Ultrastructural Study of The Detrusor in End Stage Renal Disease

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Background: *After successful renal transplantation, almost 50% of the patients complained of lower urinary tract symptoms. There is no definite conclusion to explain these voiding symptoms and ultrastructural study of detrusor muscle in end stage renal disease (ESRD) has never been carried out before.*
Objective: *To study ultrastructural changes of detrusor muscle in the specific group of patients with end stage renal disease.*
Material and Method: *Detrusor biopsy of 20 patients, including 15 in end stage renal disease and five in patients with normal creatinine, was obtained by open technique. Biopsy was done during ureteral reimplantation at the time of kidney transplantation. In normal renal function group, detrusor biopsy was done at the time of open bladder surgery from other urologic diseases. The specimens were processed for light microscopy and transmission electron microscopy using standard techniques.*
Results: *All specimens from open biopsy provided sufficient quality to be examined by electron microscope. The average creatinine level was 9.2 and 1.0 mg/dl in the ESRD group and control group, respectively. In the ESRD group, all showed hypertrophy of muscle bundles, fibrosis between muscle bundles, muscle bundle degeneration, and fragmentation of muscle cells. In ESRD group, 93% had fibrosis around nerve bundles and enlarged muscle cell nuclei. About 60% had enlarged nerve bundles, and 53% showed amorphous inclusion in muscle cells. The ESRD group displayed many more ultrastructural changes than in the control group and some appearances were not present in the control group.*
Conclusion: *There were distinct ultrastructural changes of detrusor muscles in ESRD patients. These ultrastructural changes of detrusor muscles may be associated with voiding dysfunction after kidney transplantation.*

Keywords: *Detrusor, Ultrastructural, End stage renal disease*

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The broadly accepted treatment of end stage renal disease (ESRD) is kidney transplantation[1]. For patients with end stage renal disease, some have a lot of urine volume but some have minimal. After successful kidney transplantation, most patients usually immediate have restoration of urine outputs and most of them can void spontaneously without difficulty. There are previous studies showing that 50-54% of patients have frequency in urination and 60-62% have nocturia after kidney transplantation[2,3]. This voiding dysfunction also happened in children undergoing kidney transplantation[4]. Although frequency in urination is a good sign of kidney function, it may cause bothersome symptoms and nocturia will affect their sleeping pattern[5]. Some voiding dysfunction may lead to deterioration of kidney function. Previous studies have shown that voiding dysfunction does not depend on the amount of urine before surgery, duration of hemodialysis and oral fluid intake per day[2]. There is still no clear explanation about this voiding dysfunction. Voiding dysfunction remains after three years of surgery[6]. There are several explanations such as small bladder capacity, nocturnal polyuria, fibrosis of bladder wall, and myogenic failure[2-4]. However, there is no definite proof or conclusion.

There have been ultrastructural studies of detrusor muscle in many situations such as detrusor hyperreflexia and hypoactive bladder[7-11]. However, so far, there are no ultrasturctural studies of detrusor in end stage renal disease. Therefore, the authors

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investigated changes in muscle bundles and nerve bundles by transmission electron microscopy in patients with end stage renal disease undergoing kidney transplantation to see if there might be any morphological change of these structures.

Material and Method

After obtaining local ethics committee's approval, patients with age more than 18 years and able to give informed consent for detrusor biopsy were included in the present study. Exclusion criteria included lower urinary tract diseases: neurogenic bladder, urethral stricture, BPH, history of pelvic irradiation and pregnancy.

Twenty patients were included. Fifteen patients had end stage renal disease and five had normal renal function that required open urologic surgery. Open detrusor biopsy was done in both groups. In the ESRD group, detrusor biopsy was done at the time of neoureterocystostomy during kidney transplantation. For all patients with the normal renal function, detrusor biopsies were done at the time of open urologic surgery that required entering into the bladder wall. To ensure for an adequate specimen, at least 2 mm of detrusor was obtained by a sharp cut with surgical blade to prevent thermal injury to the specimen.

For transmission electron microscopy, the detrusor specimens were first immediately fixed in 4% glutaraldehyde in phosphate buffer, pH7.4 and then in 1% Osmium tetroxide for one hour. After dehydration with alcohol and propylene oxide, the specimens were embedded in Epon block and left in an oven at 70 degree Celsius for one night. Ultrathin sections were cut with ultramicrotome before staining with 2% collodion in amyl acetate. The specimens were viewed in transmission electron microscopy by an experienced uropathologist. Observation on muscle bundles, muscle cell nulei, nerve bundles in detrusor was documented.

Results

Twenty patients were recruited into the present study. Fifteen patients belonged to the ESRD group and five patients were in normal renal function group. Table 1 shows baseline characteristics of both groups. The average creatinine (Cr) was 9.29 and 1.0 mg/dl in ESRD and normal renal function group, respectively. In the ESRD group, an average duration of chronic renal failure (CRF) was 76.47 months. An average duration of hemodialysis was 38.87 months, except for one patient that required peritoneal dialysis for 11 months with 1000 ml of urine output per day before kidney transplantation. The average amount of urine was 332 ml/day. Ten underwent living related doner kidney transplantation (LRKT) and five underwent caderveric doner kidney transplantation (CDKT) as shown in Table 2. Of the normal kidney function patients, three had prostate cancer, one had bladder cancer and one had vesico-vaginal fistula from a previous hysterectomy. The baseline BUN and creatinine in this group were 10.8 and 1.0 mg/dl, respectively.

From twenty specimens, all had adequate tissue for light and electron microscopic evaluation. Fig. 1 and 2 show detrusor cell and Schwann cell from normal creatinine group. There were specific findings that were found only in the ESRD group. All specimens in the ESRD group show hypertrophy, fibrosis, degeneration of muscle bundles and fragmentation of muscle cells as shown in Fig. 3 and 4. In the ESRD group, 93% show enlarged muscle cell nuclei (Fig. 5) and fibrosis around nerve bundles (Fig. 6). Enlarged nerve bundles could be found 60% of the ESRD group.

Table 1. Patients and clinical data

	ESRD	Normal renal function
Total No. of pts.	15	5
Males	9	4
Females	6	1
Age (years)		
Range	24-62	56-72
Mean	48.6	68.4
BUN (mg/dl)	54.33	10.8
Cr (mg/dl)	9.29	1.0

Table 2. Characteristics of ESRD patients

Clinical data	
Duration of CRF (months)	76.47
Duration of dialysis (months)	38.87
No. of hemodialysis (/wk)	2.35
Urine (ml/day)	332
Type of KT	
LRKT	10
CDKT	5

CRF = chronic renal failure; LRKT = living related donor kidney transplantation; CDKT = caderveric donor kidney transplantation

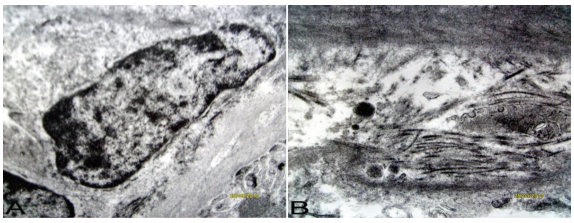

Fig. 1 Normal detrusor smooth muscle
A: Normal smooth muscle cell showing smooth nuclear edge, B: Minimal collagen fiber between smooth muscle cells (x7,000)

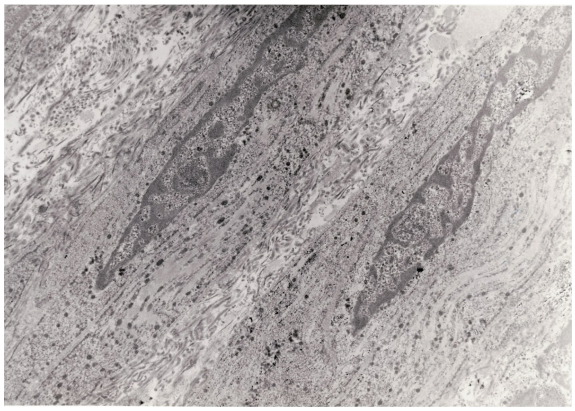

Fig. 2 Normal Schwann cell showing minimal collagen around cell (x10,000)

While there was no specific finding in normal renal function group, muscle bundle degeneration, enlarged nerve bundles, amorphous inclusion in muscle cells and fragmentation of muscle cells could be found only in the ESRD group. The most abnormal finding that could be found in normal renal function group was muscle bundle hypertrophy, which was about 60%. Other findings that could be found in normal renal function group were fibrosis between muscle bundles (40%), enlarge muscle cell nuclei (20%), clump nuclear chromatin (40%) and fibrosis around nerve bundles (20%), as in Table 3.

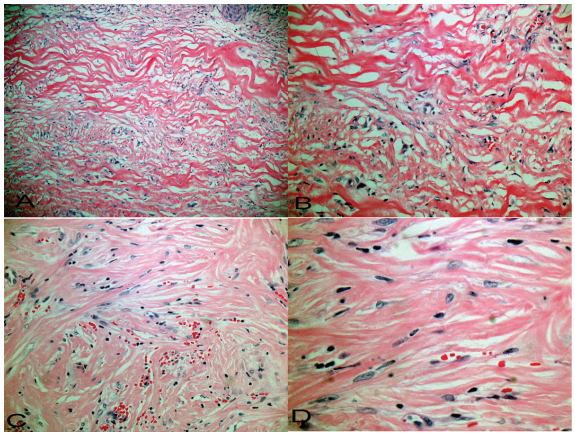

Fig. 3 Detrusor smooth muscle in ESRD from light microscope
A: Collagen bundles in fibrosis (x100), B: Collagen bundles in fibrosis (x200), C: Smooth muscle hypertrophy (x200), D: Smooth muscle hypertrophy (x400)

Discussion

There are several previous ultrastructural studies in many situations. Holm et al described a technique for detrusor biopsy and found that transabdominal abdominal approach is recommended as an efficient method for obtaining biopsies of the detrusor for ultrastructural study in voiding dysfunction[7]. Therefore, in the present study, the authors used open biopsy in both groups to ensure adequate tissue biopsy. In normal renal function group, the authors included prostate cancer patients due to limited number of cases that required open bladder surgery. However, all of them were clinical stage T1c and not in the high-risk group so this will not alter the detrusor muscle.

The present findings revealed that there were specific changes in the ESRD group. Changes in muscle bundles (hypertrophy, degeneration, and fibrosis), enlargement and fibrosis around nerve bundles were seen in nearly all specimens in the ESRD group. The authors tried to explain "what does it mean?". There are previous ultrastructural studies in neurogenic bladder dysfunction. They found that upper motor neuron neurogenic bladder dysfunction is associated with intrinsic neuromuscular defect in the detrusor[8].

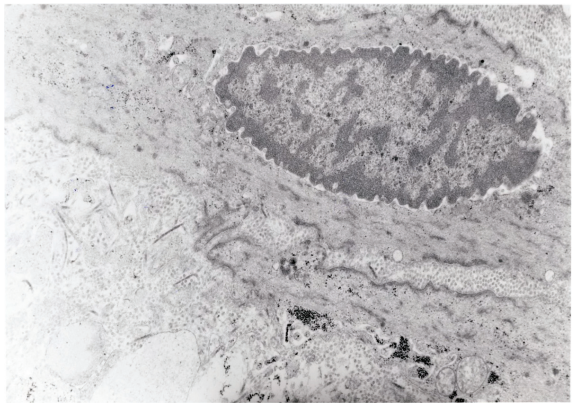

Fig. 4 Hypertrophic smooth muscle cells in ESRD (electron microscope, x20,000)

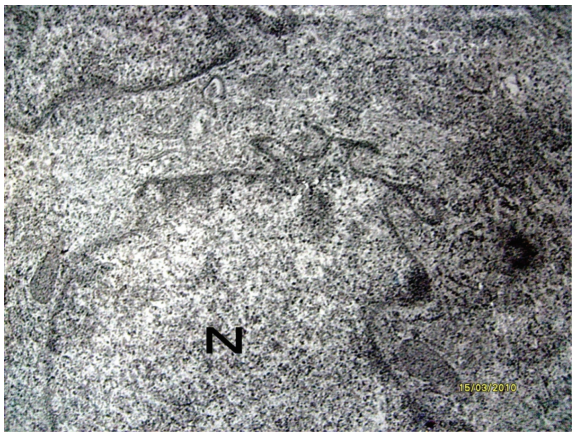

Fig. 5 Enlarged muscle cell nuclei (N), irregular and protrusion of nuclear membrane (x30,000)

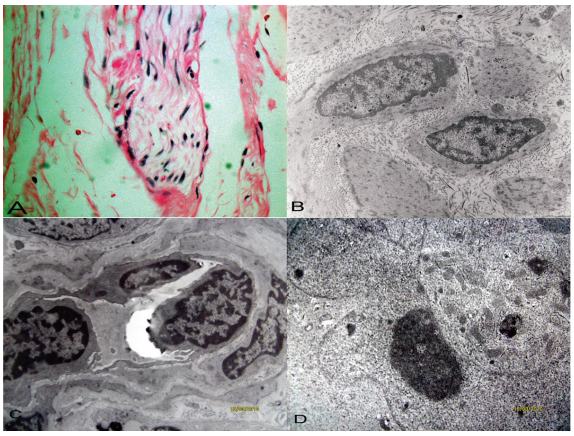

Fig. 6 Detrusor muscle in ESRD
A: Hypertrophic nerve bundle (light microscope, x400), B: Fibrosis around hypertrophic nerve (x9000), C: Thick wall small blood vessels between muscle cells (x7000), D: Amorphous inclusion in muscle cell (x7000)

In neurogenic bladder with urodynamically confirmed detrusor overactivity with 74% can be found of muscle cell degeneration[9]. From the study of Hindley et al, degeneration pattern of detrusor with disruption of muscle cell can be found in all cases of severe hypocontractility[10]. Therefore, the myogenic changes in ESRD patients were similar to detrusor changes in neurogenic bladder patients. Haferkamp et al found that combined degeneration and regeneration are the characteristic changes in intrinsic nerves of detrusor in upper motor neurone neurogenic bladder dysfunction[11]. In the present study, enlarged nerve bundles were found in 60% and fibrosis around nerve bundles were found in 93%. There were the same ultrastructural changes of detrusor in ESRD and neurogenic bladder dysfunction. These myogenic and neurogenic changes in detrusor muscle may be associated with voiding dysfunction after kidney transplantation.

Recent data about urothelium show that the urothelium is a highly specialized structure involved in the micturition reflex and sensory afferent functioning[12,13]. There are many sensory neurons and receptors in urothelium such as nicotinic/muscarinic receptors, transient receptor potential channel vanilloid 1 (TRPV1), transient receptor potential melastatin 8 (TRPM8) and purinergic receptor that play roles in detrusor overactivity[14,15]. In chronic kidney disease, many uremic toxins are found and proved that

Table 3. EM findings in ESRD and normal renal function group

	ESRD group																Normal renal function group					
Patients' number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	%	1	2	3	4	5	%
Hypertrophy of muscle bundles	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	100	√	√	√	-	-	60
Fibrosis between muscle bundles	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	100	√	√	-	-	-	40
Muscle bundle degeneration	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	100	-	-	-	-	-	0
Enlarged muscle cell nuclei	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	93	√	-	-	-	-	20
Clumped nuclear chromatin	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	6	-	√	√	-	-	40
Fibrosis around nerve bundles	√	√	√	√	√	√	√	√	√	√	√	√	√	-	√	93	-	-	√	-	-	20
Enlarged nerve bundles	-	√	-	√	√	√	-	-	√	√	√	√	√	-	-	60	-	-	-	-	-	0
Amorphous inclusion in muscle cells	√	√	-	-	-	√	√	√	-	-	-	√	√	-	√	53	-	-	-	-	-	0
Fragmentation of muscle cells	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	100	-	-	-	-	-	0

these solutes have roles in multiple organ involvement and uremic syndrome[16]. Some uremic toxins can cause specific disease such as amyloidosis from excess β2-microglobulin[16]. Some uremic toxins have effect on vascular smooth muscle such as phenilacetic acid[16,17]. Since the authors' findings are correlated with ultrastructural changes in neurogenic bladder, some uremic toxins in the urine may cause some changes in urothelium mechanoafferent transduction and have an effect on detrusor muscles. Thus, urothelium and uremic toxins in ESRD may be important for a new research, which can explain abnormal detrusor muscle and voiding dysfunction in ESRD patients.

Conclusion

There were distinct ultrastructural changes of detrusor muscles in ESRD patients. These ultra-structural changes of detrusor muscles may be associated with voiding dysfunction after kidney transplantation.

Funding source

Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Potential conflicts of interest

None.

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การศึกษาโครงสร้างระดับย่อยของกล้ามเนื้อกระเพาะปัสสาวะในผู้ป่วยไตวายระยะสุดท้าย

เปรมสันติ์ สังฆ์คุ้ม, พนัส เฉลิมแสนยากร, กิตติณัฐ กิจวิกัย, วิสูตร คงเจริญสมบัติ, วชิร คชการ

ภูมิหลัง: *ภายหลังการผ่าตัดปลูกถ่ายไตร้อยละ 50 ของผู้ป่วยจะมีอาการผิดปกติของการถ่ายปัสสาวะ ยังไม่มีข้อสรุปถึงสาเหตุของอาการดังกล่าว และยังไม่เคยมีการศึกษาโครงสร้างระดับย่อยของกล้ามเนื้อกระเพาะปัสสาวะในผู้ป่วยไตวายระยะสุดท้ายมาก่อน*

วัตถุประสงค์: *เพื่อศึกษาถึงโครงสร้างระดับย่อยของกล้ามเนื้อกระเพาะปัสสาวะในผู้ป่วยไตวายระยะสุดท้าย*

วัสดุและวิธีการ: *ศึกษาโดยการตัดชิ้นเนื้อกล้ามเนื้อกระเพาะปัสสาวะในผู้ป่วย 20 ราย โดยใน 15 ราย ทำในผู้ป่วยไตวายระยะสุดท้าย และในผู้ป่วยที่มีการทำงานของไตปกติ 5 ราย ในผู้ป่วยไตวายระยะสุดท้ายทำการตัดชิ้นเนื้อขณะทำการผ่าตัดฝังท่อไตใหม่ในระหว่างการผ่าตัดปลูกถ่ายไตในผู้ป่วยที่มีการทำงานของไตปกติ ทำการตัดชิ้นเนื้อขณะทำการผ่าตัดเปิดกระเพาะปัสสาวะด้วยโรคอื่นในระบบทางเดินปัสสาวะ ชิ้นเนื้อที่ได้จะถูกเตรียมด้วยวิธีมาตรฐานเพื่อการตรวจทางกล้องจุลทรรศน์และกล้องจุลทรรศน์อิเล็กตรอน*

ผลการศึกษา: *ทุกชิ้นเนื้อที่ได้รับมีคุณภาพเพียงพอในการนำไปตรวจโดยจุลทรรศน์อิเล็กตรอน ค่า creatinine เฉลี่ยคือ 9.2 และ 1.0 มิลลิกรัม/เดซิลิตร ในกลุ่มผู้ป่วยไตวายและกลุ่มผู้ป่วยที่มีการทำงานของไตปกติตามลำดับ ในกลุ่มผู้ป่วยไตวายทั้งหมดตรวจพบ hypertrophy of muscle bundles, fibrosis between muscle bundles, muscle bundle degeneration และ fragmentation of muscle cells พบ fibrosis around nerve bundles and enlarged muscle cell nuclei ได้ร้อยละ 93 พบ enlarged nerve bundles ได้ร้อยละ 60 พบ amorphous inclusion in muscle cells ได้ร้อยละ 53 กลุ่มผู้ป่วยไตวายระยะสุดท้ายมีการเปลี่ยนแปลงของโครงสร้างระดับย่อยของกล้ามเนื้อกระเพาะปัสสาวะมากกว่ากลุ่มผู้ป่วยที่มีค่าการทำงานของไตที่ปกติ และการเปลี่ยนแปลงบางลักษณะตรวจไม่พบในกลุ่มผู้ป่วยที่มีค่าการทำงานของไตปกติ*

สรุป: *จากการศึกษานี้ พบว่ามีการเปลี่ยนแปลงที่ชัดเจนของโครงสร้างระดับย่อยของกล้ามเนื้อกระเพาะปัสสาวะในผู้ป่วยไตวายระยะสุดท้าย มีความเป็นไปได้ว่าการเปลี่ยนแปลงที่เกิดขึ้นนี้อาจเกี่ยวข้องกับอาการผิดปกติของการถ่ายปัสสาวะในผู้ป่วยที่ได้รับการผ่าตัดปลูกถ่ายไต*