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Increased Expression of Hypoxia Inducible Factors in Detrusor Fibrosis Due to Partial Bladder Outlet Obstruction in Male Rats

Yitao Tang[1], Qi Tang[1], Ning Xiao[1,2,3*]

[1]Department of Urology, The Second Affiliated Hospital of Guilin Medical University, Guilin, China
[2]Continence Research Clinic, Department of urology, Shaoyang Central Hospital, Shaoyang, China
[3]Department of Urology, Shaoyang Hosptial Affiliated to University of South China, Shaoyang, China

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***Corresponding author:** Ning Xiao
Department of Urology, The Second Affiliated Hospital of Guilin Medical University, Guilin, China

Abstract **Original Research Article**

Objectives: The aberrant expression of related-fibrosis factors were proved in detrusor of female rats model with bladder outlet obstruction (BOO), but the relationship between hypoxia inducible factors and male gender and related regulation pathways over detrusor fibrosis has were uncommonly explored resulting to the purpose of this study. ***Methods***: A total of 12 Sprague-Dawley (SD) male rats weighing 200 to 250 g were used to induce the partial BOOO (PBOO) model of male rats. After 3 weeks of PBOO construction in male rats, those rats bladder of PBOO and Sham groups were excised and weighed. The mRNA expression of Piezo1, HIF-1α, connexin43, and TGF-β1 in detrusor were measured by Quantitative reverse transcription-polymerase chain reaction (qRT-PCR). ***Results:*** After 3 week of PBOO construction in male rats, significant heavier bladder weight was found in PBOO rats compared to that of sham operation (710.7mg±45.5 vs 218.1mg±26.7, $P < 0.001$) and the mRNA expression of Piezo1, HIF-1α, connexin43, and TGF-β1 was significantly increased in detrusor of PBOO rats compared to that of sham operation. ***Conclusions***: It was suggested that those fibrosis markers in detrusor might be not impacted by different background of sexual hormones between male and female rats. More research should focus on the influence of interaction between ncRNA and fibrosis factors, particularly HIF1A-AS1/HIF-1α pathway and TGF-β1/Smads pathway, on detrusor stability and contractility due to BOO in patients with BPH.

Keywords: Benign Prostate Hyperplasia, Bladder Outlet Obstruction, Detrusor Fibrosis, Hypoxia Inducible Factor, Regulation Pathways.

INTRODUCTION

Benign prostate hyperplasia (BPH) is the most common urological disease for 60 years and over male elderly and causes bladder outlet obstruction (BOO), initially presenting nocturia, frequency, urgency, and urinary incontinence, namely storage phase symptoms of lower urinary tract symptoms (LUTS), and gradually complicating with difficulty in micturition, feeling of non-ability in empting bladder, large post-voiding residual (PVR), and eventual urinary retention (UR), namely voiding and post-voiding phase symptoms of LUTS [1, 2]. Some morphological and pathological changes have been reported to be involved in the clinical process from compensation stage to decompensation phase of bladder detrusor due to BOO [3, 4]. However, the detailed mechanisms in the pathogenesis of these morphological and pathological changes have needed to completely be elucidated although some genes and pathways have considered involvement in it.

Acute urinary retention (AUR) is a landmark of decompensation of detrusor resulted from BOO, detrusor underactivity (DU) has been proved to play an important role in occurrence of AUR beside chronic BOO [5]. Fibrosis in smooth muscle cell of bladder detrusor has been regarded as an eventful pathological state resulting in severe decreased compliance of bladder detrusor, atonic detrusor, large PVR, chronic and acute UR, impaired function of upper urinary tract, and even uremia [3-6]. Blocking of detrusor fibrosis process has been considered a key role in treatment for DU and some target genes have been explored to prove the reasonability of the hypothesis, but the effective drugs for treating DU was not available up to date.

Notably, the female rats were used in mostly previous studies about aberrant expression of some genes in partial BOO (PBOO) model [7-10]. However, there is not the tissue of prostate gland in female rats and different background in sexual hormones between male

and female rats or human being also do not present identical condition of lower urinary tract, which may lead to a bias in understanding of pathogenesis in detrusor dysfunction due to BOO derived from BPH in male elderly using female PBOO rats model. Therefore, we induced the PBOO model in male rats to detect the expression of hypoxia inducible factors and related fibrosis markers in bladder detrusor of PBOO male rats to initially explore the role of those pathways or markers in pathogenesis of detrusor fibrosis due to BOO resulted from BPH.

MATERIAL AND METHODS

Bladder Outlet Obstruction Model of Male Rats

A total of 12 Sprague-Dawley (SD) male rats weighing 200 to 250 g were used for this study. The rats were divided into two groups: a sham-operated control group (n=6), and a 3-week PBOO group (n=6). A suprapubic midline incision was conducted to expose the proximal urethra under intraperitoneally pentobarbital sodium anesthesia (30 mg/kg) (Figure 1). A 4-0 nylon suture was used to ligate the urethra together with indwelling a 3F diameter of drainage tube in urethra. The tube was removed with closing the abdominal incision after finishing the PBOO model of male rats (PBOO group). Similar operation was performed in sham operated rats without urethral ligation (Sham group). After 3 weeks of PBOO construction in male rats, those rats bladder of PBOO and Sham groups were excised and weighed. The experimental protocol was reviewed and approved by the Animal Ethics Committee of Guilin Medical University (GLMC202106004).

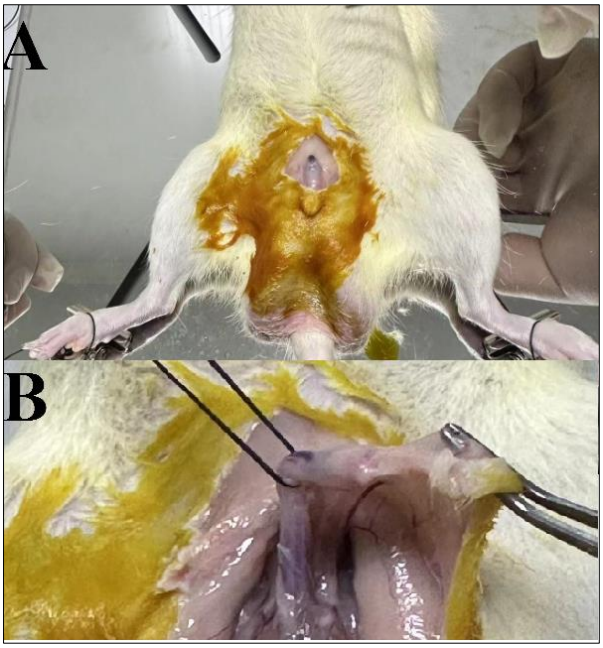

Figure 1: The suprapubic midline incision was conducted (A) and the proximal urethral was exposed (B) in Sprague-Dawley (SD) male rats.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

The bladder body was dissected out and weighed. The mRNA expression of Piezo1, HIF-1α, connexin43, and TGF-β1 in detrusor were measured by qRT-PCR. Total RNAs were extracted from the bladder tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and then reverse transcribed to cDNA with a PrimeScript™ RT Reagent Kit (Takara, Tokyo, Japan) according to the manufacturer's protocols. Quantitative PCR was performed with a 7900 Real-Time PCR Detection System (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Data were analyzed by the $2^{-\Delta\Delta Ct}$method [11]. All Primer sequences used in this study were designed according to previous studies [12-15].

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Differences between the two groups were analyzed using the student's t-test or with ANOVA if more than two groups were assessed. Differences were considered statistically significant at $P<0.05$. SPSS version 27.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all calculations.

RESLUTS

The excised bladders were weighed after 3 week of PBOO construction in male rats, significant heavier bladder weight was found in PBOO group compared to that of sham group (710.7mg±45.5 vs 218.1mg±26.7, $P<0.001$) (Table 1). Although the mRNA expression of Piezo1, HIF-1α, connexin43, and TGF-β1 was significantly increased in detrusor of PBOO

rats compared to that of sham operation after 3 weeks, the extent of statistical significance of difference between two groups in Piezo1 (p=0.021) and TGF-β1 (p=0.023) was numerically weaker than that of HIF-1α (P＜0.001) and connexin43 (P＜0.001) (Figure 2).

Table 1: Comparison of bladder weight between Sham and PBOO rats

Bladder weight (mg)	3wk	*t*	*P*-value
Sham(n=6)	218.1±26.7	22.859	＜0.001
PBOO(n=6)	710.7±45.5		

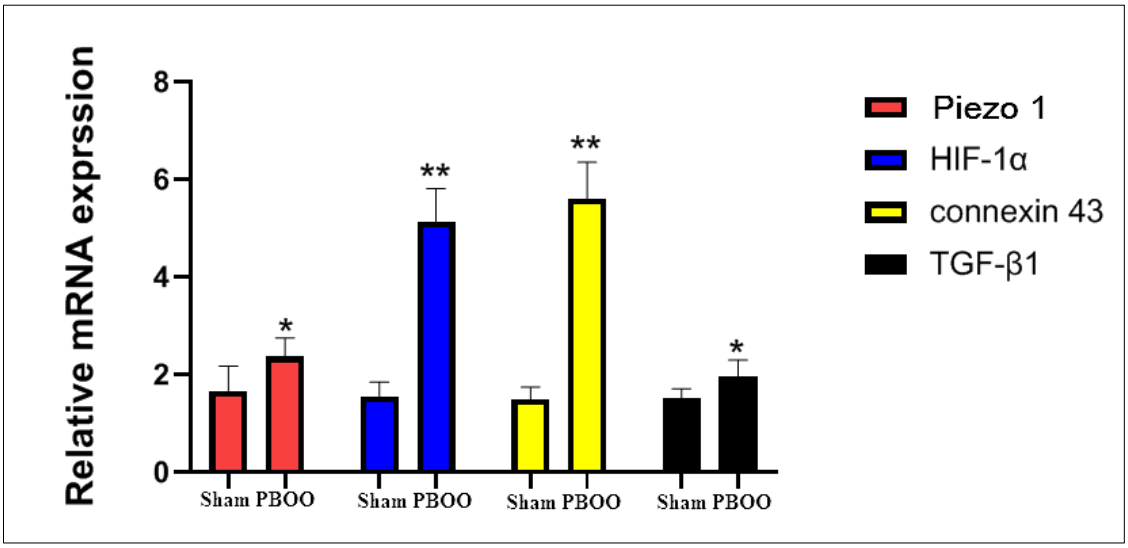

Figure 2: The comparison in the mRNA relative expression of Piezo 1, HIF-1α, connexin43, and TGF-β1 in detrusor between Sham and PBOO male rats. Each bar represents the mean 6 SEM. Single asterisk (*) indicates P＜0.05 vs sham operation. Double asterisks (**) indicate P＜0.001 vs sham operation.

PBOO: Partial bladder outlet obstruction formed by ligating proximal urethra of rats.
Sham: Sham operated rats;

DISCUSSIONS

The mRNA expression of Piezo 1, HIF-1α, connexin43, and TGF-β1 in detrusor of PBOO male rats were significantly higher than that of Sham operation, which was consistent to that of PBOO female rats. It was indicated that fibrosis markers in detrusor might be not impacted by different background of sexual hormones between male and female rats. Therefore, there would be a potential reasonability in the identical prescription for male and female patients with BOO due to various causes, including BPH, urethral stenosis, and neurogenic bladder, according to the finding of this study, although varying levels of metabolic enzymes in different gender, age and hormone states have reported to influence the speed and pathways of metabolism of drugs and foods.

Recently, Piezo1 as a mechanically activated ion channel expressed in various organs including lung, skin, kidney, endothelial cells, and bladder and commonly was considered playing an important role in sensing stretch. In bladder, little has been reported on the role of Piezo2, the other in Piezo family, but there is Piezo1 in the urothelium of normal mice and humans, and then Piezo1 has been indicated to contribute to sensing bladder distention similar to SACs[12]. We hypothesized that the increased mRNA expression of Piezo1 in bladder would cause the increased protein expression of Piezo1 attributing to detrusor overactivity (BOO), which was diagnosed with urodynamic studies (UDS) and presented nocturia, frequency, urgency, and urgent urinary incontinence in BPH/BOO patients.

Consistent to previous report, the increased expression of connexin43 also found in those of male rats in this study [9]. According to previous studies using human and animal bladders, Connexin43 is expressed not only in the urothelium but also in the bladder muscle, and connexin43-containing gap junctions acted as an integrating network for signals to afferent pathways and from motor pathways [16]. Hence, overactivity of bladder (OAB) complained by patients with BOO was partly derived from increasing expression of connexin43 in urothelium and detrusor, and detrusor fibrosis would be ensued without alleviation of BOO.

Although emerging evidence indicates that hypoxia-inducible factor-1α (HIF-1α) plays a key role in carcinogenesis, particularly in hypoxic environments, it also was identified to play important roles in formation of fibrosis in various tissues, especially in ischemia state [17, 18]. AUR and chronic UR have been regarded as the ischemia state for bladder detrusor due to excessive

inflation of bladder. Therefore, it may be the fact that long or chronic urinary retention might place bladder mucosa and detrusor under a detrimental recycle of from ischemia to reperfusion generating oxidative and hypoxia-inducible factors, for example HIF-1α, which has been reported to activate the fibrosis process by interaction with hypoxia-inducible factor-1 alpha-antisense RNA 1 (HIF1A-AS1) [19].

Transforming growth factor-β (TGF-β) is a family of factors, including TGF-β1, that drives fibrosis in many forms of chronic fibrosis disease, including chronic kidney disease (CKD), bladder detrusor fibrosis and liver fibrosis and so on [20]. Given the complex role of Smad proteins in the process of fibrosis with competing profibrotic and antifibrotic actions, it was suggested that complex interplay between TGF-β1/Smads and other signaling pathways including short and long ncRNA (LncRNA) and epigenetic modifications of DNA and histone proteins might be involved in regulation of tissue fibrosis. Given more numerically increased expression of HIF-1α in detrusor of PBOO male rats than that of TGF-β1 indicating ischemia ensued by fibrosis in detrusor, elevated expression of TGF-β1 in detrusor in this study ascertained that there was a promising future in exploration the relationship between detrusor fibrosis due to BOO and hypoxia related LncRNA(HIF1A-AS1)/ TGF-β1/Smads pathway.

However, there were some limitations in this study. First, the sample of male SD rats was relatively small. Second, the measure time from construction of PBOO rats might be short, more obvious fibrosis should be ensued with longer PBOO duration. Finally, there was only bladder weight and qRT-PCR were used to determine the effects of PBOO on detrusor fibrosis, and more assess methods would be utilized in the further research.

CONCLUSIONS

The weight of bladder body and mRNA expression of Piezo 1, HIF-1α, connexin43, and TGF-β1 was significantly increased in male SD PBOO rats compared to that of Sham operation. It was suggested that those fibrosis markers in detrusor might be not impacted by different background of sexual hormones between male and female rats. More research should focus on the influence of interaction between ncRNA and fibrosis factors, particularly HIF1A-AS1/HIF-1α pathway and TGF-β1/Smads pathway, on detrusor stability and contractility due to BOO in patients with BPH.

Competing Interests: The authors report no conflicts of interest in this work.

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