Journal of Pediatric Urology (2019) **15**, 393–398

Assessment of serum levels of copeptin and corticotropin-releasing factor in children with monosymptomatic and non-monosymptomatic nocturnal enuresis

İ. Girisgen [a], E. Avcı [b], S. Yüksel [a,*]

[a]Pamukkale University School of Medicine, Department of Pediatric Nephrology, Denizli, Turkey

[b]Pamukkale University School of Medicine, Department of Biochemistry, Denizli, Turkey

* Corresponding author. Pamukkale Universitesi Tip Fakultesi Hastanesi Cocuk Nefroloji BD, Mavi Bina 1.kat Kınıklı, 20070, Pamukkale. Denizli, Turkey. Tel.: +90 258 296 60 00x5410, +90532 664 55 22(mobile); Fax: +90 258 296 60 01.
selcukyuksel.nephrology@gmail.com (S. Yüksel)

Keywords
Nocturnal enuresis; Non-monosymptomatic; Copeptin; Corticotropin-releasing factor

Received 17 January 2019
Accepted 14 May 2019
Available online 6 June 2019

Summary

Background
Nocturnal enuresis is defined as bed-wetting in children from the age of five years that occurs during sleep; if untreated, the condition can result in social and psychological problems both for the children and their parents. Nocturnal enuresis is a complicated disease that includes multiple pathogenetic factors. Nocturnal enuresis is divided into two subgroups: monosymptomatic and non-monosymptomatic. The role of some biomarkers in patients with monosymptomatic enuresis has been reported in a small number of the studies.

Objective
The aim of this research was to evaluate the serum levels of copeptin and corticotropin-releasing factor (CRF) in monosymptomatic and non-monosymptomatic nocturnal enuresis cases. Although these markers were previously examined in children with monosymptomatic enuresis, there is no study that has evaluated these markers in non-monosymptomatic children.

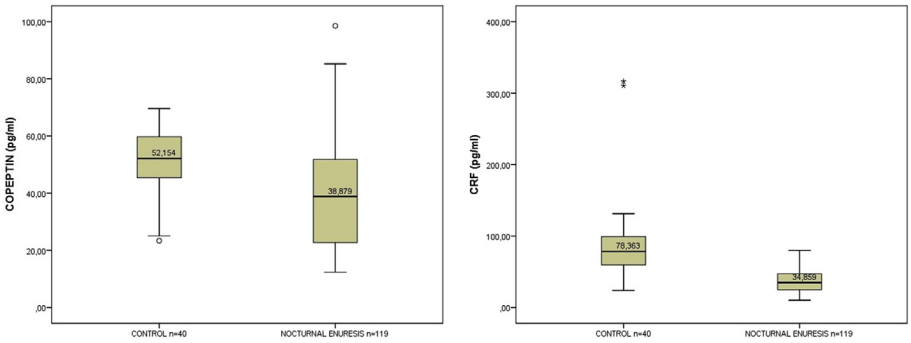
Study design
One hundred nineteen children with nocturnal enuresis (5–16 years) and forty healthy children (5–17 years) were enrolled to the study. Of the nocturnal enuresis group, forty-nine were monosymptomatic and seventy were non-monosymptomatic. Copeptin and CRF were measured by a competitive inhibition method with enzyme-linked immunosorbent assay.

Results
The serum copeptin levels were significantly lower in children with monosymptomatic and non-monosymptomatic nocturnal enuresis than in the controls.(median, 34.7 [interquartile range (IQR): 34 pg/ml], 39.8 [IQR: 29 pg/ml] vs 52.1 [IQR: 14 pg/ml], respectively, $P < 0.05$). The serum CRF levels were significantly lower in children with monosymptomatic and non-monosymptomatic nocturnal enuresis than in the controls (median, 35.1 [IQR: 19 pg/ml], 34.05 [IQR: 24 pg/ml] vs 78.3 [IQR: 39 pg/ml], respectively, $P < 0.05$). There was no significant difference in copeptin and CRF levels between the children with monosymptomatic and non-monosymptomatic nocturnal enuresis.

Discussion
Copeptin is presumed to be a sensitive surrogate biomarker for arginine vasopressin release. To date, there are only two studies in the literature that assess the relationship between copeptin and monosymptomatic enuresis. The only study in the literature demonstrated significantly decreased levels of CRF in monosymptomatic enuretic children. It was demonstrated that the levels of copeptin and CRF differ in both children with monosymptomatic and non-monosymptomatic nocturnal enuresis from the control groups. It was also demonstrated that copeptin and CRF levels were not different between the children in monosymptomatic and non-monosymptomatic groups.

Conclusion
Those changes in both copeptin and CRF which were shown in this study in monosymptomatic and non-monosymptomatic enuretic children may contribute to the pathogenesis of nocturnal enuresis. Further case–control studies can evaluate the copeptin and CRF levels before treatments in monosymptomatic and non-monosymptomatic patients to decide potential effectiveness of treatment.

https://doi.org/10.1016/j.jpurol.2019.05.017



Summary Fig Copeptin and CRF levels (according to median values) in children with nocturnal enuresis and those in the control groups. CRF, corticotropin-releasing factor. Circle and "*" in summary figure are outlier.

Introduction

According to the International Children's Continence Society (ICCS), nocturnal enuresis is defined as bed-wetting that occurs during sleep among children from the age of five years. Such a condition can cause social and psychological problems, for the child and the parents, if it is left untreated [1]. The ICCS classifies nocturnal enuresis as monosymptomatic or non-monosymptomatic. Monosymptomatic nocturnal enuresis (MNE) has no symptoms other than bed-wetting at night during sleep. Non-monosymptomatic enuresis is associated with daytime wetting and daytime symptoms that include urgency, leakage, frequency, and hesitancy. The prevalence of nocturnal enuresis in children between the age of 5 and 15 years was reported to be 10—15% in recent studies [2—4].

Nocturnal enuresis is a complicated disease including multiple pathogenetic factors. The importance of two neurohormones and biomarkers in patients with monosymptomatic enuresis has been reported in a small number of studies [5—7]. The relationship between arginine vasopressin (AVP) as a neurohormone and nocturnal enuresis is well known. Arginine vasopressin is unstable, so determining plasma AVP concentrations is not reliable with many laboratory assays [8]. In addition, some current observations have revealed the importance of two neurohormones in MNE. One of them, copeptin, is a 39—amino acid glycopeptide and a precursor of AVP. Copeptin is also a stable and sensitive marker of AVP release [5—7]. There are only two studies in the literature that have assessed the relationship between copeptin and enuresis [5,7]. Corticotropin-releasing factor (CRF) is another novel marker, and the relationship between enuresis and CRF has been demonstrated in a single human study [6]. The role of CRF in micturition has been demonstrated in a few animal studies in the literature [9,10]. Corticotropin-releasing factor has been shown to play an important role in coordinating micturition and arousal [6].

The aim of this research was to evaluate the serum levels of copeptin and CRF in monosymptomatic and non-monosymptomatic nocturnal enuresis cases. Although these markers were previously examined in children with monosymptomatic enuresis, there is no study that has evaluated these markers in non-monosymptomatic children.

Materials and methods

Patients were selected prospectively between December 2017 and December 2018 and cross-sectionally among patients with nocturnal enuresis who met the study criteria. One hundred nineteen children with nocturnal enuresis and forty healthy children were enrolled into the study. The nocturnal enuresis group was divided into two subgroups: monosymptomatic and non-monosymptomatic nocturnal enuresis. Children with no symptoms other than bed-wetting at night during sleep were taken into the MNE group. The children with daytime wetting and daytime symptoms that include urgency, leakage, frequency, and hesitancy were taken into the non-monosymptomatic nocturnal enuresis group. A bladder diary was used for all patients before classifying. Amount of water drunk (ml) and time, amount of urine, time taken to wet the underwear, and urgency were recorded during daytime.

Primary enuresis is described as mentioned previously in a child who was never 'dry' for longer than six consecutive months. Secondary enuresis exists when the child with bed-wetting was previously dry for more than six consecutive months. The patients' past medical history, in terms of using desmopressin and/or anticholinergic treatment or having urinary tract infections, within the last six months were questioned. None of them have used any medical treatment such as desmopressin and anticholinergic treatment in the last six months.

Physical examination, urine analysis, fasting blood glucose and electrolyte levels, and renal function tests (urea and creatinine; the glomerular filtration rates were calculated using the Schwartz formula) were evaluated in all patients and the controls. The physical and neurologic examinations of all children were normal. Ultrasonographic evaluation was performed in all study groups. Children with secondary nocturnal enuresis, systemic disease, and abnormal neurologic examination findings were excluded from the study. The

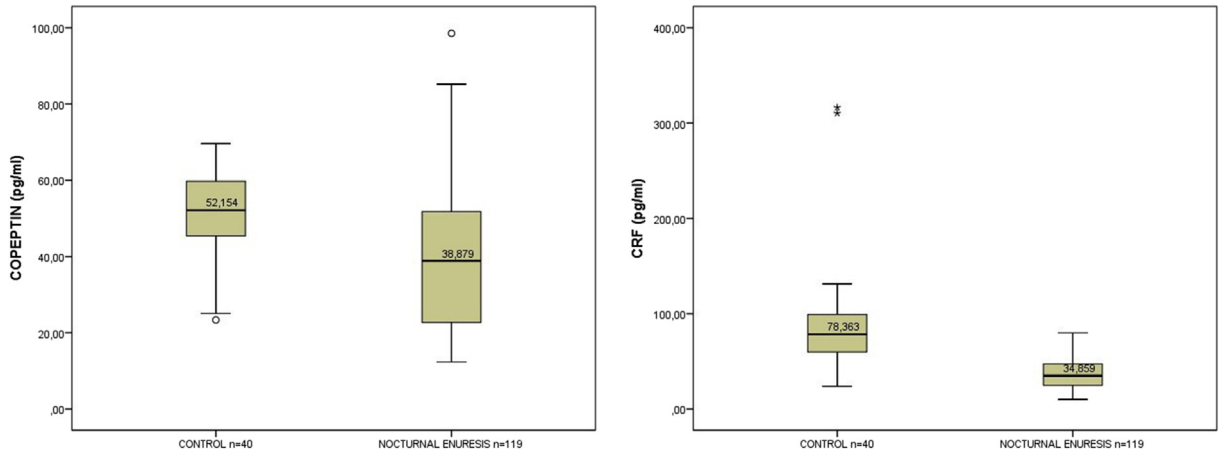


Fig. 1 Copeptin and CRF levels (according to median values) in children with nocturnal enuresis and those in the control groups. CRF, corticotropin-releasing factor. Circle and "*" in figure artwork 1 are outlier.

patients and controls who had abnormal urine and blood analysis and abnormal findings in urinary ultrasonography were excluded.

Biochemical analyses

From each volunteer, 5 ml of blood specimens were drawn from antecubital veins at 8 AM. After 30 min, all samples were centrifuged. Serum specimens were aliquoted to Eppendorf tubes and stored at −80 °C until the analysis.

Copeptin was measured by a competitive inhibition method with enzyme-linked immunosorbent assay (ELISA) for antigen detection by using a USCN (Wuhan USCN Business Co., Ltd., USA) kit. The USCN kit is a commercial ELISA kit. This assay has a lower detection limit of 24.69 pg/L, with intra-assay coefficient of variation (CV) < 10% and interassay CV<12%. All samples were assayed as a batch and analyzed in one run (24.69−2,000 pg/mL linearity).

Corticotropin-releasing factor was measured by a competitive inhibition method with ELISA by using a USCN (Wuhan USCN Business Co., Ltd., USA) kit. This assay has a lower detection limit of 12.35 pg/L; with intra-assay CV< 10% and interassay CV<12%. All samples were assayed as a batch and analyzed in one run (12.35−1.000 pg/mL linearity).

Statistical methods

Continuous variables were expressed as mean ± standard deviation, median, and interquartile range (IQR), and categorical variables, as numbers and percentages. Shapiro−Wilk tests were used for testing normality. If parametric test conditions were satisfied, independent-samples *t*-test was used for comparisons among groups. If parametric test conditions were not satisfied, the Mann−Whitney U Test was used for comparisons among groups. The differences between categorical variables were examined using Chi-squared analysis. Analysis of covariance (ANCOVA) was performed according to age, and the comparisons between groups were repeated. All statistical analyses were analysed using SPSS 24.0, and a *P* value less than 0.05 was considered statistically significant.

Table 1 Descriptive data and comparison between patients with nocturnal enuresis (monosymptomatic and non-monosymptomatic) and controls.

Variables	Patients with nocturnal enuresis (n: 119)	Monosymptomatic (n: 49)	Non-monosymptomatic (n: 70)	Controls (n: 40)	*P*[a]
Age (years)	8.5 ± 2	8.9 ± 2	8.2 ± 2	11.4 ± 3	0.001
Median	8 (5−16)	9 (5−15)	8 (5−16)	11.5 (5−17)	
Sex (m/f)	40/79	22/27	18/52	16/24	0.075
Copeptin levels					
Mean ± SD	40.2 ± 20	41.4 ± 21	39.3 ± 20	51.01 ± 11.0	0.001
Median (IQR)	38.8 (29)	34.7 (34)	39.8 (29)	52.15 (14)	0.02
CRF levels					
Mean ± SD	37.5 ± 17	38.1 ± 17	37.1 ± 18	87.7 ± 59	0.001
Median (IQR)	34.8 (22)	35.1 (19)	34.05 (24)	78.3 (39)	0.000

SD, standard deviation; IQR, interquartile range; CRF, corticotropin-releasing factor.
[a] Patients with nocturnal enuresis and controls.

Results

The study group consisted of 119 children with nocturnal enuresis (median age; 8 [5—16] years, 79 girls and 40 boys). Forty-nine of them were monosymptomatic, and seventy were non-monosymptomatic. The control group composed of 40 healthy children. The number of excluded patients due to secondary nocturnal enuresis, systemic disease, and abnormal neurologic examination findings was 40. The descriptive patient and control group data are presented in Table 1.

The copeptin levels were significantly lower in the nocturnal enuresis group than in the control group (median, 38.8 [IQR: 29 pg/ml] vs 52.1 [IQR: 14 pg/ml], $P = 0.02$; Table 1 and Fig. 1). Moreover, the copeptin levels were significantly lower in both monosymptomatic and non-monosymptomatic types of nocturnal enuresis than in the control groups ($P = 0.008$ and 0.001, respectively; Table 1). There was no significant difference in the copeptin levels between monosymptomatic and non-monosymptomatic enuresis ($P = 0.7$; Table 1).

The CRF levels were significantly lower in the nocturnal enuresis group than in the control group (median, 34.8 [IQR: 22 pg/ml] vs 78.3 [IQR: 39 pg/ml], $P = 0.000$; Fig. 1). The CRF levels were significantly lower in both monosymptomatic and non-monosymptomatic types of nocturnal enuresis than in the control groups ($P = 0.001$ and 0.001, respectively; Table 1). No significant differences were found in the levels of CRF between the monosymptomatic and non-monosymptomatic nocturnal enuresis cases ($P = 0.6$; Table 1).

Because there was statistical difference between groups according to the age, ANCOVA was performed. When the comparisons between groups were repeated using ANCOVA, copeptin and CRF levels were lower in children with nocturnal enuresis in comparison with those in the control groups.

Discussion

Nocturnal enuresis is a complicated disease that includes multiple pathogenetic factors [11]. Nocturnal polyuria that is attributed to AVP deficiency is the most common reason why monosymptomatic enuresis occurs, whereas a lower age-related bladder capacity and an associated overactive bladder are the common cause of non-monosymptomatic enuresis [11,12]. To be successful in the treatment of nocturnal enuresis, differentiation of monosymptomatic and non-monosymptomatic nocturnal enuresis and an understanding of pathogenesis are necessary. This study showed that the serum levels of copeptin and CRF were significantly lower in children with monosymptomatic and non-monosymptomatic nocturnal enuresis in comparison with those in the control groups.

Arginine vasopressin is produced in the hypothalamus; nocturnal AVP deficiency is a major cause of monosymptomatic enuresis. It contributes to the regulation of osmotic and cardiovascular homeostasis in the human body. As AVP is an unstable molecule, the measurement in serum is very difficult to achieve. Arginine vasopressin can also largely bind to platelets and is rapidly cleared [5]. However, copeptin, a 39—amino acid glycopeptide, is an AVP precursor (CT-proAVP) and is more stable [5]. It directly reflects AVP concentration in serum via a situation that is similar to that of C-peptide and insulin [8]. Therefore, copeptin is presumed to be a sensitive surrogate biomarker for AVP release [8]. Copeptin as a biomarker is also used for the diagnosis and prognosis of some diseases, such as diabetes insipidus, autosomal dominant polycystic kidney disease, hyponatremia, and vasodilatory shock [13]. To date, there are only two studies in the literature that assess the relationship between copeptin and enuresis [5,7]. A study indicated the presence of decreased levels of copeptin in children with nocturnal enuresis, but the AVP levels were the same in children with nocturnal enuresis and healthy children [5]. In addition, the research suggested that copeptin decreases with the increasing severity of the disease. Another study demonstrated that the day/night ratio of plasma copeptin before treatment is predictive of desmopressin response [7]. In this study, decreased levels of copeptin in children with monosymptomatic and non-monosymptomatic nocturnal enuresis in comparison with those in the control group were found.

Corticotropin-releasing factor is a neurohormone that is released from the Barrington's nucleus neurons in the hypothalamus. These neurons can regulate the parasympathetic tone of the bladder [6]. Corticotropin-releasing factor is known to play a role in the overactive bladder [14]. The only study in the literature demonstrated significantly decreased levels of CRF in monosymptomatic enuretic children. In this study, CRF levels were measured between morning and evening in children with nocturnal enuresis. No significant difference was found between morning and evening levels [6]. The results of animal studies show that the role of CRF in micturition regulation is conflicted. An animal study in the literature demonstrated that intrathecal application of CRF in rats was found to decrease bladder contractions [9]. Other animal studies suggested that administration of CRF in rats was found to induce bladder overactivity and facilitate micturition [10]. This study revealed decreased levels of CRF in children with monosymptomatic and non-monosymptomatic nocturnal enuresis in comparison with those in the control group.

The findings and treatment of monosymptomatic and non-monosymptomatic nocturnal enuresis are different. Although desmopressin acetate and alarm systems are the mainstays of treatment in monosymptomatic enuresis, anticholinergic drugs and urotherapy are main treatments in non-monosymptomatic enuresis [12]. Although desmopressin has been a standard treatment for MNE, 20—60% of children within that group are still desmopressin resistant [15]. In the studies that assessed low functional bladder capacity, it appeared that the intrinsic renal circadian clock system seems to play a role in resistance to desmopressin treatment [3,16]. The treatment of this condition includes desmopressin acetate with an alarm system and an anticholinergic treatment combination. Nowadays, the search for prognostic factors is continuing for patients with desmopressin resistance. In addition, diagnostic and prognostic markers are needed for treatment and to differentiate between monosymptomatic and non-monosymptomatic nocturnal enuresis. The aim of this study was to determine whether copeptin and CRF are predictive factors that differentiate between monosymptomatic and

non-monosymptomatic nocturnal enuresis. It was shown that copeptin and CRF indicate the pathogenesis of both monosymptomatic and non-monosymptomatic nocturnal enuresis. It was also demonstrated that copeptin and CRF levels were not different between the monosymptomatic and non-monosymptomatic groups.

This study has some limitations. There was statistical difference between groups according to the age and the prevalence; nocturnal enuresis was higher in girls than in boys, but this difference was not statistically significant. More boys have nocturnal enuresis than girls, although this difference tends to diminish after the age of 10 years. In this study, the female/male ratio was contrary to the expected. The patients were selected prospectively and cross-sectionally among patients with nocturnal enuresis who met the study criteria. The control group has been selected cross-sectionally from non-enuretic children who are older than five years. Gender distinction was not specifically made. Actually, the authors did not think that the *P* value of CRF and copeptin could be due to age, gender, and pubertal status because, in both groups, there were prepubertal and pubertal children. The control group consists of children aged 5—17 years, whereas the nocturnal enuretic group consists of patients aged 5—16 years. When ANCOVA was performed according to age and the comparisons between groups were repeated (eliminating the effect of age), copeptin and CRF levels were lower in children with nocturnal enuresis in comparison with those in the control groups. In addition, no correlation has been determined between CRF and copeptin with age, according to the statistical study.

The second limitation was that the circadian rhythm for copeptin and CRF levels was not considered, and evaluation of these biomarkers was performed in a single sample. Third, the fluid intake of patients with nocturnal enuresis and control groups was not recorded. Arginine vasopressin levels were not measured at the same time with copeptin.

Conclusion

In conclusion, the neurohormones and biomarkers that were previously studied in MNE were researched in monosymptomatic and non-monosymptomatic nocturnal enuresis in this study. To the authors' knowledge, there are no studies concerning CRF and copeptin in non-monosymptomatic enuresis. Those changes in both copeptin and CRF that was shown in this study in monosymptomatic and non-monosymptomatic enuretic children may contribute to the pathogenesis of nocturnal enuresis. The authors think further case—control studies can evaluate the copeptin and CRF levels before treatments in monosymptomatic and non-monosymptomatic ones to decide potential effectivity of treatment. A study in the literature demonstrated that the day/night ratio of plasma copeptin before treatment is predictive of desmopressin response. Additional case studies are needed to determine whether copeptin and CRF can show desmopressin resistance.

Author statements

Ethical approval

The study was reviewed by the Pamukkale University Medical Ethics Committee (METC) (reference number; 60116787-020/22669). Written consent was obtained from parents and their children.

Funding

This project was supported by the Scientific Research Projects Unit of Pamukkale University, project no. 2018HZDP028.

Competing interests

None declared.

References

[1] Neveus T, Eggert P, Evans J, Macedo A, Rittig S, Tekgül S, et al. Evaluation of and treatment for monosymptomatic enuresis: a standardization document from the International Children's Continence Society. J Urol 2010;183:441—7. https://doi.org/10.1016/j.juro.2010.04.006.

[2] Bayne AP, Skoog SJ. Nocturnal enuresis: an approach to assessment and treatment. Pediatr Rev 2014;35(8):327—34. https://doi.org/10.1542/pir.35-8-327.

[3] Haid B, Tekgül S. Primary and secondary enuresis: pathophysiology, diagnosis, and treatment. Eur Urol Focus 2017;3(2—3):198—206. https://doi.org/10.1016/j.euf.2017.08.010.

[4] Yüksel S, Yurdakul AÇ, Zencir M, Çördük N. Evaluation of lower urinary tract dysfunction in Turkish primary schoolchildren: an epidemiological study. J Pediatr Urol 2014;10:1181—6. https://doi.org/10.1016/j.jpurol.2014.05.008.

[5] Nalbantoğlu B, Yazıcı CM, Nalbantoğlu A, Güzel S, Topçu B, Güzel EÇ, et al. Copeptin as a novel biomarker in nocturnal enuresis. Urology 2013;82:1120—3. https://doi.org/10.1016/j.urology.2013.05.047.

[6] Motawie AA, Abd Al-Aziz AM, Hamed HM, Fatouh AA, Awad MA, El-Ghany AA. Assessment of serum level of corticotropin-releasing factor in primary nocturnal enuresis. J Pediatr Urol 2017;13:46.e1—46.e5.

[7] Hara T, Ohtomo Y, Endo A, Niijima S, Yasui M, Shimizu T. Evaluation of urinary aquaporin 2 and plasma copeptin as biomarkers of effectiveness of desmopressin acetate for the treatment of monosymptomatic nocturnal enuresis. J Urol 2017;198:921—7. https://doi.org/10.1016/j.juro.2017.04.088.

[8] Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem 2006;52:112—9. https://doi.org/10.1373/clinchem.2005.060038.

[9] Kiddoo DA, Valentino RJ, Zderic S, Ganesh A, Leiser SC, Hale L, et al. Impact of state of arousal and stress neuropeptides on urodynamic function in freely moving rats. Am J Physiol Regul Integr Comp Physiol 2006;290:1697—706. https://doi.org/10.1152/ajpregu.00742.2005.

[10] Klausner AP, Stoeng T, Na YG, Raju J, Batts TW, Tuttle JB, et al. The role of corticotrophin releasing factor and its

antagonist, astressin, on micturition in rat. Auton Neurosci 2005;123:26—35. https://doi.org/10.1016/j.autneu.2005.08.003.

[11] Bayne AP, Skoog SJ. Nocturnal enuresis: an approach to assessment and treatment. Pediatr Rev 2014;35:327—34. https://doi.org/10.1542/pir.35-8-327.

[12] Vande Walle J, Rittig S, Bauer S, Eggert P, Marschall-Kehrel D, Tekgul S. American academy of pediatrics; european society for paediatric urology; european society for paediatric nephrology; international Children's continence society. Practical consensus guidelines for the management of enuresis. Eur J Pediatr 2012;171:971—83. https://doi.org/10.1007/s00431-012-1687-7.

[13] Morgenthaler NG, Struck J, Jochberger S, Dünser MW. Copeptin: clinical use of a new biomarker. Trends Endocrinol Metabol 2008;19:43—9. https://doi.org/10.1016/j.tem.2007.11.001.

[14] Wróbel A. Overactive bladder syndrome pharmacotherapy: future treatment options. Prz Menopauzalny 2015;14:211—7. https://doi.org/10.5114/pm.2015.56403.

[15] Van Herzeele C, Walle JV, Dhondt K, Juul KV. Recent advances in managing and understanding enuresis. F1000Res 2017;24:1881. https://doi.org/10.12688/f1000research.11303.1.

[16] Dossche L, Walle JV, Van Herzeele C. The pathophysiology of monosymptomatic nocturnal enuresis with special emphasis on the circadian rhythm of renal physiology. Eur J Pediatr 2016;175:747—54. https://doi.org/10.1007/s00431-016-2729-3.