ENURESIS SUBTYPES BASED ON NOCTURNAL HYPERCALCIURIA:
A MULTICENTER STUDY

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ABSTRACT

Purpose: Desmopressin may not be effective for nocturnal enuresis associated with polyuria
and hypercalciuria. Nighttime hypercalciuria in an enuretic population from 5 centers and its
correlation with nighttime polyuria were verified.

Materials and Methods: A total of 450 enuretic patients (278 males, 172 females, mean age 9.7
years) were evaluated with 72-hour micturition charts, urinalysis, serum creatinine and osmo-
larity, diurnal and nocturnal electrolytes with fractional Na\(^+\) and K\(^+\) urinary excretion, and
nocturnal (4 a.m.) plasma vasopressin. Creatinine electrolytes and osmolality were measured in
daytime (8 a.m. to 8 p.m.) and nighttime (8 p.m. to 8 a.m.) urine volumes. Patients were divided
into group 1 with nocturnal polyuria and group 2 without nocturnal polyuria. Hypercalciuria was
defined as urinary calcium-to-urinary creatinine ratio greater than 0.21. Statistic evaluation was
performed using chi-square, Pearson correlation and ANOVA tests.

Results: Nighttime polyuria was demonstrated in 292 bedwetters (65% group 1). Nocturnal
hypercalciuria was present in 179 of the 450 children (39.7%), including 125 in group 1 (42.8%)
and 54 in group 2 (34.2%), which was statistically significant (chi-square p = 0.008, Pearson
correlation test r = 0.157). Daytime calciuria was not statistically modified in either group (group
1 p = 0.054, group 2 p = 0.56). Adrenocorticotropic hormone (ADH) was normal in 18.5% and low
in 81.5% of enuretics with nocturnal hypercalciuria. ADH levels and nocturnal hypercalciuria
significantly correlated (p = 0.003, r = 0.148). Conversely, the group 2 patients had normal ADH
levels.

Conclusions: Nocturnal hypercalciuria has a pivotal role in nocturnal enuresis, as it is significantly
associated with low ADH levels and nocturnal polyuria. A new classification of nocturnal enuresis
subtypes based on nighttime calciuria levels is mandatory to address treatment properly.

Key Words: enuresis, polyuria, vasopressin, urinary incontinence

Nocturnal enuresis (NE) is characterized by urine loss
during nighttime sleeping by children or adolescents of the
age at which bladder control is supposed to be present. Re-
duced functional bladder capacity and lower nocturnal vaso-
pressin level have been indicated as 2 major causes of NE.\(^1,2\)
Therefore, desmopressin and/or anticholinergic drugs are
commonly used for NE but their efficacy rate is estimated at
60% to 75%.\(^3,4\)

Kutzenetsova et al suggested decreased reabsorption of osmoti-
cally active solute has a main role in inducing NE.\(^5\) Desmo-
pressin has been recently demonstrated to be poorly effective in
enuretic bedwetters with nocturnal hypercalciuria.\(^6\) Valenti et
al suggested that NE could be caused by absorptive hypercal-
iciuria correlated with increased nighttime urine output.\(^7\) We
evaluate a possible correlation of NE with nocturnal hypercal-
iciuria associated with nocturnal polyuria, and recommend a
new classification of NE based on nocturnal hypercalciuria and
polyuria to address treatment accuracy.

MATERIALS AND METHODS

At 5 pediatric centers 278 boys and 172 girls, 6 to 15 years
old (mean age 9.7 years), with primary NE without daytime
symptoms of urgency, frequency and urge incontinence, were
evaluated. The frequency of nocturnal bedwetting was 4
nights a week or greater and 1 to 2 episodes a night. Study
exclusion criteria were history of heart disease, endocrinop-
athy, psychiatric problems, urinary tract abnormalities and
neuropathic bladder dysfunction. Detailed medical history,
72-hour micturition chart and physical examination were
performed, as well as urinalysis and culture. The evaluation
was completed by serum electrolytes (Na\(^+\), K\(^+\) and Ca\(^+\))
, creatinine and osmolality, nocturnal (4 a.m.) plasma vaso-
pressin, and kidney and bladder ultrasound, uroflowmetry
and, if requested, cystometry.

Diurnal (8 a.m. to 8 p.m.) and nocturnal diuresis (8 p.m. to
8 a.m.) were separately collected. Creatinine, electrolytes (Na\(^+\),
K\(^+\) and Ca\(^+\)) and osmolality were measured in daytime

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(8 a.m. to 8 p.m.) and nighttime urine volumes. The Na\(^+\) and K\(^+\) excretion fraction and the calcituria-to-creatinine ratio were calculated in daytime and nighttime urine volumes.

Hypercalciuria was defined as urinary calcium-to-creatinine ratio greater than 0.21. Nocturnal polyuria was defined as daytime/nighttime urine output ratio less than 1. Adrenocorticotropic Hormone normal values were considered greater than 3.5 pg/ml. Urine volumes during nighttime sleep were collected using bags to avoid urethral catheterization.

Statistical evaluation was performed with SPSS 10.1 version for Windows and the results are expressed as percent value or mean ± standard deviation (SD). Analysis of variance (ANOVA) and chi-square tests were used. Correlations were analyzed by Pearson’s test. For all statistical tests 2-tailed \(p < 0.05\) was considered statistically significant.

### RESULTS

Bedwetting children were divided into 2 groups based on nocturnal polyuria. Group 1, presenting with daytime-to-nighttime diuresis ratio less than 1, included 292 patients with nocturnal urine overproduction classified as nocturnal polyuria (65%). Group 2 consisted of 158 patients with daytime-to-nighttime diuresis ratio greater than 1 defined as nonnocturnal polyuria (35%). The 24-hour urine output was not significantly different between the 2 groups, while daytime and nighttime diuresis were inversely correlated and statistically different between groups 1 and 2 (\(p = 0.000\), table 1).

Nocturnal hypercalciuria was present in 179 patients (39.8%). Of the 292 group 1 children nocturnal hypercalciuria was high in 125 (42.8%) and normal in 167 (57.2%). Of the 158 group 2 patients 54 (34.2%) had high levels of nocturnal calcituria and 104 (65.8%) had normal nighttime calcituria. The difference in nighttime hypercalciuria between the 2 groups was statistically significant (\(p = 0.006\)), while daytime calcituria was not statistically modified. The mean values of diurnal and nocturnal calcituria-to-creatininuria ratio in both groups are shown in table 2. Furthermore, we found in all of the study children a strong positive correlation of nocturnal calcituria-to-creatinine ratio versus nighttime diuresis per 24-hour diuresis (\(p = 0.008\), \(r = 0.157\)) versus nighttime diuresis (\(p = 0.003\), \(r = 0.148\)). Daytime calcituria did not significantly correlate with diuresis.

Urine osmolarity did not indicate any statistical difference between the 2 groups when comparing hypercalciuria versus normocalciuria (table 3). A significant difference was noted when considering nocturnal polyuria (\(p = 0.049\) group 1). Nocturnal values of ADH were statistically different between the 2 groups (group 1 1.02 ± 1.10 pg/ml, group 2 3.53 ± 1.50 pg/ml, \(p = 0.000\)). The ADH values related to nocturnal hypercalciuria as 81.5% of group 1 and 70.6% of group 2 patients had low nocturnal ADH values, which was statistically significant (\(p = 0.026\)).

### DISCUSSION

Prevalence of NE ranges from 7% to 20% in healthy children until the age of 10 years but nocturnal bedwetting is still present at puberty or adolescence in 0.8 to 3.0% of children according to several epidemiological studies.\(^{1,8-10}\) Nighttime bedwetting is responsible for a significant decrease in self-esteem in the adolescent.\(^{9,11}\)

The pathogenesis of primary NE is not yet fully defined. In normal subjects nocturnal increase in vasopressin secretion is associated with decreased urine production at night, which commonly results in nocturnal urine volume output of about half that during the day.\(^{2}\) Polyuria has been demonstrated as a major causal factor in nocturnal NE in the last 5 decades\(^{12}\) but its role and pathogenesis have been defined only recently.\(^{13,14}\) Decreased ADH level during the night with loss of its circadian rythym and reduced functional bladder capacity are considered the 2 principal etiological mechanisms responsible for nighttime bedwetting.\(^{2,4}\) Therefore, the recommended pharmacological treatment of primary NE is based on the administration of desmopressin, an ADH analogue, and/or oxybutin in or other anticholinergic drugs.\(^{6,4}\) Results of the pharmacological approach to NE are reported as 65% to 75% successful in large multicenter series\(^{15}\); but little information is available on the pathogenesis and treatment of nonresponding bedwetters.\(^{4,16}\)

Recent data suggest that some enuretic children and adolescents might have a molecular defect on the vasopressin receptor or pathway of the signal transduction, which correlates with receptor stimulation by vasopressin.\(^{7}\) As a result, decreased resorption of water and electrolytes may produce osmotically active tubular outflow, with secondary urine overproduction.\(^{5}\) Several etiological factors are responsible for NE and, thus, different subtypes of this condition might exist. Genetics, arousal defects, nocturnal polyuria exceeding bladder capacity and low nocturnal ADH are more frequently indicated as causal factors.\(^{17,18}\)

We reported recently a strong correlation between increased nocturnal urine outflow and absorptive hypercalciuria in a population of prepubertal bedwetters.\(^{6}\) The role of aquaporins and nocturnal hypercalciuria has also been investigated. Pace et al showed a correlation between enuretic episodes and circadian high hypercalciuria levels using the Pack test and osteocalcin dosage, which demonstrated the absorptive origin of nocturnal hypercalciuria in a cohort of enuretic children.\(^{6}\) A second study showed that ADH may regulate renal water excretion by aquaporin (AQP) 2 and 3, acting in 2 separate pathways of short-term and long-term regulation.\(^{7}\) In enuretic children daytime/nighttime AQP2 was higher than normal even in presence of normal vasopressin levels. Moreover, the authors underlined the correlation between hypercalciuria and AQP2 excretion.\(^{7}\)

Our results indicated no difference in 24-hour diuresis between patients with and without nocturnal polyuria. Furthermore, we showed that nocturnal urine calcium-to-creatinine ratio positively correlated with nocturnal urine output in our population of enuretic children. We also found

### Table 3. Nighttime urine osmolarity in groups 1 and 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Osmolarity ± SD (mOsm/l)</th>
<th>p Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>855.35 ± 212.25</td>
<td>Not significant</td>
</tr>
<tr>
<td>Hypercalciuria</td>
<td>818.85 ± 246.83</td>
<td>0.049</td>
</tr>
<tr>
<td>Normocalciuria</td>
<td>892.33 ± 263.84</td>
<td>Not significant</td>
</tr>
<tr>
<td>Group 2</td>
<td>860.33 ± 290.48</td>
<td>Not significant</td>
</tr>
<tr>
<td>Hypercalciuria</td>
<td>888.29 ± 218.86</td>
<td>Not significant</td>
</tr>
<tr>
<td>Normocalciuria</td>
<td>850.85 ± 321.35</td>
<td>Not significant</td>
</tr>
</tbody>
</table>
that nocturnal urine calcium-to-creatinine ratio was significantly higher in patients with nocturnal polyuria than in the other children, while during the day this ratio was not significantly different. In addition, we noted a higher incidence of nocturnal hypercalciuria in children with than in those without nocturnal polyuria (42.8% vs 34.2%). Therefore, our results indicate that nocturnal hypercalciuria acts as a frequent pathogenetic factor and seems to be responsible for the severity of NE in a large number of children.

Higher calcium levels in nocturnal urine may cause an increase in nocturnal diuresis. It is noteworthy that daytime diuresis did not increase in our patients but nighttime urine output did significantly. This mechanism is confirmed by the statistical evidence that in both groups calciuria was higher at night than during the day. Recently, Neveus et al performed a study on 28 enuretic children who were (diuresis dependent enuresis) and 15 who were not (nondiuresis dependent enuresis) responsive to desmopressin therapy.\(^\text{19}\) No difference was found concerning urine osmolality, vasopressin and calcium urinary excretion (calcium-to-creatinine ratio) between the 2 groups and a group of 51 dry controls. The desmopressin responders had larger urine production. The authors concluded that all enuretics with nocturnal polyuria do not present with vasopressin deficiency and urinary calcium excretion does not differ between enuretic and daytime wetting children. We must note that in the study of Neveus et al urine output, calcium and urinary creatinine were not differentiated between daytime and nighttime, as was done in our study, and daytime and nighttime calciuria probably would have differed significantly in the nocturnal polyuric bedwetting population.

In enuretics with nocturnal polyuria nocturnal hypercalciuria correlated with lower levels of nocturnal urine osmolality, while there was no difference in the nonpolyuric nocturnal population. Moreover, 81.5% of our group 1 children had pathological levels of ADH versus 70.6% in group 2. We conclude that nocturnal vasopressin deficiency can be the single pathogenetic factor responsible for enuresis but this condition is worsened by the presence of hypercalciuria. Low nocturnal ADH increases diuresis by decreased renal reabsorption of water and electrolytes (especially sodium and calcium), and the biochemical mechanism seems to be mediated by AQP2 and AQP3.\(^\text{20}\)

Our results demonstrate that NE might depend on decreased nocturnal AQP2 caused by low ADH levels stimulated by nocturnal hypercalciuria. Accordingly, children with nocturnal polyuria have lower ADH levels than those without nocturnal polyuria.\(^\text{7}\) Comparing ADH secretion and nocturnal hypercalciuria, we found that nocturnal polyuria, hypercalciuria and low vasopressin are likely to be related among themselves as a unique pathophysiological mechanism in the etiology of NE.

Our data show a different spectrum of severity of NE in patients with nocturnal hypercalciuria. In these children osmolality is lower than that in others with an increase in nocturnal urine volume (nocturnal polyuria) but without an increase in 24-hour diuresis. The nighttime polyuric and hypercalciuric bedwetter group seemed to respond poorly to single treatment with desmopressin. Modified dietary regimens, especially at dinner, with low sodium and calcium content, and a decrease in absorptive nocturnal hypercalciuria seem to be paramount to achieve results in treating nocturnal bedwetting.\(^\text{20}\)

**CONCLUSIONS**

Nocturnal hypercalciuria has a pivotal role in primary NE. High levels of overnight calciuria are significantly associated with low nocturnal ADH and polyuria during the night. Absorptive hypercalciuria alone could be responsible for NE if high urinary calcium levels are associated with increased nocturnal diuresis in enuretic patients who often do not respond to desmopressin.

Our multicenter study results indicate that urinary calcium-to-creatinine ratio, ADH levels and polyuria are the 3 parameters to consider in the nocturnal metabolic balance of any enuretic child. Nocturnal diuresis and nocturnal calciuria should be evaluated in the diagnostic approach to NE, as correct identification of these factors allows for more accurate and cause specific treatment. Our proposed new classification of NE subtypes, based on nighttime polyuria and calciuria levels is shown in the figure.

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