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Change of Urinary Nitrite Excretion in Primary Enuresis after Indomethacin Treatment

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Various treatment modalities have been used in primary Nocturnal Enuresis (PNE). Inhibition of prostaglandin synthesis may have value in the management of PNE. The effect of PGs on the urinary system are similar to those of Nitrous oxide (No), so there might be a link between No production and PNE. We can use nitrite as a good indicator of both PG and No because it is a stable metabolite of No. Our objective in this study was to assess urinary nitrite excretion in patients with enuresis and to evaluate the effect of indomethacin (a potent prostaglandin synthesis inhibitor) on urinary nitrite excretion. Sixty children participated in this study with age range 5-14 years and were divided into three groups: Group A comprised 20 children with PNE and were given 50 mg indomethacin suppositories each night for 1 month, group B comprised also 20 children with PNE not receiving treatment. Both groups were assessed by frequency of bed wetting episodes as well as by measuring urinary nitrites. In addition, 20 normal comparable controls were assessed as regards their urinary nitrites to show the difference in its values between enuretics and normal individuals. The results showed increase in nitric acid level in enuretic children than controls with marked decrease in its levels after receiving Indomethacin and marked improvement in the frequency of bed-wetting.

Keywords: Indomethacin; Primary enuresis; Urinary nitrite.

Nocturnal enuresis affects 15% to 20% of 5-years – old children Nocturnal enuresis delays early autonomy and socialization by decreasing in self-confidence and self-esteem. Classification of nocturnal enuresis is the preliminary step to correct therapy. Enuresis is classified as primary (never acquired nocturnal control) or secondary (at last 6 months of dry nights) [1].

There are various effects of PGs on the urethral, vesical, renal and sympathetic nervous system, PGs decrease aldosterone secretion, inhibit tubular reabsorption and ADH, so causes natriuresis, glomerular vasodilatation and diuresis[2]. They also relax the urethra, reduce intraurethral pressure and enhance micturition. In addition, they activate capsaicin-sensitive afferents in urinary bladder and increase acetylcholine from nerves[3].

PGs are proposed to be important in the pathogenesis of primary enuresis; accordingly, strong inhibitors of PG synthesis (e.g. indomethacin and diclofenac) were used to treat primary enuresis [4, 5]. We used Carbamazepine to treat PNE and those not responding, responded well to stronger Prostaglandin inhibitors[6].

In enuretic patients urinary and serum PGE2 concentrations are higher than in controls[7].

There is also evidence of increased production of autacoids in PNE, which can cause diuresis and naturesis[8].

Most of the effects of No are similar to those of PGs on the urinary system[9]. It regulates the paracelluar permeability of proximal tubular cells, Na+/K+ ATPase and Na+/H+ exchanger. It also inhibits sodium reabsorption, mediating pressure diuresis and natriuresis [10]. There are also a relationship between PG production and No biosynthesis, so there might be a link between No production in the urinary system and PNE[11].

We used nitrite as a marker for No production in vivo because it is a stable metabolite of No, and it is the major nitric oxide oxidation products in biological fluids. So nitrites can be used as a good indicator of both PG and NO levels[12, 13].

The aim of our study was to assess urinary nitrite excretion in patients with primary nocturnal enuresis (PNE) and to evaluate the effects of indomethacin (a potent PG synthesis inhibitor) on urinary nitrite excretion.

Patients and Methods

The study comprised forty children (21 girls and 19 boys) recruited from Enuresis clinic of specialized pediatric Hospital of Cairo University and Child Health Clinic, National Research Center. They were divided into two groups:

Group A: Comprised of 20 Children with primary enuresis and were given 50 mg indomethacin suppositories each night for 1 month.

Group B: Comprised of 20 children with PNE not receiving treatment just fluid restriction by night.

Inclusion criteria

1- Age 5-14 years.
2- Parents' consent to participate in the study.
3- Bed wetting each night and completely continent during the day.
4- No evidence of any other systemic disease, neurological disease or urinary tract abnormalities.
5- No medications used within 1 month before the study.

All patients were subjected to the following:

1- History of frequency of bed wetting episodes, frequency of voiding at daytime and voiding abnormalities.
2- Complete clinical examination.
3- Laboratory investigations:

a. Complete urine analysis to exclude bacteriuria, proteinuria or glucosuria.
b. Urea and creatinine using Kone lab automated analyzer (Kone lab Corporation, Rukintie 18, FIN – 02320, ESPOO, Finland).
c. Total nitric oxide: The first urine of the day (mid-stream) was aseptically collected, voided directly into a sterile container. Samples were centrifuged, then diluted 10-folds with reaction buffer and ultra-filtered through a 10.000 molecular weight cut off filter to eliminate proteins.

Samples were analyzed for nitric oxide by colorimetric method (R & D system, Inc. 614 McKinley place N.E. Minneapolis, USA) according to the following principle (R): nitrate is converted to nitrite by nitrate reductase enzyme, nitrite is detected as an azo dye product of the Griess reaction in which acidified nitrite produces a nitro sating agent that reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphtly) ethylene diamine to form the chromophoric azo-derivative, which absorbs light at 450-570 nm[14].

- The previously mentioned tests were assessed at the beginning of the study and at the end after 1 month.
- In addition, 20 normal comparable controls were assessed as regards their urinary nitric acid concentrations to show the difference in its value between enuretics and normal individuals.

Statistical analysis

All values were expressed as the mean (SD) and student's t-test used for the statistical analysis and ANOVA test for comparison between three variables, with $P<0.05$ taken to indicate significant differences.

RESULTS

Our study included 40 children with PNE who were divided into two groups; Group A (11 girls and 9 boys with a mean age of 8.6 ± 2.3) receiving 50 mg Indomethacin suppositories each night for 1 month and group B (9 girls and 11 boys with a mean age of 8.9 ± 2.1) not receiving treatment, just fluid intake restriction by night (Table 1).

Twenty normal comparable controls were assessed at the beginning of the study as

Table 1. Data of the studied groups A and B

	Group A	Group B
Age	8.6 ± 2.3	8.9 ± 2.1
Sex	11 girls and 9 boys	9 girls and 11 boys
Frequency of bed wetting	1-4 / night	1-4 / night
Frequency of day voiding	5-7/ day	4-6/ day

regards only their urinary nitric acid just to show the difference in its levels in normal children from those with PNE (Table 2).

There was a much higher levels of urinary nitric acid levels in groups A and B (enuretic children) than found in normal comparable controls which was highly statistically significant (P.0.001).

After 1 month of treatment by Indomethacin, Group A patients showed a marked

Table 2. Differences in urinary nitric acid levels between enuretic and normal children before the study

	Group A		Group B		Controls	
	Mean	SD	Mean	SD	Mean	SD
Urinary nitric acid	318.7	66.33	323.8	75.43	33.0	7.6
p-value				0.001		

Table 3. Urinary nitric acid levels and frequency of bed-wetting in-group A before and after the study

	Nitric acid µmol/L		Frequency of bed wetting number/night	
	Mean	SD	Mean	SD
Before	318.7	66.33	2.25	0.35
After	146.3	41.58	0.91	0.48
p-value	0.001	0.04		

Table 4. Urinary nitric acid levels and frequency of bed-wetting in Group B before and after the study

	Nitric acid µmol/L		Frequency of bed wetting number/night	
	Mean	SD	Mean	SD
Before	323.8	75.43	2.2	1.45
After	275.0	90.68	1.89	0.75
p-value	0.487		0.366	

Table 5. Urinary nitric acid levels and frequency of bed-wetting in both groups A and B after the study

	Nitric acid µmol/L		Frequency of bed wetting number/night	
	Mean	SD	Mean	SD
Group A	146.3	41.58	0.91	0.48
Group B	275.0	90.68	1.89	0.75
p-value	0.001		0.001	

decrease in urinary nitric acid and the frequency of bed-wetting is markedly decreased (Table 3).

While in Group B patients who did not receive Indomethacin; there was an improvement but it was not statistically significant as regards both nitric acid levels and frequency of bed wetting (Table 4).

On comparing the results of both Groups A and B after the study time, analysis of the results showed a highly statistically significant in the urinary nitric acid and frequency of bed wetting with a much better improvement in group A (Table 5).

DISCUSSION

Our study showed that patients with PNE have high nitrite concentration that markedly decreased after indomethacin treatment and this was associated with a significant decrease in the frequency of bed-wetting.

The means of nitric acid excretion in the normal control was 33 ± 7.6 while it was 318.7 ± 66.3 in group A (Patients with PNE receiving indomethacin) and 323.8 ± 75.4 in group B (enuretics not receiving treatment) at the beginning of the study which is a highly significant difference. While comparing the nitric acid excretion by the end of the study in both of group A and B; their means were 146.3 ± 41.58 and 275 ± 90.68 respectively with a p-value of 0.001, which is a highly statistically significant difference. The decrease in the nitrite excretion was associated with a great improvement in the frequency of bed-wetting in-group A receiving indomethacin, while in-group B (not receiving indomethacin, just fluid restriction) there was an improvement in bed wetting, but was not of statistically significant difference.

So the percentage of improvement in bed wetting and the decrease in the nitric acid excretion in group A after taking indomethacin was 95% white it was only 35% in group B.

It was suggested that there is a major interaction between PG and No in mediating the renal response to various situations. Rajapakse et al., (2004) reported that over production of No increases urine production, decrease urethral pressure and solute excretion allowing passage of little urine in urethra and initiating contraction of the bladder, which is exaggerated by high PG production, encountered to enuresis [15].

Our findings agree with a study done by Jabbour et al., (2019) who stated that urinary excretion of nitrite, nitrate and No production were inhibited by indomethacin treatment and so enuresis is improved by inhibiting No and PG production in urinary system [16].

In addition, a study done by Al-Waili et al., (2005) reported that high urinary nitrite excretion in UTIs and cystitis cause enuresis, and this provide an evidence of the possible role of No in the pathogenesis of enuresis [17].

A report done by Kawauchi et al., (2000) stated that decrease in nocturnal bladder capacity was related to nocturnal voiding frequency in elderly patients, as volumes did not change significantly[18]. Another study done by Al-Waili (2002) showed that indomethacin decreased the nocturnal voiding frequency [19].

Kamperis et al., (2017) stated that inhibiting PGs with indomethacin could increase the bladder capacity and decrease voiding frequency in patients with a small bladder capacity, as PGs prompt bladder contractions and responsible for an unstable bladder[20].

CONCLUSION

In patients with PNE, there is a significant increase in urinary nitrite excretion, indomethacin markedly reduced bed-wetting and decreased the frequency of voiding in enuretics, which was associated with significant decrease in urinary nitrite excretion. Drugs reducing urinary nitrite excretion should be considered as successful therapies in primary nocturnal enuresis.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Neveus T, Fonseca E, Frano I, et al., Management and treatment of nocturnal enuresis – an updated standardization document from the International

children's continence society. *J Pediatr Urol,* 16-10 (2020).
2. Kuznetsova AA, Shakhmatoava EL, Prutskova NP, Nato Chin YV. Possible role of prostaglandins in pathogenesis of nocturnal enuresis in children. *Scandinavian journal of urology and nephrology*, **34**(1): 27-31 (2000).
3. Rahnama'i, M. S., Van Kerrebroeck, P. E., De Wachter, S. G., Van Koeveringe, G. A. The role of prostanoids in urinary bladder physiology. *Nature Reviews Urology*, **9**: 283-290 (2012).
4. Kiddoo D. Nocturnal enuresis. BMJ Clin Evid. 2007 Oct; 1: 0305.
5. Vande Walle J, Rittig S, Bauer S. et al. Practical Consensus guidelines for the management of enuresis. *Eur J Pediatri,* 171: 971 (2012).
6. Al-Waili NS, Al-Waili H, Saloom KY, AL-Waili A, Akmal M, Al-Waili F. Effect of carbamazepine on urinary volume and osmolality, water clearance and serum osmolality in patients with primary enuresis. *Eur Urol,* **50**(4): 844-9 (2006).
7. Zhang ZH, Yu Y, Wei SG, Nakamura Y, Nakamura K, Felder RB. EP3 receptors Mediate PGE2- induced hypothalamic paraventricular nucleus excitation and sympathetic activation. *Am J Physiol Heart Circ Physiol.;* **301**(4): 1559-69 (2011).
8. Lamas S, Rodriguez –Puyol D. Endothelial control of vasomotor tone: The kidney perspective. *Semin Nephrol.*; **32**(2): 156-66 (2012).
9. Robson WL. Clinical Practice. Evaluation and management of enuresis. *N Engl J Med.;* **360**: 1429 (2009).
10. Caldwell PH, Deshpande AV, Von Gontard A. Management of nocturnal enuresis. *BMJ;* **347**: f6259 (2013).
11. Robin A walker. *Nocturnal Enuresis Prim Care.;* **46**(2):243-248 (2019).
12. Deshpande AV, Caldwell PH, Suresh Kumar P. Drugs for nocturnal enuresis in children (Other than desmopressin and tricyclics). *Cochrane Data base Syst Rev.;* **12**: CD002238 (2012).
13. Chan IHY, Wong KKY. Common urological problems in children: primary nocturnal enuresis. *Hong Kong Med J.;* **25**(4): 305-11 (2019).
14. Miles AM. Determination of nitric oxide using fluorescence spectroscopy. *Methods Enzymol.;* **268**: 105-120 (1996).
15. Rajapakse NW, Flower RL, Eppel GA, Denton KM, Malpas SC, Evan RG. Prostaglandins and nitric oxide in regional kidney blood flow responses to renal nerve stimulation *Pflugers Arch*.; **449**(2): 143-9 (2004).
16. Jabbour M, Abou Zahr R, Boustangy M. Primary Nocturnal Enuresis: A Novel Therapeutic strategy with higher Efficacy. *Urology;* 124-241 (2019).
17. AL-Waili NS, Al-Waili TN, AL- Waili AN, Saloom KY. Urinary nitrite excretion and urinary variables in patients with primary nocturnal frequency of micturition, effects of indomethacin suppositories. *World J Urol.;* **23**(4): 287-94 (2005).
18. Kawauchi A, Tanaka Y, Soh J, Ukinura O, Kojima M. Causes of nocturnal frequency and reasons for its increase with age in healthy older men. *J Urol.;* **163**:81-4 (2000).
19. Al – Waili NS. Increased urinary nitrite excretion in primary enuresis: effects of indomethacin treatment on urinary and serum osmolality and electrolytes, urinary volumes and nitrite excretion. *BJU Int*. **90**(3): 294-301 (2002).
20. Kamperis K. Hagstroem S, Faerch M, Mahler B, Rittigs, Djurhuus JC. Combination treatment of nocturnal enuresis with desmopressin and indomethacin. *Ped Nephrol.;* **32**(4): 627-633 (2017).