 (3.36 – 8.31)</td>
</tr>
<tr>
<td>Microalbuminuria (&gt;30 mg/g)</td>
<td>4 (10.5%)</td>
<td>3 (3.6%)</td>
<td>6 (10.7%)</td>
</tr>
<tr>
<td>NGAL/cr (ng/mg)</td>
<td>7.10 (4.52 – 18.62)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.13 (3.49 – 19.46)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.43 (2.41 – 10.00)</td>
</tr>
</tbody>
</table>

Values are shown in median (interquartile range), or n (%).

ACR=albumin/creatinine ratio, cr=creatinine. For a conversion from mg/g of creatinine to mg/mmol creatinine, divide by 8.84.

<sup>a</sup> significant difference between hypertensive overweight and normotensive non-overweight children (<i>p</i>&lt;0.05), <sup>b</sup> significant difference between normotensive overweight and non-overweight children (<i>p</i>&lt;0.05).

Girls had significantly higher urinary NGAL/creatinine levels than boys: 15.54 ng/mg (6.32–27.03) versus 3.18 ng/mg (2.28–5.38) (median, interquartile range), <i>θ</i> 1.78, CI 1.60–1.99, <i>p</i>&lt;0.001. Age and BMI were no confounders in this association (Table 3).

There was no significant association between NGAL/creatinine levels and age (<i>p</i>=0.106), or ethnicity (<i>p</i>=0.121) (data not shown).
Table 3 Difference in NGAL/creatinine levels between overweight and non-overweight girls and boys.

<table>
<thead>
<tr>
<th></th>
<th>Girls (n=84)</th>
<th></th>
<th>Boys (n=94)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overweight</td>
<td>Non-overweight</td>
<td>Overweight</td>
<td>Non-overweight</td>
</tr>
<tr>
<td></td>
<td>(n=66)</td>
<td>(n=18)</td>
<td>(n=58)</td>
<td>(n=38)</td>
</tr>
<tr>
<td>NGAL/cr (ng/mg)</td>
<td>16.14</td>
<td>13.33</td>
<td>4.18</td>
<td>3.31</td>
</tr>
<tr>
<td></td>
<td>(6.87–29.05)</td>
<td>(4.72–21.29)</td>
<td>(2.50–5.64)</td>
<td>(2.26–5.38)</td>
</tr>
</tbody>
</table>

Values are shown in median (interquartile range). cr=creatinine.

Discussion

This cross-sectional study showed no significant difference in urinary NGAL/creatinine values between hypertensive overweight children, overweight children with normal blood pressure, or non-overweight children with normal blood pressures. In addition, no significant difference in urinary albumin/creatinine ratio between the three groups was found. There was no significant association between NGAL/creatinine levels and the presence of microalbuminuria. Remarkably, there was a significant difference in NGAL/creatinine values between boys and girls.

Early detection and treatment of kidney injury is important in order to prevent chronic kidney injury, or worse, end-stage renal failure (14). Microalbuminuria serves as an important marker for kidney injury. We found a low prevalence of microalbuminuria (7%), and no difference between hypertensive and non-hypertensive children, or overweight and non-overweight children. Findings from previous studies were inconsistent. Similar to our results, several studies did not find a significant difference in levels of urinary microalbumin excretion between children with hypertension and children without hypertension (30–32), nor between obese and non-obese children (24;33). However, several
other studies did find a significant positive association between microalbuminuria and hypertension (17;34), or microalbuminuria and BMI (35;36). Remarkably, other studies found that urinary albumin levels were higher in normal-weight children than overweight or obese children (37;38). Our results and the inconsistent literature data contribute to the discussion at what age kidney injury in obesity can first be demonstrated, and if so, whether microalbuminuria or NGAL are suitable markers for early detection of kidney injury in these children.

Urinary NGAL has been presented as a promising novel biomarker for kidney injury (14;18;19). However, only three studies examined urinary NGAL levels in children with hypertension and obesity. A study by Blumczynsky et al. found a significant association in children between elevated systolic blood pressure and higher urine NGAL concentrations, however, no association with BMI was found (14). A study by Tomczak et al. found a significant positive correlation between NGAL concentrations and body weight, as well as with systolic and diastolic blood pressure (39). We did not find significant differences in urinary NGAL levels between overweight children with hypertension, overweight children without hypertension, and non-overweight children without hypertension. This is in accordance with a study by Goknar et al. (24).

Remarkably, we found significantly higher urinary NGAL/creatinine levels in girls than boys, independent of age or BMI. To our knowledge only Blumczynsky et al. compared NGAL levels between boys and girls, finding no difference (14). Although the difference was considerable, it is unknown whether this difference is clinically relevant. More studies are needed to determine if this is an incidental finding or a real difference. Several studies measured NGAL in a control group of healthy children. The mean values of
NGAL in these children showed great variance with a range from 5.87 (0.30–33.2) ng/ml (14) and 191.51±101.55 ng/mg creatinine (24).

Strengths and limitations

A major strength of this study is the comparison of three study groups: hypertensive overweight children, overweight children without hypertension, and non-overweight children without hypertension. This provides insight into early markers of kidney injury in obesity-associated hypertension. A limitation of this study is that blood pressure was measured three consecutive times on one occasion only and not at multiple occasions as would be preferred. In addition, no 24-hour ambulatory blood pressure monitoring was performed to confirm the diagnosis of hypertension. Furthermore, only random samples of urine were collected, instead of morning urine or 24-hour urine samples. Another limitation is the cross-sectional study design, since kidney injury most likely develops over time.

Conclusion

There was no significant difference in presence of microalbuminuria nor NGAL between overweight and non-overweight children, nor between children with or without hypertension. Based on the results, we can conclude that in our sample there was no evidence of kidney injury. We cannot conclude on the use of NGAL as marker for chronic kidney injury in hypertensive overweight children. Remarkably, girls had significantly higher NGAL levels than boys, independent of age or BMI.

Longitudinal studies are needed to evaluate early effects of overweight and obesity on the kidneys and the process of development of kidney injury in overweight and obese children with and without hypertension. In addition,
more research should be done on the use of urinary NGAL as an early marker for kidney injury.
Reference List


CHAPTER 6


