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# Measurement of Pubic Symphysis Joint Width in the Evaluation of Patients with Primary Nocturnal Enuresis: A Case-Control Study

### Primer Enürezis Nokturnalı Hastaların Değerlendirilmesinde Simfizis Pubis Eklem Genişliğinin Ölçümü: Vaka-Kontrol Araştırması

<sup>10</sup> Uğur Ufuk IŞIN<sup>a</sup>, <sup>10</sup> Emin ÇAKMAKCI<sup>b</sup>, <sup>10</sup> Nuray Gülden TANGAL<sup>b</sup>, <sup>10</sup> Numan İlteriş ÇEVİK<sup>c</sup>

<sup>a</sup>University of Health Sciences Ankara Atatürk Sanatoryum Training and Research Hospital, Department of Pediatrics, Ankara, Türkiye <sup>b</sup>University of Health Sciences Ankara Atatürk Sanatoryum Training and Research Hospital, Department of Radiology, Ankara, Türkiye <sup>c</sup>University of Health Sciences Ankara Bilkent City Hospital, Department of Radiology, Ankara, Türkiye

ABSTRACT Objective: The aim of this study was to investigate whether pubic symphysis width (PSW) and renal pelvis diameter (RPD) are associated with the presence of monosymptomatic primary nocturnal enuresis (MPNE) in children. Material and Methods: A total of 80 children, including 40 with MPNE and 40 without (controls) were included in this prospective original article conducted between May 2024 and June 2024. Age, sex, height, weight and body mass index were obtained. PSW and RPD were measured using an ultrasound device. To assess the associations between the measurements and MPNE, patients with and without MPNE were compared via various analyses. Results: The median age of the MPNE group was 8 (7-10), and that of the control group was 9 (7,5-11) years (p=0.039). In the MPNE group 55.0% were boys, while only 32.5% of the control group were boys (p=0.071). PSW, left RPD and average RPD were significantly lower in the MPNE group. Diagnostic analysis showed that a PSW value of <8 mm had 76.3% overall accuracy to distinguish children with MPNE from controls (80% sensitivity, 72.5% specificity, 74.4% positive predictive value and 78.4% negative predictive value). Multiple logistic regression showed that children with decreased PSW (<8 mm) had 8.527fold higher likelihood of enuresis, after adjusting for age, sex, BMI and presence of spina bifida (p<0.001). Conclusion: Lower PSW (<8 mm) was found to be an independent risk factor and a significant predictor for MPNE, after matching for age, sex, BMI and spina bifida presence. RPD was not associated with enuresis or PSW-apart from a minor correlation.

Keywords: Monosymptomatic primary nocturnal enuresis; pubic symphysis width; diameter of the renal pelvis; ultrasound ÖZET Amac: Bu çalışmanın amacı, Simfizis pubis eklem genişliği (SPG) ve renal pelvis capının (RPC) cocuklarda monosemptomatik primer noktürnal enürezis (MPNE) varlığı ile ilişkili olup olmadığını araştırmaktır. Gerec ve Yöntemler: Mayıs 2024-Haziran 2024 tarihleri arasında yürütülen bu prospektif vaka-kontrol calışmasına 40'ı MPNE olan ve 40'ı kontrol grubu olmak üzere toplam 80 çocuk dâhil edilmiştir. Yaş, cinsiyet, boy, kilo ve beden kitle indeksi (BKİ) elde edilmiştir. SPG ve RPÇ, USG cihazı kullanılarak ölçülmüştür. Ölçümler ile MPNE arasındaki ilişkileri değerlendirmek için MPNE olan ve olmayan hastalar tek değişkenli ve çok değişkenli analizlerle karşılaştırılmıştır. Bulgular: MPNE grubunun ortanca yaşı 8 (7-10), kontrol grubununki ise 9 (7,5-11) idi (p=0,039). MPNE grubunun %55,0'i erkek iken kontrol grubunun yalnızca %32,5'i erkekti (p=0,071). SPG, sol RPÇ ve ortalama RPÇ, MPNE grubunda anlamlı derecede düşüktü. Tanısal analiz, SPG değerinin <8 mm olmasının MPNE'li çocukları kontrol grubundan ayırt etmede %76,3 genel doğruluğa sahip olduğunu göstermiştir (%80 duyarlılık, %72,5 özgüllük, %74,4 pozitif prediktif değer ve %78,4 negatif prediktif değer). Çoklu lojistik regresyon, yaş, cinsiyet, BKİ ve spina bifida varlığı ayarlandıktan sonra, SPG'si azalmış (<8 mm) cocukların enürezis olasılığının 8,527 kat daha yüksek olduğunu göstermiştir (p<0,001). Sonuç: Düşük SPG (<8 mm), yaş, cinsiyet, BKİ ve spina bifida varlığı için eşleştirme yapıldıktan sonra bağımsız bir risk faktörü ve MPNE için anlamlı bir belirleyici olarak bulunmuştur. RPC, küçük bir korelasyon dışında enürezis veya SPG ile ilişkili saptanmamıştır.

Anahtar Kelimeler: Monosemptomatik primer nokturnal enürezis; simfizis pubis genişliği; renal pelvis çapı; ultrasonografi

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Correspondence: Uğur Ufuk IŞIN University of Health Sciences Ankara Atatürk Sanatoryum Training and Research Hospital, Department of Pediatrics, Ankara, Türkiye E-mail: ugurufuk@yahoo.com

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2587-0483 / Copyright © 2024 by Türkiye Klinikleri. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Nocturnal enuresis, simply described as "enuresis", is one of the most common childhood problems.<sup>1</sup> It is defined as involuntary urine discharge , which occurs discrete amounts during sleep, in children older than a developmental age of 5 to 7 years.<sup>2,3</sup> It has been reported that about 25% of children aged 5 years have experienced episodes of nocturnal enuresis.<sup>2</sup> This prevalence then demonstrates a steep decreases to 10% at 7 years, followed by 3% at 11-12 years and 0.5-1% at 16-17 years.<sup>4</sup> If left untreated, enuresis can lead to low self-esteem, social isolation, poor school performance, psychological impairment, chronic sleep disorders, as well as decreased quality of life in the whole family.<sup>4-6</sup>

Primary enuresis describes cases in which an organic or psychological cause for enuresis cannot be identified (around 80% of cases).<sup>2,3</sup> Monosymptomatic enuresis refers to the absence of any symptoms other than wetting of bed or clothes.<sup>3</sup> The enuresis referred in this study is termed as monosymptomatic primary nocturnal enuresis (MPNE).<sup>7</sup> A number of possible etiological factors have been put forth, including genetic factors, dysfunctions in arousal mechanisms, increased nocturnal detrusor activity, nocturnal low bladder capacity, nocturnal polyuria, non-inhibition of the micturition reflex during sleep, and altered diurnal rhythm of vasopressin secretion.7-14 Although most cases of MPNE are easily diagnosed with basic examinations and can be managed with first-line treatments, some patients may be resistant to treatment. This may be due to the unexplained aspects of MPNE etiopathogenesis.

Radiological assessment, especially ultrasound (US) imaging, is often used in MPNE research. Results are conflicting with respect to the benefit of US examination of the lower urinary tract, and also, renal US studies have reported inconclusive findings.<sup>1,7,15-18</sup> Despite having a completely different pathophysiological background, stress incontinence in women has been associated with variations of the bony pelvis.<sup>19,20</sup> In this context, parameters such as pubic arch angle, pubic ramus width, pubic symphysis length, interobturator foramina line–inferior pubic symphysis distance, interobturator foramina distance, interspinous and intertuberous diameters have been

investigated, and some have been found to have utility in distinguishing women with and without stress incontinence.<sup>19,20</sup> Although it is well established that these conditions are greatly different from each other, we hypothesized that bone-related measurements [e.g., pubic symphysis width, (PSW)] might be associated with MPNE in children.

We postulate that, since PSW can be a potential indicator of skeletal maturation abnormality and congenital urogenital abnormalities, it also may be associated with known or unknown enuresis-related abnormalities and may be a precursor sign of possible pathologies associated with enuresis.<sup>21-24</sup> Therefore, in this study we aimed to investigate whether PSW is associated with MPNE presence in children. As a secondary aim, we also investigated whether the renal pelvis diameter (RPD) was associated with enuresis or PSW.

### MATERIAL AND METHODS

#### ETHICAL CONSIDERATIONS

The protocol for this study was approved by the Ethics Committee of Ankara Atatürk Sanatoryum Training and Research Hospital (date: May 08, 2024, no: 64). The study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki and its later amendments. Written informed consent from parents or legal guardians of each child was received after detailed explanation of the purpose of the study and its requirements. The children also received age-appropriate explanations and, when possible, verbally confirmed their voluntary participation.

# STUDY DESIGN, SETTING AND POPULATION (ANALYTICAL RESEARCH)

This was a prospective, single-centered case-control study conducted May 2024 and June 2024 at the Radiology Department of Ankara Atatürk Sanatoryum Training and Research Hospital, Ankara, Türkiye.

A total of 80 participants were included in the study, 40 children diagnosed with MPNE (patient or enuresis group) and 40 children without enuresis (control group). Volunteer participants in the control group were randomly selected from children who were admitted to the outpatient clinic for any reason other than urological/nephrological disorders and who were to undergo US examination (mostly thyroid or abdomen US). Additionally, whether participants in the control group had any history of enuresis after 5 years of age was questioned by a specialist pediatrician who obtained detailed anamneses. The common exclusion criteria for both groups were: less than 5 years of age, older than 15 years, history of prior pelvic surgery or trauma, presence of congenital pelvic bone or renal anomaly, midline fusion defects, skeletal dysplasia, and inability to undergo an acceptable US evaluation. In addition, for the MPNE group, those who received previous or current treatment for enuresis, secondary enuresis and nonmonosymptomatic enuresis cases were excluded from the study.

The diagnosis of enuresis was made according to the enuresis definition criteria of the International Children's Continence Society.<sup>25</sup> Briefly, nocturnal enuresis was defined as recurrent and intermittent urinary incontinence during sleep in a child 5 years or older-given that there were no congenital anomalies of the urinary system or congenital or acquired defects of the central nervous system. For the diagnosis, a period of 3 consecutive months and a frequency of at least three episodes per week were required.<sup>26</sup> Primary nocturnal enuresis was defined as the absence of nocturnal dryness period lasting longer than 6 months in a child older than 5 years who was not found to have any organic or psychological cause.<sup>2</sup> Secondary nocturnal enuresis was defined (for exclusion) as reoccurrence of nocturnal enuresis after at least 6 months without events.<sup>2</sup> Monosymptomatic enuresis was defined as the absence of any additional symptoms other than wetting of the bed or clothes at night.<sup>3,4</sup> Non-monosymptomatic enuresis was defined (for exclusion) as having any of the following lower urinary tract symptoms (in addition to bedwetting): daytime incontinence, urge, increased or decreased voiding frequency, voiding postponement, and holding maneuvers.3,4

Age and sex information of all participants were recorded. Medical history about enuresis and its treatment, hydronephrosis and spina bifida were questioned and also checked from hospital records if available. Height (cm) and weight (kg) measurements were made. Body mass index (BMI) was calculated as weight/height<sup>2</sup> (kg/m<sup>2</sup>).

#### ULTRASONOGRAPHY

PSW and RPD were measured using a routinely-used US device (Toshiba Aplio PowerVision; Toshiba Medical Systems; Tokyo, Japan) equipped with a 7-12 Mhz linear probe (Toshiba XG; Toshiba Medical Systems; Osaka, Japan) by the same experienced radiologist who was unaware of the patients' enuresis status.

The subject was placed on their back with hips in approximately 15-degree flexion. The probe was placed at the upper border of the symphysis with a caudad angle. After obtaining a clear image of the upper part of the pubic symphysis and the surrounding pubic bones in the axial section, the distance between the medial edges of the superior pubic rami at the upper border of the symphysis was measured to obtain PSW.<sup>27</sup> RPD was measured from the right and left sides and was defined as the anteroposterior diameter on a transverse plane scan, with the child in the prone position.<sup>28</sup> Average RPD was also calculated in each patient.

#### OUTCOME

The primary outcome of the study was to investigate whether there was a relationship between enuresis and PSW. The secondary outcome of the study was to investigate the relationship between RPD and enuresis.

#### STATISTICAL ANALYSIS

All analyses were performed on IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) and results were interpreted according to a p<0.05 significance threshold (two-tailed). The Shapiro-Wilk test was used to determine whether variables were normally distributed. Data are given as median (1<sup>st</sup> quartile-3<sup>rd</sup> quartile) for continuous variables according to non-normality of distribution, and as frequency (absolute and relative) for categorical variables. Continuous variables were compared with the Mann-Whitney *U* test, and Spearman correlation coefficients were calculated to evaluate directional relationships between continuous variables. PSW performance to discriminate children with enuresis and healthy controls was evaluated by using Receiver Operating Characteristic curve analysis. The optimal cut-off was determined by the Youden *J* index. Logistic regression (enter method) was performed to determine factors independently associated with enuresis. Multiple linear regression (enter method) was performed to determine factors independently associated with RPD.

# RESULTS

The median age of the MPNE group was 8 (7-10), while the control group had a median age of 9 (7.5-11) years (p=0.039). Males represented 55.0% of the MPNE group and 32.5% of the control group (p=0.071). Although height was significantly lower in the MPNE group compared to controls (p=0.041), the groups were similar in terms of weight (p=0.058) and BMI (p=0.505). PSW values were significantly lower (p<0.001) in the MPNE group [8.70 (6.00-7.75) mm] compared to the control group [8.75 (7.15-10.00) mm]. Left RPD [3.45 (3.0-4.0) mm vs. 4.25 (3.0-5.0) mm] and average RPD [3.50 (3.0-4.5) mm vs. 4.38 (3.5-5.0) mm] were significantly lower (p=0.010 and p=0.022, respectively) in patients with enuresis compared to the control group (Table 1).

<b>TABLE 1:</b> Summary of children's characteristics with regard to groups.						
	Groups					
	Control (n=40)	p value				
Age	9 (7.5-11)	8 (7-10)	0.039			
Sex						
Male	13 (32.5%)	22 (55.0%)	0.071			
Female	27 (67.5%)	18 (45.0%)				
Height, cm	140.5 (129-148.5)	130 (121-140)	0.041			
Weight, kg	35.5 (30-47)	30 (25.5-38)	0.058			
Body mass index, kg/m <sup>2</sup>	18.67 (17.20-21.32)	18.92 (16.39-20.81)	0.505			
Hydronephrosis	0 (0.0%)	0 (0.0%)	N/A			
Spina bifida	3 (7.5%)	9 (22.5%)	0.117			
Pubic symphysis width, mm	8.75 (7.15-10.00)	7.00 (6.00-7.75)	<0.001			
Renal pelvis diameter, mm						
Right	4.25 (3.0-5.5)	3.05 (3.0-5.0)	0.090			
Left	4.25 (3.0-5.0)	3.45 (3.0-4.0)	0.010			
Average	4.38 (3.5-5.0)	3.50 (3.0-4.5)	0.022			

Data are given as median (1st quartile-3rd quartile) for continuous variables according to non-normality of distribution and as frequency (percentage) for categorical variables; N/A: Not applicable.

TABLE 2: The performance of pubic symphysis width to   discriminate children with monosymptomatic primary nocturnal   enuresis from healthy controls.				
Cut-off	<8 mm			
Sensitivity	80.0%			
Specificity	72.5%			
Positive predictive value	74.4%			
Negative predictive value	78.4%			
Accuracy	76.3%			
Area under ROC curve (95.0% CI)	0.758 (0.648-0.867)			
p value	<0.001			

CI: Confidence intervals

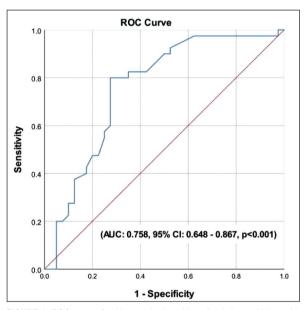


FIGURE 1: ROC curve of pubic symphysis width to discriminate children with monosymptomatic primary nocturnal enuresis and healthy controls.

With a cut-off value of <8 mm, PSW had 80.0% sensitivity, 72.5% specificity, 74.4% positive predictive value and 78.4% negative predictive value to distinguish patients with enuresis from controls. Overall correct classification frequency (accuracy) was 76.3% (AUC: 0.758, 95% CI: 0.648–0.867; p<0.001) (Table 2, Figure 1).

According to multiple logistic regression, children with decreased PSW (<8 mm) had an 8.527-fold higher risk to have enuresis (OR: 8.527, 95% CI: 2.783-26.129; p<0.001) after adjusting for age, sex, BMI and history of spina bifida-which were not found to have any independent impact on the likelihood of MPNE (Table 3).

TABLE 3: Factors independently associated with enuresis, multiple logistic regression analysis.						
	β coefficient	Standard error	<b>p value</b> 0.190	Exp(β)	95.0% Cl for Exp(β)	
Age	-0.189	0.145		0.827	0.623	1.098
Sex, Female	-0.956	0.588	0.104	0.384	0.121	1.217
Body mass index, kg/m <sup>2</sup>	0.007	0.089	0.937	1.007	0.845	1.200
Pubic symphysis width, <8 mm	2.143	0.571	<0.001	8.527	2.783	26.129
Spina bifida	0.582	0.815	0.476	1.789	0.362	8.844
Constant	0.786	1.956	0.688	2.194		

Nagelkerke R<sup>2</sup>=0.397; CI: Confidence interval

Correlations between RPD and several variables are depicted in Table 4. Accordingly, a significant moderate positive correlation was found between average RPD and age (r=0.402, p<0.001). Other correlations were either insignificant or too weak to be noted.

According to multiple linear regression analysis, age was the only factor independently associated with RPD values (p=0.001). Other variables included

<b>TABLE 4:</b> Correlations between renal pelvis diameter and other variables.					
		Renal pelvis diameter			
		Right	Left	Average	
Age	r	0.379	0.399	0.402	
	р	0.001	<0.001	<0.001	
Sex, Female	r	-0.173	-0.161	-0.161	
	р	0.125	0.154	0.154	
Body mass index, kg/m <sup>2</sup>	r	0.217	0.239	0.239	
	р	0.055	0.034	0.034	
Pubic symphysis width	r	0.057	0.128	0.090	
	р	0.617	0.258	0.425	
Spina bifida	r	0.028	-0.093	-0.022	
	р	0.808	0.414	0.846	

r: Spearman correlation coefficient.

in the analysis were found to be non-significant (Table 5).

### DISCUSSION

In the current study, we investigated whether PSW values in children could be associated with MPNE. We found that, compared to controls, children with enuresis had significantly lower PSW values. Also, a PSW value of < 8 mm was found to be associated with a 8.5-fold greater likelihood of MPNE. RPD was not associated with enuresis.

Despite all diagnostic and therapeutic efforts, some children with nocturnal enuresis cannot be treated effectively (therapy-resistant patients).<sup>3</sup> Since the population with MPNE are children, invasive methods and advanced radiological examinations cannot be freely utilized in research. However, since US assessments do not pose instrument-related risks, various studies have utilized US examinations in patients with MPNE. Similarly, we aimed to determine whether PSW was related to enuresis, and demonstrated lower PSW values among children with MPNE compared to those with normal nocturnal con-

Age	Unstandardized β 0.255	Standard error 0.076	Standardized β 0.391	p value 0.001	95.0% CI for β	
					0.103	0.407
Sex, Female	-0.412	0.309	-0.146	0.187	-1.028	0.204
Body mass index, kg/m <sup>2</sup>	0.024	0.045	0.063	0.594	-0.066	0.114
Pubic symphysis width	-0.017	0.073	-0.027	0.815	-0.163	0.129
Spina bifida	0.105	0.424	0.026	0.805	-0.741	0.951
Enuresis	-0.454	0.326	-0.162	0.168	-1.105	0.196
Constant	2.046	1.034		0.052	-0.016	4.108

Adjusted R<sup>2</sup>=0.194; F=4.138; p=0.001; CI: Confidence interval.

tinence. To our knowledge, available literature is devoid of any studies exploring the clinical use of PSW in this context.<sup>21</sup> Our results showed that the cut-off value associated with enuresis was <8 mm; however, in an exploratory study by McAlister et al., mean PSW was reported to be 6.8±1.6 mm (median: 7.0 mm, range: 0.52-0.84) in the pediatric population, regardless of age or sex, and PSW values were found to demonstrate a gradual but marginal increase with age.<sup>21</sup> The reasons for differences among populations are many, such as racial variations, growth & development-related differences, the inclusion of children within a certain age group (in the current study), and measurement methodology. Notably, our MPNE patient group had significantly lower age compared to controls, and thus, these patients would have been expected to have relatively lower PSW values based on the data reported by McAlister et al.<sup>21</sup> Notwithstanding the fact that our overall measurements appear to be higher compared to the aforementioned data, our MPNE group had lower age and lower PSW values, indicating a likely confounder. However, regression analysis excluded an independent relationship between age and MPNE. Taken together, these findings could suggest that MPNE might be associated with early pelvic bone maturation or variations in bone development that can have an impact on PSW. These results must be supported by further studies.

The diagnosis of enuresis can mostly be made with a basic evaluation and first-line treatment methods are usually successful in management.<sup>1,3</sup> Some clinical and physioanatomical data about the lower urinary tract can also contribute to enuresis management, determination of the need for further examinations, and making decisions on patient-specific treatment.<sup>7,29</sup> Unfortunately, some of these clinical and physioanatomical features, such as daytime voiding symptoms, frequency and timing of enuresis, micturition habits and bladder capacity, can be affected by external variables including defecation characteristics, urogenital abnormalities, urinary tract infections, and psychological and neurological disorders.<sup>5,30</sup> In addition, studies have often reported conflicting results concerning the benefit of lower urinary tract US parameters in the management of MPNE.<sup>1,7,15</sup> On the other hand, genetic factors may

contribute to the likelihood of enuresis, and studies have uncovered some potential loci on chromosomes 12, 13, and 21 -without identification of any specific gene variants or mutations.<sup>2-4</sup> The genetic background is demonstrated by increased likelihood of nocturnal enuresis among children with parents who had a history of the condition themselves. When a single parent has a history of enuresis, the probability of their child having enuresis is 44%. If both parents have it, this likelihood increases to 77%. If neither parent has a history of enuresis, this risk drops to 15% -which is lower than the prevalence of nocturnal enuresis in the general population.<sup>8-10</sup> However, the inconsistency between genotype and phenotype and the fact that somatic and psychosocial environmental factors have a significant regulatory effect causes parental enuresis history to provide limited information about the prediction of enuresis in the child.<sup>31</sup> Therefore, objective and easily obtainable instruments that will not be affected by external factors can make important contributions to enuresis management. Measuring the PSW with US can meet this need with following advantages: (i) because it is not an elastic component, it will not be affected by external pressures -unlike bladderrelated measurements, (ii) it can provide objective and repeatable numerical data, (iii) measurement via US is a strong advantage in terms of both convenience and lack of irradiation. Furthermore, PSW can be a potential indicator of skeletal maturation abnormality, an individual with skeletal maturation abnormality is also likely to have neurological and/or other abnormalities of related systems, including the urogenital system, as shown previously.<sup>21-23</sup> It is also debatable that there may be an association between PSW and known or unknown congenital abnormalities that might lead to MPNE.<sup>24</sup> Therefore, PSW may be a precursor sign of the known and possible pathologies associated with nocturnal enuresis.

The main purpose in the initial evaluation of a child suffering from enuresis is to confirm the diagnosis of enuresis and to look for a possible organic or psychological cause.<sup>2-4</sup> Invasive urological diagnostic procedures are not primarily used because an organic form of urinary incontinence is rare in a child older than 5 years.<sup>4</sup> Instead, a detailed case history, bladder and bowel diary, physical examination, psy-

chological screening, urinalysis and, if necessary, US of the urinary tract are recommended for a basic assessment.<sup>4</sup> US examination provides very useful information when an organic cause of enuresis needs to be investigated.<sup>7,25,32,33</sup> However, although some researchers have argued that lower urinary tract US examination is useful and necessary in the management of MPNE others have argued that these parameters do not differ between children with MPNE and normal children, and have advised against routine lower urinary tract US.<sup>1,7,15,18,29,34</sup> While opinions about lower urinary tract US examination seem contradictory, the recommendation of upper urinary tract US examination seems clear. In the present study, we did not evaluate the lower urinary tract. We only investigated the relationships between PSW, RPD and MPNE. Although the average RPD of the MPNE group was found to be significantly lower in univariate analysis, RPD was not found to be associated with MPNE or PSW in multivariable analysis. This is consistent with most previously published studies, in suggesting that routine renal US may not be necessary in MPNE patients, albeit our data is limited to a single variable in this respect (RPD).7,15,16,18

The study is the first to investigate the relationship between PSW and MPNE and we found a significant relationship between PSW and MPNE. However, some limitations should be taken into account when interpreting the results. It is a single-center study with relatively small sample size. Since the patient population consisted of children, the difficulty of forming the control group and the random selection of patients in order to avoid a possible bias prevented enrollment of an age-matched control group. This limitation was largely addressed with the multivariable regression analysis that demonstrated the absence of an independent relationship between age and PSW. Nonetheless, because PSW changes with age, it can be difficult to define an overall cut-off value. For this reason, studies with more participants can be planned in which cut-off values will be investigated separately according to age ranges and racial differences. Another notable limitation of this study was that the frequency and severity of enuresis were not studied. Finally, considering that the current study did not assess treatment response, it would be very interesting to investigate whether PSW is associated with treatment success or other parameters.

### CONCLUSION

Lower PSW (<8 mm) was found to be an independent risk factor and a significant predictor for MPNE, after matching for age, sex, BMI and spina bifida presence. RPD was not associated with enuresis or PSW-apart from a minor correlation. US-based PSW measurement seems to be a useful parameter with a potential role in the diagnosis of MPNE and, possibly, may contribute to the elucidation of other possible pathophysiological mechanisms of MPNE.

#### Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

#### **Conflict of Interest**

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

#### Authorship Contributions

Idea/Concept: Uğur Ufuk Işın, Emin Çakmakcı; Design: Uğur Ufuk Işın, Emin Çakmakcı; Control/Supervision: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal, Numan İlteriş Çevik; Data Collection and/or Processing: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal, Numan İlteriş Çevik; Analysis and/or Interpretation: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal, Numan İlteriş Çevik; Literature Review: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal, Numan İlteriş Çevik; Writing the Article: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal, Numan İlteriş Çevik; Critical Review: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal; References and Fundings: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal; Materials: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal, Numan İlteriş Çevik; Other: Uğur Ufuk Işın, Emin Çakmakcı, Nuray Gülden Tangal; Refer-

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