THE JOURNAL OF UROLOGY®

Printed in U.S.A.
DOI: 10.1097/01.ju.0000091221.55082.17

Enuresis/Incontinence

ENURESIS SUBTYPES BASED ON NOCTURNAL HYPERCALCIURIA: A MULTICENTER STUDY

GABRIELLA ACETO, ROSA PENZA, MARIA SUSANNA COCCIOLI, FABRIZIO PALUMBO, LORENZO CRESTA, MARCELLO CIMADOR, MARIA LAURA CHIOZZA AND PAOLO CAIONE*

From the Departments Biomedicina Età Evolutiva, (GA, RP, MSC), and Nefrologia e trapianti d'organo (FP), University, Bari, Department Pediatria (LC), Ist. G. Gaslini, Genova, Department Chirurgia Pediatrica (MC), E. Albanese Hospital, Palermo, Department Pediatria (MLC), University, Padova and Department Urologia Pediatrica (PC), "Bambino Gesù" Children's Hospital, Rome, Italy

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 osmolarity, diurnal and nocturnal electrolytes with fractional Na^+ and K^+ urinary excretion, and nocturnal (4 a.m.) plasma vasopressin. Creatinine electrolytes and osmolarity 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. Reduced functional bladder capacity and lower nocturnal vasopressin 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]

Kutznetsova et al suggested decreased reabsorption of osmotically active solute has a main role in inducing NE.[5] Desmopressin 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 hypercalciuria correlated with increased nighttime urine output.[7] We evaluate a possible correlation of NE with nocturnal hypercalciuria associated with nocturnal polyuria, and recommend a new classification of NE based on nocturnal hypercalciuria and polyuria to address treatment accuracy.

Supported by Club Italiano Enuresi Notturna.

* Corresponding author: Department of Pediatric Urology, "Bambino Gesù" Children's Hospital, Piazza S. Onofrio, 4 - 00165 Rome, Italy (telephone: +39-06-68592337, FAX: +39-06-68592430, e-mail: caione@opbg.net).

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, endocrinopathy, 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 osmolarity, nocturnal (4 a.m.) plasma vasopressin, 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 osmolarity were measured in daytime

TABLE 1. *Daytime and nighttime urine output*

	Mean ± SD		p Value (ANOVA)
	Group 1	Group 2	
Diuresis/24 hrs (ml)	919.17 ± 309.75	971.27 ± 339.36	Not significant
Daytime diuresis (ml/12 hrs)	401.15 ± 134.72	642.33 ± 305.01	0.000
Nighttime diuresis (ml/12 hrs)	518.02 ± 193.80	346.93 ± 131.47	0.000

TABLE 2. *Mean values of calciuria-to-urinary creatinine ratio (normal value less than 0.21) in groups 1 and 2*

Calciuria-to-Urinary Creatinine Ratio	Mean ± SD		p Value (ANOVA)
	Group 1	Group 2	
Daytime	0.13 ± 0.10	0.11 ± 0.07	Not significant
Nighttime	0.22 ± 0.15	0.18 ± 0.14	0.006

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

Hypercalciuria was defined as urinary calcium-to-urinary 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 was 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 calciuria 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 calciuria and 104 (65.8%) had normal nighttime calciuria. The difference in nighttime hypercalciuria between the 2 groups was statistically significant ($p = 0.006$), while daytime calciuria was not statistically modified. The mean values of diurnal and nocturnal calciuria-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 calciuria-to-urinary creatinine versus nighttime diuresis per 24-hour diuresis ($p = 0.008$, $r = 0.157$) versus nighttime diuresis ($p = 0.003$, $r = 0.148$). Daytime calciuria 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 oxybutinin or other anticholinergic drugs.[3,4] Results of the pharmacological approach to NE are reported as 65% to 75% successful in large multicenter series[3,15] but little information is available on the pathogenesis and treatment of nonresponding bedwetters.[4,16]

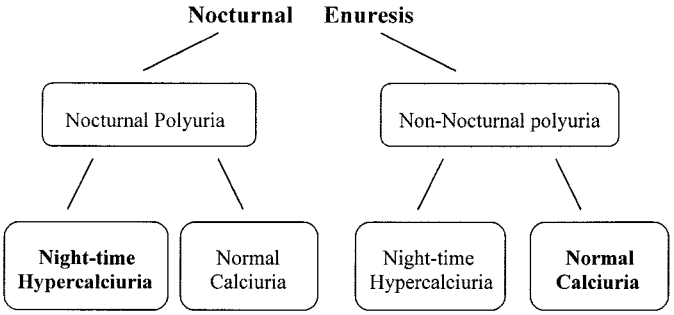
Recent data suggest that some enuretic children and adolescents might have a molecular defect on the vasopressin receptor or pathway of the signal transduction, which correlates with receptor stimulation by vasopressin.[7] As a result, decreased resorption of water and electrolytes may produce osmotically 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 osteocalcine 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*

	Mean Osmolarity ± SD (mOsm/l)	p Value (ANOVA)
Group 1:	855.35 ± 212.25	
Hypercalciuric	818.85 ± 246.83	
Normocalciuric	882.33 ± 263.84	0.049
Group 2:	860.33 ± 298.48	
Hypercalciuric	888.29 ± 218.86	
Normocalciuric	850.85 ± 321.35	Not significant
Group 1 vs 2		Not significant



Enuresis subtypes based on nocturnal hypercalciuria

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.[19] No difference was found concerning urine osmolarity, 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, calciuria 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 bedwetter population.

In enuretics with nocturnal polyuria nocturnal hypercalciuria correlated with lower levels of nocturnal urine osmolarity, 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.

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.[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 osmolarity 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.[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.

REFERENCES

1. Mark, S. D. and Frank, J. D.: Nocturnal enuresis. Br J Urol, **75:** 427, 1995
2. Ritting, S., Shaumburg, H., Schmidt, F., Hunsballe, J. M., Hansen, A. F. et al: Long-term home studies of water balance in patients with nocturnal enuresis. J Urol Nephrol, suppl., **183:** 25, 1997
3. Swedish Enuresis Trial group (SWEET): Long term treatment with desmopressin in children with primary monosymptomatic nocturnal enuresis: an open multicentre study. Br J Urol, **82:** 704, 1998
4. Koff, S. A.: Why is desmopressin sometimes ineffective at curing bedwetting? Scand J Urol Nephrol, suppl., **173:** 103, 1995
5. Kutznetsova, A. A., Natochin, Y. V. and Papayan, A. V.: Osmoregulatory function of kidney in enuretic children. Scand J Urol Nephrol, **32:** 132, 1998
6. Pace, G., Aceto, G., Cormio, L., Traficante, A., Tempesta, A., Lospalluti, M. L. et al: Nocturnal enuresis can be caused by absorptive hypercalciuria. Scand J Urol Nephrol, suppl., **33:** 111, 1999
7. Valenti, G., Laera, A., Pace, G., Aceto, G., Lospalluti, M. L., Penza, R. et al: Urinary aquaporine 2 and calciuria correlate with severity of enuresis in children. J Am Soc Nephrol, **11:** 1873, 2000
8. Alon, U. S.: Nocturnal enuresis. Pediatr Nephrol, **9:** 94, 1995
9. Nappo, S., Del Gado, R., Chiozza, M. L., Biraghi, M., Ferrara, P. and Caione, P.: Nocturnal enuresis in the adolescent: a neglected problem. BJU Int, **90:** 912, 2002
10. Lackgren, G., Neveus, T. and Senberg, A.: Diurnal plasma vasopressin and urinary output in adolescents with monosymptomatic nocturnal enuresis. Acta Paediatr, **86:** 385, 1997
11. Chiozza, M. L., Bernardinelli, L., Caione, P., Del Gado, R., Ferrara, P., Giorgi, P. C. et al: An Italian epidemiological Multicentre Study on nocturnal enuresis. Br J Urol, suppl., **81:** 86, 1998
12. Puolton, M. E.: Relative nocturnal polyuria as a factor in enuresis. Lancet, **2:** 906, 1952
13. Norgaard, J. P., Pedersen, E. B. and Djurhuus, J. C.: Diurnal antidiuretic hormone levels in enuretics. J Urol, **134:** 1029, 1985
14. Hunsballe, J. M., Hansen, T. K., Rittig, S., Norgaard, J. P., Pedersen, E. B. and Djurhuus, J. C.: Polyuric and non-polyuric bedwetting—pathogenic differences in nocturnal enuresis. Scand J Urol Nephrol, suppl., **173:** 77, 1995
15. Lackgren, G., Hyalmas, K., Van Gool, J., von Gontard, A., de Gennaro, M., Lottmann, H. et al: Nocturnal enuresis: a suggestion for a European treatment strategy. Acta Paediatr, **88:** 679, 1999
16. Moffatt, M. E. K., Harlos, S., Kirshen, A. J. and Burd, L.: Des-

mopressin acetate and nocturnal enuresis: how much do we know? Pediatrics, **92:** 420, 1993
17. Norgaard, J. P., Jonler, M. and Rittig, S.: Pharmacodynamic study of desmopressin in patients with nocturnal enuresis. J Urol, **153:** 1984, 1995
18. Watanabe, H. and Azuma, Y.: A proposal for a classification system of enuresis based on overnight simultaneous monitoring of electroencephalography and cystometry. Sleep, **12:** 257, 1989
19. Neveus, T., Hansell, P. and Stenberg, A.: Vasopressin and hypercalciuria in enuresis: a reappraisal. BJU Int, **90:** 725, 2002
20. Valenti, G., Laera, A., Gouraud, S., Pace, G., Aceto, G., Penza, R. et al: Low-calcium diet in hypercalciuria enuretic children restores AQP2 excretion and improves clinical symptoms. Am J Physiol Renal Physiol, **283:** F895, 2002