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Indian J Nephrol. 2012 Mar-Apr; 22(2): 88–93. PMCID: PMC3391829
doi: 10.4103/0971-4065.97110

Is there any correlation between hypercalciuria and nocturnal enuresis?

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Abstract

Nocturnal enuresis is a common problem among children. Hypercalciuria has been proposed as an important etiology of bedwetting. We investigated the incidence of hypercalciuria among children with nocturnal enuresis and age- and sex-matched healthy controls. In this case–control study 118 children with nocturnal enuresis and 100 age-, sex-, and educational district-matched healthy controls were recruited. Urine samples were obtained from each subject twice: immediately after awakening and 2 hours later at school. Urinary calcium and creatinine levels were measured and the subjects with a urinary calcium/creatinine ratio more than 0.2 were considered as hypercalciuric. Serum Ca, P, Na, K, and PTH levels were measured for all hypercalciuric subjects. The mean urine calcium to creatinine ratio in the second urine sample was 0.070±0.06 mg/mg and 0.050±0.046, respectively in the case and control groups (P=0.008). There were 12/118 and 3/100 hypercalciuric subjects in the case and control groups respectively (P=0.032). The serum Ca, P, Na, K, and PTH levels were in normal range in all hypercalciuric subjects. In our study there was a significant difference in the frequency of hypercalciuria among children with nocturnal enuresis and healthy controls, so we can suggest adding the measurement of urine electrolytes especially the calcium level in patients with nocturnal enuresis.

Keywords: Children, hypercalciuria, nocturnal enuresis

Introduction

According to the recent terminology of lower urinary tract function in children, enuresis (which is also called nocturnal enuresis to add extra clarity) is defined as intermittent incontinence while sleeping regardless of the presence or absence of concomitant daytime symptoms.[1] Nocturnal enuresis is a multifactorial disease. Hypercalciuria has been considered as an important pathogenic factor of nocturnal enuresis. This idea was first proposed by Pace *et al*.,[2] who noted that a proportion of enuretic children had absorptive hypercalciuria. Since that time some strategies were made to measure urinary calcium excretion in the evaluation of nocturnal enuresis.[3,4]

Other pathogenic factors that have been suggested are nocturnal desmopressin deficiency,[3] disorder of circadian rhythm of renal functions (sodium and osmotic excretion),[5] dysregulation of prostoglandins excretion, etc. Treatment with desmopressin is effective only in 30–60%[6] which demonstrates that other pathogenic factors may play a role in development of nocturnal enuresis. Several Italian studies[4,7–9] demonstrated a strong association between desmopressin resistance and hypercalciuria, with desmopressin responsiveness increasing when a calcium restricted diet was implemented.

Conflicting evidence has been published about the possible relationship between hypercalciuria and enuresis. Neveus *et al*. in a study concluded that the urinary calcium excretion does not differ between enuretic and dry children.[3] Kamperis *et al*. in another study observed no significant difference among calcium excretion of children with or without nocturnal enuresis.[10] Although there are some reports about the role of hypercalciuria in polysymptomatic enuresis, but it is insufficient in monosymtomatic enuresis.

Considering the controversy surrounding the role of hypercalciuria in the pathogenesis of nocturnal enuresis, we investigated the incidence of hypercalciuria (calcium/creatinine ratio greater than 0.20 mg/mg) among children with monosymptomatic nocturnal enuresis and age- and sex-matched controls.

Materials and Methods

This case–control study was approved by the University scientific and ethical review boards. The research team conducted cluster sampling in elementary schools in five different educational districts.

Considering the prevalence rate of 8% and 20% for hypercalciuria, respectively, in healthy and enuretic children, with a statistical power of 0.8 and statistical significance level of 0.05, calculation of sample size indicated that a minimum sample size of ≈ 100 enuretic and 100 controls would be adequate to investigate any probable correlation between hypercalciuria and nocturnal enuresis.

Nocturnal enuresis was defined as the involuntary loss of urine that occurs only at night and without a history of bladder dysfunction is defined as monosymptomatic enuresis (MSE).[11] Bedwetting several nights a week is less likely to resolve without intervention,[12] so we selected patients with MSE with bedwetting 3 times a week.

By an announcement, parents of 1800 school students were asked to inform the study group if their child wet the bed at least three times per week by filling a checklist. Checklists were sent back by 1610 parents and the cooperation rate was 89.4%. One hundred thirty parents claimed their children wet their beds.

Our exclusion criteria were the presence of vesico-ureteral reflux, history of urinary tract infection during the last month, nutrition with ketogenic diet, any treatment with corticosteroids, or diuretics in the last month, or high-dose vitamin D in the last 6 months (medications like drops, syrup, or ampul), major fracture in lower extremities or bed rest for a long time, symptoms (including urgency, frequency, dysuria) or abdominal pain, untreated constipation (to avoid coincidence of above-mentioned urinary symptoms), height and body weight outside normal reference frame (less than 5^{th} and greater than 95^{th} percentile), usually wearing tights pants (this may predispose children to develop urinary symptoms or urinary tract infection), or children who did not undergo circumcision. The presence of exclusion criterion was determined by interviewing the parents of the subjects, and no other clinical investigation was done in this regard. None of our participants received desmopressin or used any alarm system for the treatment of enuresis. They were screened via a questionnaire and none of them had a history of seeking any clinical consultation for the complaint of nocturnal enuresis.

None of the subjects had day-time incontinence. All subjects were investigated by urine analysis and urine culture. Six subjects were excluded because of urinary tract infection. All remaining 124 subjects were asked to collect two urine samples one at home in the morning and the other at school. We asked the parents to deliver the urine samples to the laboratory of Motaharri hospital to be investigated for their calcium and creatinine level. Six subjects were refused to continue their participation. Finally 118 subjects with nocturnal enuresis were enrolled. One hundred age- and sex-matched healthy children without any history of nocturnal enuresis were recruited as the control group from the same educational district from which the case group was selected in order to prevent probable diversity in socioeconomic status.

Hypercalciuria was defined as urinary calcium/creatinine ratios higher than 0.2 mg/mg.[13,14] All laboratory studies were performed by only one laboratory technician who was expert in the investigation of urine calcium level using the standard Hitachi 9/2 autoanalyzer device. For investigating urine creatinine level according to the Jaffe method, first we diluted the urine sample 50 times, and then evaluated it by the autoanalyzer device and the number was multiplied in 50 to yield the urine random creatinine level. All laboratory investigations were performed during the maximum time of 3 hours after urine collection.

After determining the urine calcium/creatinine ratio for subjects in both case and control groups, subjects with hypercalciuria were investigated for the etiology of their hypercalciuria via blood samples including measuring alkaline phosphatase, phosphorus,

calcium, potassium, sodium, blood urea nitrogen, serum creatinine, and parathyroid hormone (PTH) (using the chemoluminescence method). The laboratory technician was blinded regarding the cases and controls.

Informed consent was obtained from all participants and their parents, but limited data regarding the details of urine analysis were provided for parents in order to prevent their misunderstanding about possible consequences of high calcium (milk) intake for children. Collected data were analyzed by independent and paired t-, chi-square, and Pearson correlation tests using SPSS software ver. 16.

Results

The information regarding the age and sex distribution, and mothers' employment and educational status in both case and control groups is depicted in Table 1.

The urine samples were obtained from each subject on the two above-mentioned occasions. The mean urine calcium levels in the morning sample were 7.58±5.01 and 5.85±3.49 mg/dl respectively in the case and control groups. The mean urine creatinine level in the morning urine sample was 114.2±52.4 and 117.7±54.7 mg/dl respectively in the case and control groups. The mean urine calcium level in the second sample was 5.14±4.11 and 3.89±3.03 mg/dl respectively, in the case and control groups. The mean urine creatinine level in the second sample was 84±37.7 and 91.1±36.8 mg/dl respectively, in the case and control groups.

The mean urine calcium to creatinine ratio in the morning urine sample was 0.077±0.056 and 0.061±0.057 mg/mg respectively, in the case and control groups (P=0.047). The mean urine calcium to creatinine ratio in the second urine sample was 0.070±0.06 and 0.050±0.046 mg/mg respectively in the case and control groups (P=0.008). By considering a urine calcium to creatinine ratio higher than 0.2 in the second urine sample as hypercalciuria, there were 12/118 in the case group and 3/100 in the control group. Chi-square analysis revealed a significant difference in the frequency of hypercalciuria among children with and without nocturnal enuresis (P=0.032). The absolute frequency of hypercalciuria based on the 8:00 am samples was 3 in the case and 1 in the control group (P=0.562), applying a 0.2 mg/mg cutoff point for determining hypercalciuria cases. The frequency of hypercalciuria is much higher in the second samples (10:00 am) compared to the first samples in the morning (8:00 am).

Applying a paired t-test in order to compare the urine calcium to creatinine ratio between two sampling occasions (after wakening and at school) suggested a significant difference (0.069±0.057 in the morning vs. 0.061±0.055 in second sample; P=0.025). However the same analysis for each subject group revealed no significant difference among the Ca/Cr ratio of two urine samples (in the morning and at school) of each subject. It yields a *P value* of 0.153 and 0.087, respectively, in the case and control groups.

Five of 55 girls and 10 of 163 boys were documented to have hypercalciuria, but there was no correlation seen between sex and hypercalciuria. Such correlation was not seen in each group, too.

Six of 151 subjects (3.9%) whose mothers were homemakers, and 9 of 67 subjects (13.4%) whose mothers were employed outside the house, demonstrated to have hypercalciuria. Pearson correlation analysis revealed a significant correlation among hypercalciuria and mothers' employment status (P=0.015).

Considering mothers' educational status, among children of 130 mothers with diploma or lower educational degree, 12 (9.2%) demonstrated to have hypercalciuria, whereas only 2 cases of 35 mothers (5.7%) with diploma from college (Fogh-e diploma), and 1 of 53 mothers with a bachelor or higher degree, demonstrated to have a child with hypercalciuria. However there was no significant correlation between mothers' educational status and hypercalciuria (P=0.196), even with categorizing mothers' educational level into two groups of primary and higher education (P=0.078).

All 15 hypercalciuric subjects (12 in case and 3 in control groups) were tested for their serum BUN, Cr, Na, K, Ca, P, alkaline phosphatase and parathyroid hormone levels, and all laboratory tests were in normal range [Table 2].

Discussion

Nocturnal enuresis is a common problem in children.[15] Recently hypercalciuria has been considered to be important in the pathogenesis of enuresis.[2,16,17] Some other studies claimed that urinary calcium excretion does not differ among children with and without enuresis.[3]

In 2006, extensive discussions were held by the board of the International Children's Continence Society (ICCS), which yielded new definitions and terminology of lower urinary tract function in children and adolescent.[1] Before the consensual definitions, different publications did not differentiate clearly enuresis from other dysfunctional voiding. The discrepancy between studies such as the frequency of the effect of DDAVP or incidence of hypercalciuria could be attributed to this fact.

The current study was performed considering the conflicting evidence about the possible relationship between hypercalciuria and bedwetting. Also these new ICCS definitions could be the aim of the current article to clearly demonstrate the true frequency of hypercalciuria in enuresis.

In this study 1800 questionnaires were distributed among students in five different educational districts. Many studies have a selected population of patients consulting for enuresis in a medical center. Other important recent studies, with the same model (school population), are lacking for comparison.[18–20]

Parental feedback was received in 1600 cases. Among them 130 claimed their child to have nocturnal enuresis. This yields a prevalence rate of 8.12% for bedwetting in the elementary school students' population. This rate is to some extent lower than the rate which was reported by Gur *et al*. from Turkey (12.4%)[15] or Raes *et al*. form Sweden (12%).[17]

The majority of children with enuresis were males. This was similar to findings of Gur *et al*.,[15] but we should not forget the impact of cultural problems (some traditions) while public community is usually antipathic to report the bedwetting of their girl children in Iran.

In our study 12 subjects in the case group (10.1%) had hypercalciuria. The study of Azhir *et al*.[21] in Isfahan, Iran, has reported the prevalence rate of hypercalciuria among children with enuresis to be 9.2% which is almost similar to our results. Another study by Pace *et al*. in Italy demonstrated a lower rate for hypercalciuria (5.17%) among 406 children with nocturnal enuresis.[2]

An important factor resulting in various prevalence rates among two different studies in a similar population is the cutoff point of those studies for the definition of hypercalciuria. For example, Pace *et al*. considered subjects with a urine calcium/creatinine ratio more than 2.1 mg/mg as hypercalciuric,[2] but this cutoff point in our study was 0.2 mg/mg.

All 15 hypercalciuric subjects in our study had normal serum Ca, P, Na, K, and parathyroid hormone (PTH). Thus absorptive or reabsorptive type of hypercalciuria could be ruled out and the diagnosis of idiopathic hypercalciuria (IH) was proposed in all cases. The idiopathic hypercalciuria is the most common type of hypercalciuria, worldwide.

We did not perform any ultrasound-based evaluation for the cases with enuresis to rule out anomalies of the urinary system. However according to the study of Pace *et al*., urinary tract abnormalities were observed only in 7.1% of subjects with enuresis, and the relationship among anatomic anomalies and nocturnal enuresis is rare.[2] But the study of Azhir *et al*. from Iran, reported higher urinary system abnormalities to be 33% in children with nocturnal enuresis (small bladder with a thick wall in 27%, and large bladder capacity with a thin wall in 6% of cases).[21]

In the study of Penido *et al*. in all hypercalciuric subjects with nocturnal enuresis, this problem was resolved by the treatment of hypercalciuria (high water intake, adjusting diet for sodium and protein amount, administration of potassium citrate and thiazide diuretics).[16] In a study by Valenti *et al*.; the effect of a therapeutic intervention in 46 enuretic children has been analyzed and their results indicated that urinary calcium levels modulate aquaporin 2 excretion and is likely to be useful for treatment of children with enuresis.[9] A recent report from Iran[22] indicated that hypercalciuria can negatively affect the responsiveness to desmopressin therapy, so there are several studies which pointed out the therapeutic approach of patients with and without hypercalciuria in children with nocturnal enuresis.

In our study, although all hypercalciuric cases were advised to visit a pediatric nephrologist, but unfortunately the data regarding the implemented actions and the outcome of treatment if done were not available.

Having data about the nutritional parameters (such as calcium intake) could be effective in the interpretation of findings, even though these data can be difficult to evaluate and so were not evaluated in this study. The presence of nocturnal polyuria was not investigated, as well. This may be another flaw for our study.

Some studies used orally calcium loading in order to diagnosis the etiology of hypercalciuria (absorptive or renal), but we did not use this test in our study.

In this study the subtypes of hypercalciuria were not determined among the hypercalciuric patients. There are several reports which indicated that the classic distinction among absorptive, renal, and resorptive hypercalciuria seems insufficient to explain the many cellular and tissue modifications observed in patients with primary hypercalciuria.[23] It is possible that absorptive and renal hypercalciuria may represent a continuum of a single disease. In this regard there are some studies that pointed out that renal and absorptive hypercalciuria may not be distinct entities, as indicated by the lack of increased bone turnover in hypercalciuric children.[24,25] Aladjem *et al*. have demonstrated that children initially diagnosed with having either renal or absorptive hypercalciuria had a different result when tested 3–7 years later.[26] So the authors did not use or even suggest formal assessments for subtypes of hypercalciuria in similar studies.

Considering the controversy which exists about the role of hypercalciuria in the pathogenesis of nocturnal enuresis, the authors suggest a meta-analysis to be implemented in this regard.

Conclusion

In our study there was a significant difference among the frequency of hypercalciuria among children with nocturnal enuresis and healthy controls. So we can suggest adding the measurement of urine electrolytes especially calcium level in the process of looking for the etiologies in a patient referred with nocturnal enuresis. This study confirmed the association between nocturnal enuresis and hypercalciuria, so based on accompanying enuresis and hypercalciuria, its therapeutic approach may need to be changed.

Acknowledgment

The authors would like to thank Urmia University of Medical Sciences for the grants provided for our study.

Footnotes

Source of Support: Urmia University of Medical Sciences

Conflict of Interest: The authors declared that there was no conflict of interests. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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Figures and Tables

Table 1

Subjects' characteristics	Case	Control	*P* value
Age (Mean±SD)	7.61±0.84	7.61±0.86	0.99
Age			0.994
7 years old	70 (59.3)	60 (60)	
8 years old	28 (23.7)	23 (23)	
9 years old	16 (13.6)	13 (13)	
10 years old	4 (3.4)	4 (4)	
Sex (male/female)	88/30	75/25	0.53
Mother's employment status			0.007
Employed	45 (38.1)	22 (22)	
Unemployed	73 (61.9)	78 (78)	
Mother's educational status			0.769
Diploma and lower	72 (61)	58 (58)	
Diploma from college	17 (14.4)	18 (18)	
Bachelor and higher	29 (24.6)	24 (24)	

Figures in parenthesis are in percentage

Subject characteristics in the case and control groups

Table 2

Lab test	Total (Mean±SD) N = 15	Case (Mean±SD) N = 12	Control (Mean±SD) N = 3	*P* value
BUN	14.6±2.44	15.17±2.40	12.33±0.57	0.07
Cr	0.80±0.11	0.82±0.12	0.73±0.05	0.235
Na	137.6±2.36	137.33±2.42	139.00±2.10	0.295
K	4.46±0.47	4.34±0.44	4.96±0.15	0.036
Ca	9.97±0.38	9.90±0.39	10.23±0.20	0.199
P	3.88±0.35	3.85±0.38	4.00±2.00	0.533
Alk-P	598.6±156.0	550.0±137.7	793.3±30.5	0.009
PTH	35.4±12.6	36.92±12.96	29.33±11.06	0.371

SD: Standard deviation; BUN: Blood urea nitrogen; Cr: Serum creatinine; Na: Sodium; K: Potassium; Ca: Serum calcium level; P: Phosphorus; Alk-P: Alkaline phosphatase; PTH: Parathyroid hormone

Laboratory results among subjects with hypercalciuria