Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.52.008271

High Urinary Nitric Oxide Levels May Indicate the Presence of Bladder Outlet Obstruction, But Not Overactive Bladder

Kimio Sugaya[1,2]*, Saori Nishijima[1], Katsumi Kadekawa[1,3], Katsuhiro Ashitomi[1,4], Mayuko Sakanashi[5] and Seiji Matsumoto[6]

[1]*Southern Knights' Laboratory, Okinawa, Japan*

[2]*Department of Urology, Kitakami Central Hospital, Okinawa, Japan*

[3]*Department of Urology, Okinawa Kyodo Hospital, Okinawa, Japan*

[4]*Ashitomi Urologic Clinic, Okinawa, Japan*

[5]*College of Pharmacy, Kinjo Gakuin University, Nagoya, Japan*

[6]*Headquarters for Research Promotion, Asahikawa Medical University, Japan*

***Corresponding author:** Kimio Sugaya, Southern Knights' Laboratory, 1-1-823 Miyagi, Chatan 904-0113, Japan.

ARTICLE INFO

Received: August 23, 2023

Published: August 29, 2023

Citation: Kimio Sugaya, Saori Nishijima, Katsumi Kadekawa, Katsuhiro Ashitomi, Mayuko Sakanashi and Seiji Matsumoto. High Urinary Nitric Oxide Levels May Indicate the Presence of Bladder Outlet Obstruction, But Not Overactive Bladder. Biomed J Sci & Tech Res 52(4)-2023. BJSTR. MS.ID.008271.

ABSTRACT

Objectives: Nitric oxide (NO) exerts multiple biological actions and is one of the most crucial signaling molecules. NO is measured as NO_2^- + NO_3^-, which is referred to as NOx. To date, urinary NOx has been measured only in animal studies. Therefore, we investigated the relationship between urinary NOx and lower urinary tract symptoms in urological patients.

Methods: We enrolled 30 male patients with benign prostatic hyperplasia (BPH), 34 female patients with overactive bladder (OAB), and 31 male and 36 female healthy volunteers as controls. BPH was untreated in 15 patients and treated in 15; all treated patients received alpha-1 blockers. OAB was untreated in 16 patients and treated in 18; all treated patients received antimuscarinics. Patients with BPH completed the International Prostate Symptom Score (IPSS), and those with OAB completed the OAB Symptom Score. Urine was collected, and urinary NOx and creatinine were measured. We then examined the relationship between the diseases, questionnaire responses, and urinary NOx (with creatinine correction).

Results: Urinary NOx levels were significantly higher in the untreated BPH group than in the control group, but the levels in the treated BPH group were not different from those in the control group. In male patients, urinary NOx levels correlated positively with postmicturition symptoms, voiding symptoms, and the total IPSS but not with storage symptoms. In female patients, urinary NOx levels did not differ between the control, untreated, and treated OAB groups.

Conclusions: High urinary nitric oxide levels might indicate the presence of bladder outlet obstruction, but not overactive bladder.

Keywords: Bladder Outlet Obstruction; Benign Prostatic Hyperplasia; Biomarker; Overactive Bladder; Urinary Nitric Oxide

Abbreviations: ATP: Adenosine Triphosphate; BOO: Bladder Outlet Obstruction; BPH: Benign Prostatic Hyperplasia; cGMP: Cyclic Guanosine Monophosphate; cre: Creatinine; eNOS: Endothelial Nitric Oxide Synthase; iNOS: Inducible Nitric Oxide Synthase; IPSS: International Prostate Symptom Score; mRNA: Messenger Ribonucleic Acid; nNOS: Neuronal Nitric Oxide Synthase; NO: Nitric Oxide; OAB: Overactive Bladder; OABSS: Overactive Bladder Symptom Score; QOL: Quality of Life

Introduction

Nitric oxide (NO) exerts multiple biological actions and is one of the most crucial signaling molecules [1,2]. It is endogenously synthesized from the precursor L-arginine by a family of NO synthases (neuronal [nNOS], inducible [iNOS], and endothelial NO synthase [eNOS]), with stoichiometric production of L-citrulline. NO activates soluble guanylyl cyclase in cells to synthesize cyclic guanosine monophosphate (cGMP) and relax smooth muscle. It also acts as a neurotransmitter. NO is cytotoxic, and macrophages produce NO to kill pathogens [3,4]. However, in sepsis macrophages produce such a large amount of NO that it may exacerbate inflammation and also cause hypotension (because NO relaxes vascular smooth muscle) [4,5]. NO is immediately oxidized in the living body, so it is measured as NO_2- + NO_3-, which is referred to as NOx [6]. Some animal and clinical studies investigated the relationship between lower urinary tract function and NO by measuring NOS messenger ribonucleic acid (mRNA) and protein in the bladder wall. In an animal model of overactive bladder (OAB), urinary NOx was found in rats with pelvic venous congestion. In this model, eNOS mRNA in the bladder wall and urinary NOx were decreased, and administration of alpha blockers [7,8], phosphodiesterase type 5 inhibitor [9], or antimuscarinics restored NOx levels [10]. On the other hand, animal studies of bladder outlet obstruction (BOO) showed that in the early stages of occlusion the expression of iNOS was increased in the bladder wall and in the chronic phase the expression of both nNOS and eNOS was increased [11,12].

In humans, iNOS was found to be expressed in the bladder wall in BOO associated with benign prostatic hyperplasia (BPH) [13]; another study found no difference in the expression of eNOS in the bladder wall between underactive bladder and non-underactive bladder in patients with BPH [14]. Furthermore, a reduction in NOS-containing nerves was reported in the prostate and bladder/urethra in patients with BOO [15]. However, to our knowledge no clinical studies have evaluated whether a relationship exists between urinary NOx and lower urinary tract symptoms or diseases. Therefore, in this study we investigated whether urinary NOx is associated with lower urinary tract symptoms in male patients with BPH and female patients with OAB.

Subjects and Methods

We recruited 30 male patients with benign prostatic hyperplasia (BPH) and 34 female patients with overactive bladder (OAB) from among the patients who visited the urology department at Okinawa Kyodo Hospital, Okinawa, Japan, or Kitakami Central Hospital, Okinawa, Japan, between February and August 2015. Their urinalysis and urinary sediment were normal. In addition, we recruited 31 male and 36 female volunteers from the staff at both hospitals as controls; volunteers had no or only mild micturition disorders and were not receiving any specific drug treatment: The total International Prostate Symptom Score (IPSS) in the male controls was less than or equal to 9, and the IPSS-quality of life (QOL) index was less than or equal to 2. The men with BPH were divided into an untreated BPH group (n = 15) and a treated BPH group (n = 15) (Table 1). The untreated BPH group consisted of new cases of BPH. In this study, BPH was diagnosed by prostatomegaly (over 25 mL) on transabdominal ultrasonography, a total of 8 or more points on the IPSS, and a score of 3 or more points on the IPSS-QOL index before treatment. The treated BPH group consisted of patients receiving treatment for BPH who were almost satisfied with the treatment results 3 months or more after the start of treatment. All patients with treated BPH patients received alpha-1 blockers, and some were given additional dutasteride, antimuscarinics, or botanical drugs. The women with OAB were also divided into an untreated OAB group (n = 16) and a treated OAB group (n = 18).

Table 1: Characteristics of the study participants.

	No. of Cases	Age (years old)
Males Totals	**61**	
control	31	52.6±18.8
Untreated BPH	15	70.8±13.4**
Treated BPH	15	74.6±10.2**
α1 blocker		
Silodosin	7	
Tamsulosin hydrochloride	4	
Naftopidil	4	
Others		
Dutasteride	5	
Solifenacin succinate	4	
Imidafenacin	1	
Fesoterodine fumarate	1	
Botanical drugs	3	
Females total	**70**	
Control	36	54.8±11.9
Untreated OAB	16	62.9±12.6*
Treated OAB	18	64.6±17.3**
Anti-muscarinics		
Solifenacin succinate	5	
Imidafenacin	5	
Fesoterodine fumarate	4	
Propiverine hydrochloride	4	
Others		
Botanical drugs	4	

Note: BPH: benign prostatic hyperplasia, OAB: overactive bladder, Mean ± standard deviation, *: $p<0.05$, **: $p<0.01$ vs each control.

In this study, patients with organic urinary tract diseases such as pelvic organ prolapse and bladder tumors, as well as interstitial cystitis, were excluded by interview and ultrasound. The untreated OAB

group consisted of new cases of OAB. OAB was diagnosed as a total of 3 points or more on the Overactive Bladder Symptom Score (OABSS) and 2 points or more for the item "urgency" [16]. The treated OAB group consisted of patients receiving treatment for OAB who were almost satisfied with the treatment results 3 months or more after the start of treatment. All patients with treated OAB received antimuscarinics, and some were given additional botanical drugs. The Okinawa Kyodo Hospital Ethics Committee approved the study on behalf of the 2 participating institutions, and each patient provided informed consent to participate in the study prior to enrolment (approval no. 2014-003). Male volunteers and patients with BPH completed the IPSS, and female volunteers and patients with OAB patients completed the OABSS. Urine for urinalysis was collected, and some was frozen and stored (at -80 ºC). At a later date, urinary NOx was measured by the Griess method by a high-performance liquid chromatography system with an automated NO detector (ENO-20, Eicom, Kyoto, Japan) [6]. Urinary creatinine was also measured (BML, INC. Tokyo, Japan). We then examined the relationship of BOO and OAB with questionnaire scores and urinary NOx (with creatinine correction, μM/cre). Results are reported as the mean ± SD. Student's t test was used for statistical analysis, and $P < 0.05$ was considered to indicate statistical significance.

Results

We found no relationship between age and urinary NOx levels in the male and female healthy control volunteers (Figure 1). The untreated and treated BPH groups were significantly older than the control group, but age was similar in the two BPH groups. There was no difference in ultrasound-measured prostate size between untreated (31.9 ± 5.3 mL) and treated BPH groups (33.6 ± 5.6 mL). Urinary NOx levels were significantly higher in the untreated BPH group (0.213 ± 0.205 μM/cre) than in the control group (0.097 ± 0.074 μM/cre; P = 0.019), but they were similar in the treated BPH group (0.129 ± 0.098 μM/cre) and control group (Figure 2A). In the male controls and all BPH patients, the higher the NOx level, the higher the IPSS item score for incomplete emptying (R = 0.2586, P = 0.037), intermittency (R = 0.3537, P = 0.014), straining (R = 0.3490, P = 0.017), and the total score (R = 0.2734, P = 0.047) (Figure 3); the remaining items of the IPSS were not associated with the NOx level. Urinary NOx levels were positively correlated with postmicturition symptoms, voiding symptoms, and the total IPSS, but not with storage symptoms. In the women, the untreated and treated OAB groups were significantly older than the control group, but age was similar in the 2 OAB groups. Urinary NOx levels were similar in the 3 female groups (controls, 0.119 ± 0.083 μM/cre; untreated OAB, 0.110 ± 0.062 μM/cre; treated OAB, 0.153 ± 0.193 μM/cre) (Figure 2B).

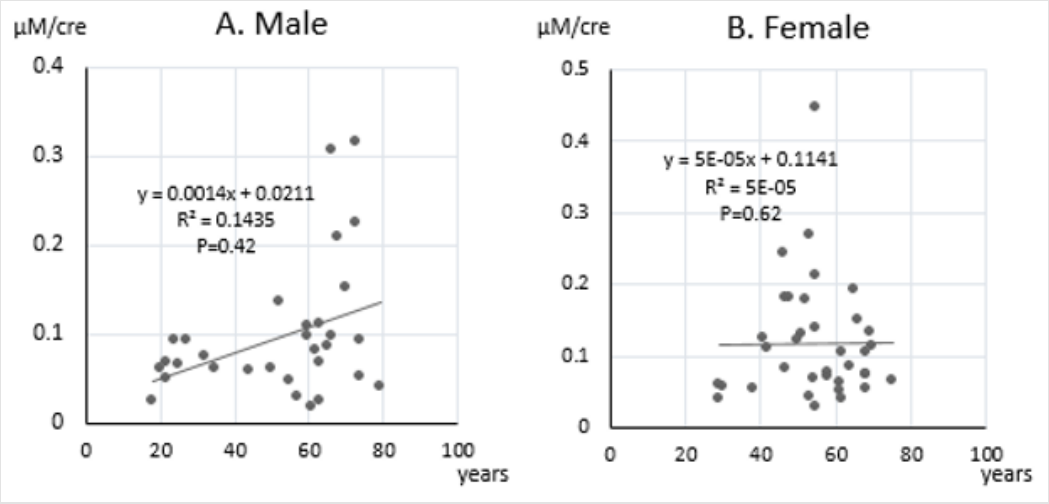

Figure 1: Relationship between urinary levels of NO_2^- + NO_3^- (referred to as NOx) and age in healthy controls
A. Men,
B. Women.

Note: The study found no relationship between age and urinary NOx levels in male and female healthy control volunteers.

 DOI: 10.26717/BJSTR.2023.52.008271

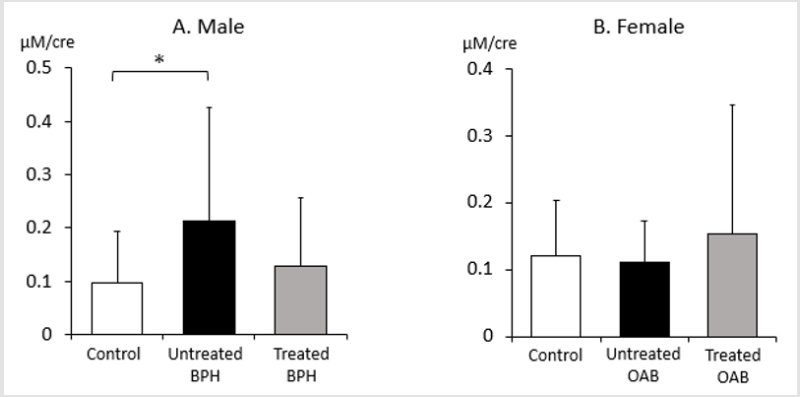

Figure 2: Comparison of urinary levels of NO_2^- + NO_3^- (referred to as NOx)
A. Men (healthy controls, patients with untreated and treated benign prostatic hyperplasia [BPH]).
B. Women (healthy control, patients with treated and untreated overactive bladder [OAB]).

Note: Details in the text. *P < 0.05

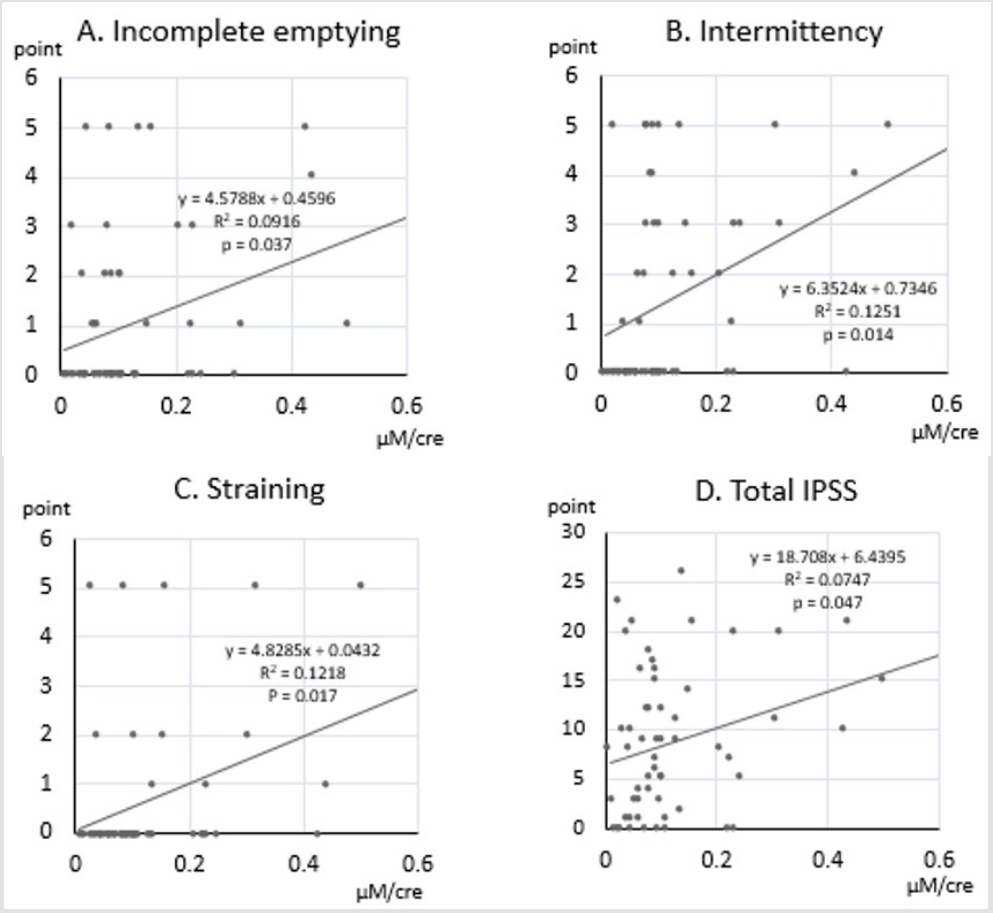

Figure 3: Relationship between urinary levels of NO_2^- + NO_3^- (referred to as NOx) and each item of the International Prostate Symptom Score (IPSS) in all male controls and patients with benign prostatic hyperplasia (BPH).
A. Incomplete emptying,
B. Intermittency,
C. Straining, and
D. Total IPSS.

Note: Details in the text.

Discussion

In the present study, urinary NOx levels were higher in patients with untreated BPH than in controls, but this was not the case in patients with treated BPH or in female patients with OAB. In all male patients, urinary NOx levels were positively correlated with postmicturition symptoms, voiding symptoms, and the total IPSS, but with not storage symptoms. Therefore, we hypothesize that urinary NOx might increase in BOO but not in OAB. To our knowledge, this is the first clinical study investigating the relationship between urinary NOx and lower urinary tract symptoms and diseases.

In a guinea pig model of BOO, after 2 weeks the bladder mucosa showed markedly greater iNOS immunoreactivity and higher iNOS mRNA expression in animals with BOO than in controls [11]. Another study reported that 1 week after the start of BOO iNOS knockout mice had a significantly larger bladder capacity and significantly smaller responses to electrical stimulation than wild type mice [17]. Additionally, 5 weeks after the start of BOO bladder capacity and contractility returned to baseline levels in knockout mice but capacity was significantly larger, and contractility was decreased in wild type mice [17]. These data suggest that in the early stage of an obstruction changes are seen in the bladder and nervous system that may lead to an overactive bladder and, at the same time, iNOS expression is enhanced and NO production increases; this increase in NO suppresses bladder smooth muscle activity, thus counteracting the overactive bladder and preventing subsequent detrusor dysfunction (low activity state). Clinically, in patients with BOO associated with BPH some superficial urothelial cells lacked the protein subunits of the asymmetric unit membrane, suggesting that the blood-urine barrier was compromised in these areas [13]. In such relatively undifferentiated urothelial zones, an accompanying increase was found in the expression of iNOS, which marks perturbed urothelial differentiation and may modulate bladder response to the outlet obstruction [13].

If BOO is chronic, the expression of eNOS and nNOS is increased in the bladder of guinea pigs [11] and rats [12]. In patients with BPH, the expression of eNOS in the bladder wall is similar in those with underactive bladder and those with non-underactive bladder [14]. Six weeks after BOO in rats, the percentage of bladder afferent neurons expressing nNOS immunoreactivity increased in the L6 and S1 dorsal root ganglia [18]. In this condition, NO of the afferent fiber may suppress the overactive bladder if the excitatory nervous system of the spinal cord is suppressed [19], but it may also contribute to the development of bladder overactivity if the inhibitory nervous system of the spinal cord is suppressed [18]. In any case, urinary NOx may be a marker for obstructive bladder.

In one of our animal models of OAB, ie, rats with pelvic venous congestion, eNOS mRNA in the bladder wall and urinary NOx were decreased, and levels were restored by administration of alpha blockers [7,8], phosphodiesterase type 5 inhibitor [9], or antimuscarinics [10]. On the other hand, in the present study urinary NOx was increased in patients with BPH with voiding disorders but not in patients with BPH treated with alpha-1 blockers. These findings of animal experiments and clinical studies might indicate that NOS and NOx do not have clinically relevant effects on the bladder wall. However, long-term NO deficiency causes impaired bladder outlet relaxation and leads to detrusor overactivity [20]. Therefore, NO might act at the bladder neck and urethra, and urinary NOx could be a clinical biomarker of obstructive bladder. The urinary adenosine triphosphate (ATP) to creatinine ratio has also been proposed as a marker of pathological bladder function because it was high in patients with BPH or OAB [21-23]. Treatment with an alpha-1 receptor antagonist or anti-muscarinic agent decreases the urinary ATP to creatinine ratio. However, the urinary ATP level is influenced by bacterial infection, renal dysfunction, voided urine volume, and sex [22,24]. This ratio shows a significant negative correlation with the voided urine volume in healthy men. Thus, urinary ATP levels may not necessarily be a useful marker of bladder pathology. Urinary NO metabolites are also associated with increased blood pressure in normotensive subjects [25], and urinary NOx predicts the progression to hypertension independent of baseline blood pressure [25]. Thus, urinary NOx is also suggested as a biomarker for individual new-onset hypertension. Because of the above findings, blood pressure, bacterial infection, renal dysfunction, and voided urine volume must be considered when evaluating urinary NOx.

This study has some limitations. The number of participants was small. The diagnosis of BOO was made from the measurement of prostate size and the IPSS, but the pressure flow study was not performed. There were multiple therapeutic agents. Future studies are needed to evaluate whether urinary NOx levels change with age or voided urine volume. Thus, our findings are preliminary.

In conclusion, urinary NOx levels were positively correlated with postmicturition symptoms, voiding symptoms, and the total IPSS in men, but not with storage symptoms in men or women. Therefore, high urinary nitric oxide levels might indicate the presence of bladder outlet obstruction. When assessing urinary NOx, physicians must consider blood pressure, bacterial infection, renal function, and voided urine volume, and it will be necessary to unify these conditions.

Disclosure

The authors declare no conflict of interest.

References

1. Ignarro LJ (1990) Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol 30: 535-560.
2. Tsutsui M, Tanimoto A, Tamura M, Mukae H, Yanagihara N, et al. (2015) Significance of nitric oxide synthases: Lessons from triple nitric oxide synthases null mice. J Pharmacol Sci 127: 42-52.
3. Stuehr DJ, Marietta MA (1985) Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide. Proc Natl Acad Sci USA 82: 7738-7742.
4. Kolb H, Kolb-Bachofen V (1992) Nitrix oxide; a pathogenetic factor in autoimmunity. Immunology Today 13: 157-160.

5. Moncada S, Palmer RM, Higgs EA (1991) Nitric oxide. physiology, pathophysiology, and pharmacology. Pharmacol Rev 43: 109-142.
6. Yamashiro S, Noguchi K, Matsuzaki T, Miyagi K, Nakasone J, et al. (2002) Beneficial effect of tetrahydrobiopterin on ischemia-reperfusion injury in isolated perfused rat hearts. J Thorac Cardiovasc Surg 124: 775-784.
7. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T, et al. (2016) Effects of silodosin on bladder activity in rats with frequent urination induced by pelvic venous congestion. Int J Urol 23: 881-887.
8. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T, et al. (2016) Naftopidil improves locomotor activity and urinary frequency in rats with pelvic venous congestion. Biomed Res 37: 221-226.
9. Nishijima S, Sugaya K, Kadekawa K, Ashitomi K, Ueda T, et al. (2019) Tadalafil improves bladder dysfunction and object recognition in rats with pelvic venous congestion. Int J Urol 26: 578-585.
10. Nishijima S, Sugaya K, Kadekawa K, Ashitomi K, Ueda T, et al. (2019) Mechanisms underlying the effects of propiverine on bladder activity in rats with pelvic venous congestion and urinary frequency. Biomed Res 40: 145-152.
11. Chertin B, Rolle U, Solari V, Cascio S, Puri P (2004) The role of nitric oxide in bladder urothelial injury after bladder outlet obstruction. BJU Int 94: 392-399.
12. Kim SO, Choi D, Song SH, Ahn KY, Kwon D, et al. (2013) Effect of detrusor overactivity on the expression of aquaporins and nitric oxide synthase in rat urinary bladder following bladder outlet obstruction. Can Urol Assoc J 7: E268-274.
13. Romih R, Korosec P, Jezernik K, Sedmak B, Trsinar B, et al. (2017) Inverse expression of uroplakins and inducible nitric oxide synthase in the urothelium of patients with bladder outlet obstruction. BJU Int 91: 507-512.
14. Cho KJ, Koh JS, Choi J, Kim JC (2017) Changes in Adenosine Triphosphate and Nitric Oxide in the Urothelium of Patients with Benign Prostatic Hyperplasia and Detrusor Underactivity. J Urol 198: 1392-1396.
15. McVary K (2006) Lower urinary tract symptoms and sexual dysfunction: epidemiology and pathophysiology. BJU Int 97(suppl 2): 23-28.
16. Homma Y, Yoshida M, Seki N, Yokoyama O, Kakizaki H, et al. (2006) Symptom assessment tool for overactive bladder syndrome--overactive bladder symptom score. Urology 68: 318-323.
17. Lemack GE, Zimmern PE, Vazquez D, Connell D, Lin VK (2000) Altered response to partial bladder outlet obstruction in mice lacking inducible nitric oxide synthase. J Urol 163: 1981-1987.
18. Zvara P, Folsom JB, Kliment JJr, Dattilio AL, Moravcíková A, et al. (2004) Increased expression of neuronal nitric oxide synthase in bladder afferent cells in the lumbosacral dorsal root ganglia after chronic bladder outflow obstruction. Brain Res 1002: 35-42.
19. Sasatomi K, Hiragata S, Miyazato M, Chancellor MB, Morris SMJr, et al. (2008) Nitric oxide-mediated suppression of detrusor overactivity by arginase inhibitor in rats with chronic spinal cord injury. Urology 72: 696-700.
20. Noguchi K, Sugaya K, Nishijima S, Sakanashi M, Kadekawa K, et al. (2019) Evaluation of a rat model of functional urinary bladder outlet obstruction produced by chronic inhibition of nitric oxide synthase. Life Sci 234: 116772.
21. Sugaya K, Nishijima S, Kadekawa K, Miyazato M, Mukouyama H (2009) Relationship between lower urinary tract symptoms and urinary ATP in patients with benign prostatic hyperplasia or overactive bladder. Biomed Res 30: 287-294.
22. Antunes-Lopes T, Cruz F (2019) Urinary Biomarkers in Overactive Bladder: Revisiting the Evidence in 2019. Eur Urol Focus 5: 329-336.
23. Chen Z, Liu Y, Zhao M, Zu S, Li Y, et al. (2020) Urinary ATP may be a biomarker for bladder outlet obstruction and its severity in patients with benign prostatic hyperplasia. Transl Androl Urol 9: 284-294.
24. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Yamamoto H (2018) Relationship between voided urine volume and urinary ATP in healthy volunteers. Open J Urol 8: 275-280.
25. Baumann M, Schmaderer C, Kuznetsova T, Bartholome R, Smits JFM, et al. (2011) Urinary Nitric Oxide Metabolites and Individual Blood Pressure Progression to Overt Hypertension. Eur J Cardiovasc Prev Rehabil 18: 656-663.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.52.008271

Kimio Sugaya. Biomed J Sci & Tech Res