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Assessment of Total Serum Immunoglobulin E and Eosinophilic Cationic Protein in Primary Mono-symptomatic Nocturnal Enuresis: Allergic Theory?

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Authors' contributions

This work was carried out in collaboration between all authors. Authors Motawie and Abd Al-Aziz designed thestudy and wrote the protocol. Authors Motawie, Hamed and Abd Al-Aziz managed the literature searches. All pediatric team managed patient's recruitment. Author Awad did the lab assessment. Author Hamed performed the statistical analysis. Authors Motawie and Hamed wrote the first draft of the manuscript. Author Abd Al-Aziz revised and edited the manuscript. Authors Motawie, Hamed and Abd Al-Aziz managed the analyses of the study and executed the manuscript in its final form. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The available evidences indicate that nocturnal enuresis has multi-factorial etiology and this may underlie the wide range of outcomes. Various pathophysiological mechanisms have been discussed and succeeded in defining many causes of enuresis such as disturbed functional bladder capacity, reversal of anti-diuretic hormone secretion rhythm and disturbed sleep architecture but still the cause could not be achieved in some enuretics. An association between allergic diseases and urinary tract disorders was reported by some authors and allergy was suggested to be one of the etiologies implicated in primary nocturnal enuresis (PNE).
Aim: is to negate or approve the allergic theory through assessment of serum level of total immunoglobulin E (IgE) and eosinophil cationic protein (ECP) in a sample of Egyptian children with

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primary mono-symptomatic nocturnal enuresis and correlate their levels with both bladder capacity and efficiency.

Patients and Methods: Seventy-three children aged 6-18 years (32 males & 41 females) complaining of primary mono-symptomatic nocturnal enuresis on ordinary diet (no restriction to any food) were recruited. Sixty-nine healthy children of the same age and sex with good toilet control day and night were also recruited**.** All children were evaluated by medical history, clinical examinations and assessment of total serum IgE and eosinophil cationic protein using ELISA technique. Bladder capacity and efficiency were assessed using abdominal and pelvic ultrasonography (for 44 patients and 21 controls).

Results: Serum levels of total IgE and ECP were higher in enuretics than controls but statistically insignificant. The bladder capacity of patients was significantly smaller than controls (p=.04) while no significant difference was found regarding bladder efficiency. Total serum IgE was significantly elevated in enuretics with small bladder capacity (p=0.01) compared to enuretics with normal bladder capacity. There was a significant negative correlation between total serum IgE and both bladder capacity (p=0.041, r=-0.3) and bladder efficiency (p=0.04, r= -0.27).

Conclusion: Immunoglobulin E induced hypersensitivity may be one of the pathophysiologic mechanisms involved in PNE through reduction of the bladder capacity and efficiency. Further proof studies on a large scale is recommended. Food elimination and challenge test could help if accused allergen is known.

Keywords: Primary Nocturnal enuresis; IgE induced allergy; bladder capacity; bladder efficiency.

1. INTRODUCTION

Nocturnal enuresis (NE) is the most frequent cause of bed wetting. It is a potentially disabling disorder for children and their families [1]. In Egypt, the prevalence of primary nocturnal enuresis (PNE) was found to be 11.5% in students aged 6-18 years [2].

The exact etiology of PNE is unknown and several pathophysiologic mechanisms appeared to contribute to its initiation, severity and presence of associated symptoms. Various theories have been proposed to explain why children with NE fail to recognize or respond to a full or contracting bladder during sleep [1,3,4,5]. A local intra-vesical allergic mechanism was suggested to be one of the etiologies of urinary incontinence [6]. An association between allergic diseases and urinary tract disorders was reported by Yamada et al. [7]. Urodynamic evaluation of children with dietary provoked enuresis revealed a reduced bladder capacity for age and dietary restriction in those children revealed an increase in their functional bladder capacity [8]. The above findings encouraged us of thinking that allergy could be one of the pathophysiologic mechanisms implicated in PNE.

Immunoglobin E (IgE) and serum eosinophil cationic protein (ECP) are essential components of allergic responses that may serve as measures of the activity of the allergic immune response [9].

Our aim in this study is to negate or approve this allergic theory through assessment of serum level of total IgE and ECP in a sample of Egyptian children with primary mono-symptomatic NE and correlate their levels to both bladder capacity and efficiency.

2. PATIENTS AND METHODS

Seventy-three children aged 6-18 years (32 males and 41 females) complaining of primary mono-symptomatic nocturnal enuresis on ordinary diet (no restriction to any) were recruited from Pediatric outpatient clinic, National Research Centre. This study was part of a project entitled" *Interplay between genetics, bladder, sleep and hormonal disorders in primary nocturnal enuresis*" ID 8040511, funded by National Research Centre.

The Patients were evaluated by means of medical history and clinical examinations using our urinary sheet. Exclusion criteria were: secondary nocturnal enuresis (spinal, brain or pelvic surgery, congenital anomalies of urological tract, urinary tract infection, diabetes mellitus and diabetes insipidus). Sixty-nine healthy children of the same age and sex with good toilet control day and night were recruited as controls. Written informed consents were taken from the guardians of all children. The study was approved by the ethical committee of the National Research Centre.

2.1 Laboratory Investigations

2 cc of venous blood were withdrawn from all enuretic cases and healthy controls by veni-puncture under aseptic technique for IgE and ECP assessments using ELISA technique available from Immuno-Spec Corporation (Los Angeles, California, USA).

2.2 The Abdominal and Pelvic Ultrasound

The abdominal and pelvic ultrasound were assessed for 44 patients and 21 controls. All of them were examined by diagnostic ultrasound nr.410477 Falco (incl. accessory kit, e- manual set, art. 410638 3.5/5.0Mhz CAHiD probe, probe holder).

Pre-voiding bladder volume (V1) and post-voiding bladder volume (V2) were assessed by obtaining sagittal and transverse images of the largest cross sections of bladder visualized. The images were measured in the three orthogonal directions: from the top to bottom of the bladder (y) and at 90° to this in the sagittal plane (z) and from left to right in the transverse plane (x) [10]. The volume was calculated using the following formula: **Formula Volume** = x× y× z × k (k is constant (0.72) [11].

- **A): Functional bladder capacity was calculated as follows:** Bladder capacity (V1) /Standard estimated bladder capacity for age (EBC) [12]. Standard EBC for age= 30 + (age in yrs×30) ml [13]. This formula is useful up to 12 years after that EBC is 390 ml [12]. Bladder over distension was arbitrary defined as bladder capacity ≥ 115 ml [14]. Small bladder capacity is considered if <70 ml [15].
- **B): The bladder emptying efficiency was calculated as follow:** Pre-voiding volume (V1) **-** Post-voiding volume (V2) / Pre-voiding volume (V1)

If >90%, efficiency was not achieved, this is considered as indication of voiding dysfunction [16].

2.3 Statistical Methods

Statistical package SPSS version 15 for Windows (SPSS, Chicago, and IL., USA) was used for statistical analysis. Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. The t-test was used to compare between 2 independent means. Correlation between various variables was done using Pearson correlation coefficient. A p-value of <=0.05 was considered statistically significant.

3. RESULTS

The study included 73 children suffering from primary mono-symptomatic nocturnal enuresis (32 males & 41 females) and 69 normal controls (33males & 36 females), their comparative demographic, laboratory and ultra-sonographic data were summarized in Table 1. The level of both total serum IgE and ECP were higher in patients compared to controls, however no significant statistical difference was detected between them. The bladder capacity of patients was significantly smaller than controls (p=.04) while no difference was found regarding bladder efficiency.

Comparing the levels of total serum IgE and ECP between small and normal bladder capacity in enuretics were presented in Fig. 1. Total IgE levels were significantly elevated in patients with small bladder capacity (p=0.01).

Comparison between normal and weak bladder efficiency regarding total serum IgE and ECP was presented in Table 2. Total serum IgE and ECP were higher in the patients with weak bladder efficiency compared to normal ones, but was statistically insignificant.

There was a negative correlation between bladder capacity and total IgE (p=0.041&r=-0.3) (Fig. 2). Also, there was a negative correlation between bladder efficiency and total IgE (p=0.04&r=-0.27) (Fig. 3). However, there was no correlation between ECP with either bladder efficiency or capacity (p=0.09, r=0.23&p=0.2, r=-0.14) respectively.

4. DISCUSSION

Investigations of enuretic children may not demonstrate anatomical defects [12,17], therefore, it is thought that this disorder may have a functional basis. The available evidences indicate that NE has multi-factorial etiology and this may underlie the wide range of outcomes [12]. Various pathophysiological mechanisms have been discussed and succeeded in defining many causes of enuresis in many patients such as disturbed functional bladder capacity [3],

reversal of anti-diuretic hormone (ADH) secretion rhythm [4], disturbed sleep architecture [5], and role of corticotropin hormone –releasing factor [18] but still the cause could not be detected in some patients.

Nocturnal enuresis was suggested to be associated with allergy although there is no objective evidence relating them [19]. Yamada et al. [7] suggested that some urinary tract diseases may be associated with allergy. It was reported that, NE was associated with a history of bronchial asthma, positive family history of allergy and skin test verified allergy [20]. The previous findings highlight the allergic theory as one of possible causes of NE.

Table 1. Comparison between enuretics and controls regarding demographic, laboratory and ultra-sonographic data

	Patients (mean ± SD)	Controls (mean ± SD)	p-value
Number	73	69	
Age (years)	11.03±3.11	12.32±2.79	0.3
Sex (m/F)*	32/41 (43.8/56.2%)*	33/36 (47.8 /52.2%)*	0.6
Weight (kg)	38.3±18.1	43.1±18.13	0.01
Height (cm)	135.4±17.6	135±0.15	0.6
BMI (Kg/m2)	19.5±5.5	22.8±6.38	0.1
Bed-wetting per week	**Daily**		
Total serum IgE (IU/ml)	85.9±84.4	67.6±66.4	0.24
ECP (ug/l)	45.54±37.22	28.5±27.37	0.1
Functional bladder capacity (ml)	46.3±27	68.1 ± 41	0.04
Bladder efficiency %	88.8±11.3	90.1±11.4	0.7

*ECP = Eosinophil cationic protein; *Number (%)*

Table 2. Comparing the levels of IgE and ECP between weak and normal bladder efficiency

	Weak bladder efficiency% mean ± SD	Normal bladder efficiency% mean ± SD	P value
Number	16	28	
IgE (IU/ml)	139.42±112.61	82.61±102.19	0.1
ECP (ug/l)	21.99±19.79	36.09±38.73	0.25

ECP = Eosinophil cationic protein

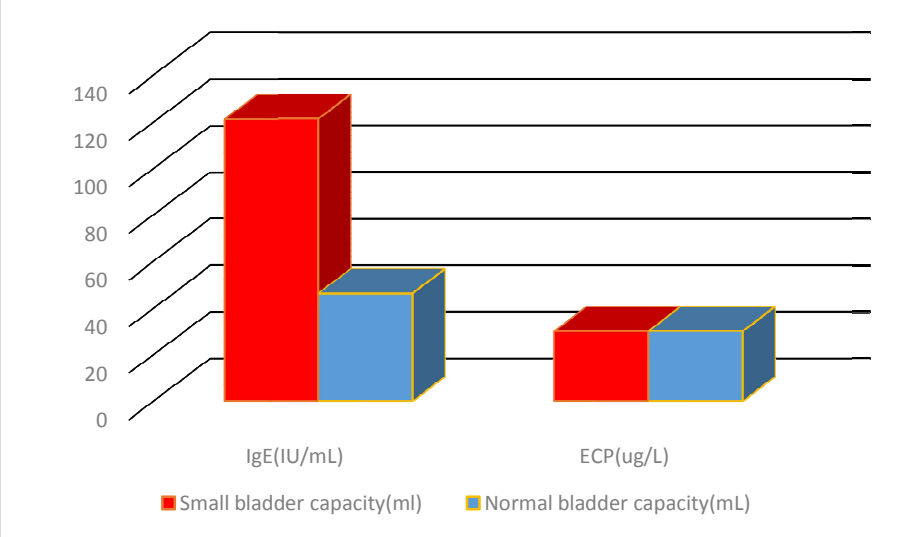

Fig. 1. Comparing the serum levels of total IgE and ECP between small bladder capacity and normal bladder capacity in enuretic group
(p=0.01 & P=0.9 respectively)

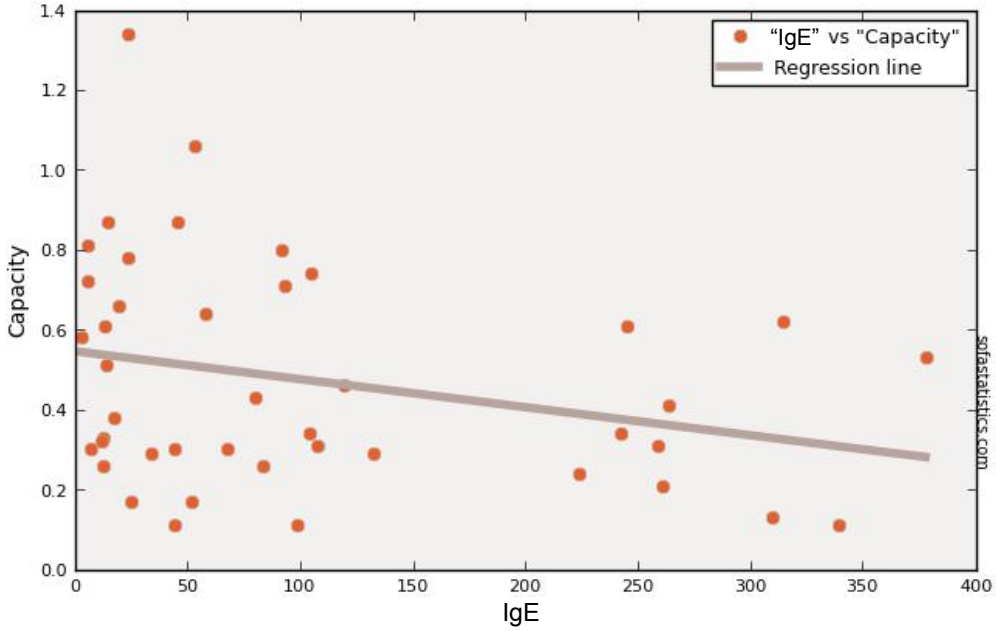

Fig. 2. The correlation between bladder capacity and IgE
(p value: 0.041 & Pearson's R statistic: -0.3)

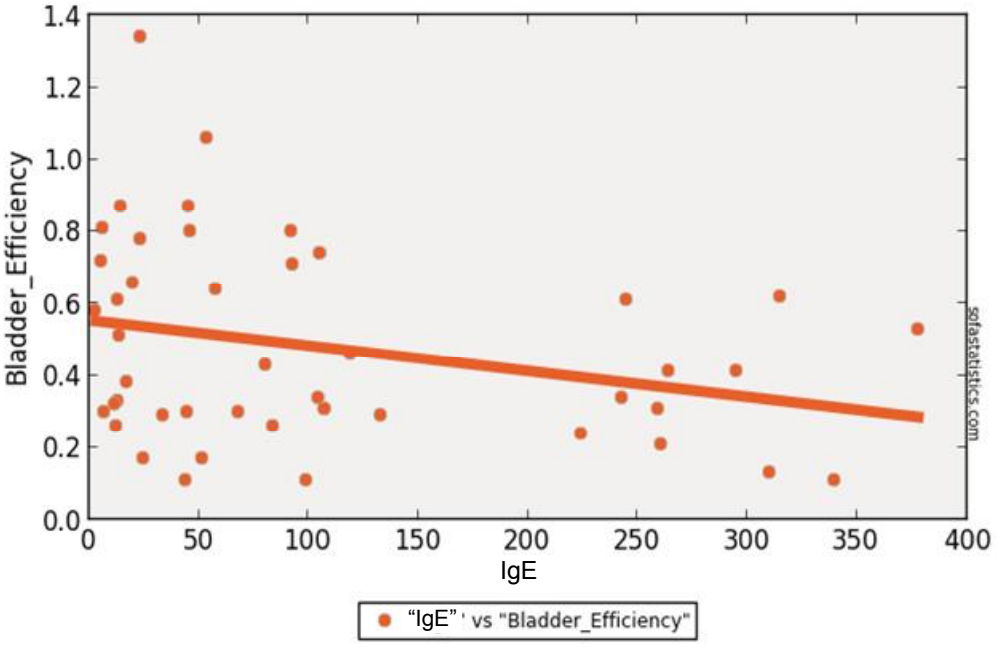

Fig. 3. The correlation between bladder efficiency (%) and IgE
(p value: 0.04 & Pearson's R statistic: -0.27)

Allergy has been defined as an inherited tendency to produce excess IgE antibody in response to common environmental allergens [21]. Although our patients had higher serum level of total IgE than control, yet no significant difference was found which go on parallel with Mungan et al. [22].

Functional bladder capacity is defined as the volume of urine accumulated in the bladder prior

to voluntary micturition; it is typically evaluated by measuring the maximum, mean, or median voided volume recorded on a bladder diary [23]. Total IgE levels were significantly higher in enuretic group with small functional bladder capacity in accordance with Esperance & Gerrard, [8]. They found that urodynamic evaluation of children with dietary provoked enuresis revealed a functionally small bladder capacity and dietary restriction to those patients. eliminate factors in the diet which can cause an increase in bladder tone leading to increase in functional bladder capacity [8].

Zhang et al. [6] and Wang et al. [24] demonstrated, that IgE and mast cell proteases contributed to neovascularization and vascular cell apoptosis by releasing histamine, proteases, cytokines and monocyte chemoattractant protein after IgE binding to macrophages which may play a role in small bladder capacity. It was also reported that, following food allergy, the food–specific antibodies on mast cells or basophiles leading to release of mediators such as histamine, prostaglandins and leukotrienes that promote vasodilatation, smooth muscle contraction and mucus secretion, resulting in the symptom of intermediate hypersensitivity and decrease in the bladder capacity [22]. The negative correlation between total serum IgE and bladder capacity in our patients was supported by Mungan et al. [22], Zhang et al. [6] and Wang et al. [24]. However, this needs further proof on a large scale.

Voiding efficiency (VE) is defined as percentage of volume voided compared to the pre-void bladder volume. It is one of the indices that provide an easy and precise way of defining an individual voiding function. Voiding efficiency is a clinically meaningful method of assessing bladder emptying function [25]. There was a significant negative correlation between serum IgE and bladder efficiency in our enuretic patients which reflect the effect of IgE on bladder efficiency. The reduction in both bladder efficiency and capacity can result in nocturnal enuresis [3].

Sugai et al. [26] had suggested that measurement of serum ECP or other eosinophil granular proteins may serve as a measure of the activity of the atopic immune response. Our results showed higher level of ECP in patients than controls, but the difference was statically insignificant. Our results contrast with Mungan et al. [22] who reported, significant ECP elevations in their enuretic patients. Studies showed that ECP, induce apoptosis in cells that may produce small bladder capacity [27,28], however our study showed no correlation between ECP and either bladder capacity or efficiency.

Our patients who had small bladder capacity were successfully treated with imipramine which has anticholinergic properties together with antihistaminic and anti-serotonin actions. Some of our patients relapsed after stoppage of this treatment which may be due to persistence of allergen in their food. Esperance & Gerrard, [8] found that the ideal management for those enuretic children was the dietary restriction of offending agents together with imipramine therapy. This finding may highlight the role of IgE on the reduction of bladder capacity and consequently may approve the allergic theory.

5. CONCLUSION

Immunoglobulin E induced hypersensitivity may be one of the pathophysiologic mechanisms implicated in PNE through reduction of the bladder capacity and efficiency.

6. RECOMMENDATION

Further proof studies on a large scale is recommended. Food elimination and challenge test could help if accused allergen is known.

7. LIMITATION OF THE STUDY

Small size of the study group, not all studied patients were subjected to abdominal ultrasonography.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Neveus T, Lackgren G, Tuvemo T, Hetta J, Hjalmas K, Stenberg A. Enuresis—background and treatment. Scand J Urol Nephrol. 2000;206:l-44.
2. Mohammad A, Mohsen D. Nocturnal Enuresis among School Children in Menofia Governorate, Egypt; a Hidden Problem. Journal of American Science. 2012;8(1):327-34. Available:http://www.americanscience.org
3. Amany MA, Hanan MH, Ayat AM, Abeer MA, Amany AF, Hala TE, Maha ME, Marwa IS. Post voiding residual urine volume and genetic assessment in Egyptian children with primary nocturnal enuresis. Research Journal of Medicine and Medical Sciences. 2011;6(1):1-9.
4. Amany AF, Ayat AM, Amany MA, Hanan MH, Mona AA, Amany AE, Hala TE, Marwa IS, Maha ME. Anti-diuretic hormone and genetic study in primary nocturnal enuresis. Available online 14 December 2012, Journal of Pediatric Urology. 2013;9: 831-837.
5. Hanan MH, Amany MA, Ayat AM, Amany AF, Mona AA, Lamia A. Polysomnography and antidiuretic hormone secretion pattern in children with primary nocturnal enuresis. The Egyptian Journal of Neurology, Psychiatry and Neurosurgery. 2015;52(4): 258–263.
6. Zhang J, Sun J, Lindholt JS, Sukhova GK, Sinnamon M. Mast cell tryptase deficiency attenuates mouse abdominal aortic aneurysm formation. Circ. Res. 2011;108: 1316–1327.
7. Yamada T, Murayama T, Mita H, Akiyama K. Bladder hypersensitivity of interstitial cystitis complicated by allergic diseases. Urology. 2001;57(Suppl. 6A):125.
8. Esperanca M, Gerrard JW. Nocturnal enuresis: Comparison of the Effect of Imipramine and Dietary Restriction on Bladder Capacity. Can Med Assoc. J. 1996;101:72.
9. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. J Allergy Clin Immunol. 2006;117:1277–1284. [PubMed]
10. Dudley NJ, Kirkland M, Lovett J. Clinical agreement between automated and calculated ultrasound measurements of bladder volume. The British Journal of Radiology. 2003;76:832-834.
11. Bih LI, Ho CC, Tasi SJ, Lai SL, et al. Bladder shape impact on the accuracy of ultrasonic estimation of bladder volume. Arch Phys Med Rehabil. 1998;79:1553-6.
12. Neveus T, von A. Gontard, Hoebeke P, Hjalmas K. The standardization of terminology of lower urinary tract function in children and adolescents: Report from the standardization committee of the international children continence society. The journal of Urology. 2006;176:314-324.
13. Koff SA. Estimating bladder capacity in children. Urology. 1983;21:248.
14. Chang SJ, Yang SS. Variability, related factors and normal reference value of post-void residual urine in healthy kindergarteners. The Journal of Urology. 2009;182(4):1933-1938.
15. Hagstroem S, Kamperis K, Rittig S, Djurhuus JC. Bladder reservoir function in children with mono-symptomatic nocturnal enuresis and healthy controls. The Journal of Urology. 2006;176:759-763.
16. Leung VYF, Wing CW, Young CK, Metreweli C. Ureteric jet Doppler waveform and bladder wall thickness in children with nocturnal enuresis. Pediatric Research. 2006;60(5):582-586.
17. Kaplan GW, Wallace WW, Orgel HA, Miller JR. Serum immunoglobulin E and incidence of allergy in group of enuretic children. Urology. 1977;10:428-30.
18. Ayat AM, Amany M Al-Aziz, Hanan MH, Amany AA, Mona AMA, Amany A El-Ghany. Assessment of serum level of corticotropin-releasing factor in primary nocturnal enuresis. Journal of Pediatric Urology (Available online 24 October 2016) xx, 1.e1e1.e5.
19. Oei HD, Pelikan-Filipek M, Pelikan Z, van Vliet AC. Enuresis and encopresis as a reaction to food. Ned Tijdschr Geneeskd. 1989;133:1555-7.
20. Rawashdeh YF, Hvistendahl GM, Kamperis K, Hansen MN, Djurhuus JC. Demographics of enuresis patients attending a referral centre. Scand J Urol Nephrol. 2002;36:348-53.

21. Morfin-Maciel BM. Correlation between renal-uretero bladder diseases and allergies. Rev Alerg Mex. 2002;49:60-5.
22. Mungan N. Aydin, Ilker Seckiner, Cetin Yesilll, Bulent Akduman, Ishak O. Tekin. Nocturnal enuresis and allergy. Scandinavian Journal of Urology and Nephrology. 2005;39:237-241.
23. Haylen BT, de Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J, Schaer GN. An international uro-gynecology association (IUGA)/International Continence Society (ICS) joint report on the international terminology of female pelvic floor dysfunction. Neurourology and Urodynamics. 2010;29:4–20.
24. Wang J, Cheng X, Xiang MX, Alanne-Kinnunen M, Wang JA, Chen H, He A, Sun X, Lin Y, et al. J Clin Invest. 2011;121(9):3564-77.
DOI: 10.1172/JCI46028. Epub 2011 Aug 8.
25. Abram P. Bladder outlet obstruction index, bladder contractility index and bladder voiding efficiency: Three simple indices to define bladder voiding function. BJU International. 1999;84:14-15.
26. Sugai T, Sakiyama Y, Matumoto S. Eosinophil cationic protein in peripheral blood of pediatric patients with allergy disease. Clin Exp Allergy. 1992;22:275-81.
27. Chang KC, Lo CW, Fan TC, Chang MD, Shu CW, Chang CH, Chung CT, Fang SL, Chao CC, Tsai JJ, Lai YK. TNF-α mediates eosinophil cationic protein-induced apoptosis in BEAS-2B cells. BMC Cell Biol. 2010;11:6.
DOI:10.1186/1471-2121-11-6.
PMC 2819994. PMID 20089176.
28. Navarro S, Aleu J, Jiménez M, Boix E, Cuchillo CM, Nogués MV. The cytotoxicity of eosinophil cationic protein/ribonuclease 3 on eukaryotic cell lines takes place through its aggregation on the cell membrane. Cell. Mol. Life Sci. 2008;65(2):324–37.
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