Urothelial Dysfunction and Chronic Inflammation are Associated With Increased Bladder Sensation in Patients With Chronic Renal Insufficiency

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Purpose: Chronic kidney disease (CKD) or end-stage renal disease (ESRD) patients usually have lower urinary tract symptoms, such as frequency and urgency. Additionally, they frequently suffer from urinary tract infections. This study investigated dysfunction and chronic inflammation of the bladder urothelium in ESRD/CKD patients.

Methods: This study enrolled 27 patients with CKD (n = 13) or ESRD (n = 14) for urodynamic studies and bladder biopsies. Patients presented with detrusor underactivity (DU; n = 8) or bladder oversensitivity (BO; n = 19). Bladder biopsies were performed in these patients and in 20 controls. The bladder mucosa was examined for E-cadherin and zonula occludens-1 (ZO-1) expression, activated mast cell count (through tryptase staining), and urothelial apoptosis (through terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling [TUNEL]). The urodynamic parameters were also compared with variables regarding urothelial dysfunction.

Results: The bladder mucosa samples of ESRD and CKD patients revealed significantly higher mast cell counts, more urothelial apoptosis, and lower levels of ZO-1 expression than the control samples. E-cadherin expression was significantly reduced in ESRD/CKD patients with DU, but not in ESRD/CKD patients with BO. Increased mast cell and apoptotic cell counts were also associated with ESRD/CKD with BO. Less expression of ZO-1 and E-cadherin was significantly associated with increased bladder sensation and a small bladder capacity.

Conclusions: Bladder urothelial dysfunction and chronic inflammation were present to a noteworthy extent in patients with ESRD or CKD. Increased inflammation and defective barrier function were more notable in ESRD/CKD bladders with BO than in those with DU. The clinical characteristics of these patients may involve urothelial pathophysiology.

Keywords: Bladder dysfunction; Kidney; Lower urinary tract symptoms; Inflammation

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INTRODUCTION

Patients with end-stage renal disease (ESRD) usually have a small bladder capacity and increased bladder sensation. Patients on chronic dialysis usually have lower urinary tract symptoms (LUTS), such as bladder oversensitivity (BO) or difficulty in urination [1]. The prevalence of urinary tract infections (UTIs) and the incidence of urothelial cell carcinoma (UCC) are also higher in patients with ESRD [2]. Among patients with ESRD, the capacity and compliance of the bladder decrease significantly with the duration of dialysis. In one study, abnormal storage function was noted in up to 71% of ESRD patients and bladder outlet obstruction in 51.6% [3]. Vesicoureteral reflux and high postvoid residual (PVR) urine volumes were observed in 110 of 622 (17.5%) and 83 of 62 patients 2 (13.6%), respectively [4]. Although the bladder capacity increased after kidney transplantation and LUTS remained present in only 31 of 622 of patients (4.9%), patients who did not receive kidney transplants still experienced bothersome bladder symptoms [4].

We previously investigated the bladder conditions of patients with ESRD. Abnormal cystoscopic findings were noted in 48.4% of patients, including mucosal fissures, trabeculated bladder, and polypoid formation [3]. These bladder characteristics imply that chronic inflammation and urothelial dysfunction are present in the bladders of patients with ESRD. Clinically, ESRD patients are usually anuric, yet they can still feel lower abdominal discomfort and urgent sensations. UTIs are also frequently encountered in patients with chronic kidney disease (CKD) or ESRD.

Previous studies of interstitial cystitis (IC) have demonstrated that urothelial dysfunction and chronic inflammation are involved in the pathophysiology of that disease [5]. Both phenomena are associated with a small bladder capacity and bladder pain [5]. However, little research has investigated urothelial dysfunction and LUTS in patients with ESRD or CKD. This study was designed to investigate urothelial dysfunction and chronic inflammation in the bladders of patients with ESRD and CKD and to identify associations with patients' clinical characteristics and urodynamic parameters.

MATERIALS AND METHODS

Bladder symptoms and lower urinary tract conditions were investigated in 27 patients with ESRD (n = 13, including 4 male and 9 female patients) or CKD (n = 14, including 10 male and 4 female patients). CKD was defined as an estimated glomerular filtration rate lower than 30 mL/min. ESRD was defined as hemodialysis or peritoneal dialysis of more than 6-month duration. The clinical characteristics of the patients with ESRD/CKD were obtained through chart review. The analytical variables included gender, CKD or ESRD, anuria or nonanuria, history of UCC of the bladder, the presence of recurrent UTIs, and clinical symptoms of bladder pain. None of the patients had current bladder UCC or an active UTI at the time of enrollment.

All patients underwent video-urodynamic studies (VUDS) to evaluate the conditions of their bladder and bladder outlet. We classified patients as having detrusor underactivity (DU) or BO based on their clinical and urodynamic parameters, following the definitions of the International Continence Society [6]. BO was defined as increased bladder sensation in the filling phase of urodynamic studies, including hypersensitive bladder and detrusor overactivity (DO). Bladder biopsies were obtained to check for bladder carcinoma or other lesions during cystoscopy. We also selected 20 female patients with stress urinary incontinence without LUTS, who had undergone suburethral sling procedures, to serve as the control group. All the control patients provided written informed consent and agreed to provide bladder specimens for this study.

The Research Ethics Committee of the Tzu-Chi General Hospital approved the study (TCGH IRB101-61). Each patient was informed of the study rationale and procedures and written informed consent was obtained before cystoscopy and bladder biopsy procedures. All experimental methods were performed in accordance with relevant guidelines and regulations.

The intravesical pressure (Pves) and intra-abdominal pressure, which were used to calculate the detrusor pressure (Pdet), were recorded using VUDS. Cystometrography and cystography were concomitantly performed and C-arm cinefluoroscopy was performed to visualize the bladder neck and urethra during the filling and voiding phases. The highest maximum flow rate (Qmax) during free uroflowmetry or pressure flow studies was selected to represent the parameter of uroflowmetry, and PVR was recorded before VUDS. The terminology used in this study is in accordance with the recommendations of the International Continence Society [6].

We performed cystoscopic bladder biopsies from the mucosal layer at the posterior wall. Four bladder biopsy specimens were taken for a pathological examination to exclude the possibility of carcinoma *in situ* (1 specimen). Three specimens were embedded in optimal cutting temperature medium (Miles,

Elkhart, IN, USA) and frozen in liquid nitrogen (−80°C) for other analyses. The same biopsy and specimen preparation procedures were also performed in the control group.

Immunofluorescence Staining and Quantification of Protein Expression

The urinary bladder specimens were immediately fixed in ice-cold 4% formaldehyde phosphate buffered saline (PBS) (pH, 7.4) solution for 1 hour. Next, they were rinsed overnight with ice-cold PBS containing 15% sucrose at 4°C. Then, the specimens were embedded in optimal cutting temperature medium (Miles) and stored at −80°C in liquid nitrogen. This procedure was the same as in our previous report [7].

Four sections per specimen were cut in a cryostat at a thickness of 8 μm and collected on Polysine glass slides (Fisher Thermo Scientific, Waltham, MA, USA). Sections were post-fixed in acetone at −20°C and blocked with blocking solution (BioGenex Laboratories Inc., Santa Cruz, CA, USA). The sections were incubated overnight at 4°C with a primary antibody. Anti-human zonula occludens-1 (ZO-1) (Invitrogen, Burlington, ON, Canada), anti-human E-cadherin (BD Biosciences, Franklin, NJ, USA), and anti-human mast cell tryptase (Chemicon, Temecula, CA, USA) were used as the primary antibodies. After rinsing the sections with 0.1% Tween-20 in PBS, rabbit anti-mouse immunoglobulin/fluorescein isothiocyanate (FITC) or swine anti-rabbit immunoglobulin/FITC (DakoCytomation A/S, Copenhagen, Denmark) were applied to the sections, which were then incubated for 1 hour at room temperature. Then, sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI) (Sigma Chemical Co., St. Louis, MO, USA). Negative controls were processed using the same procedures, but omitting the primary antibody.

Immunofluorescence-stained images of E-cadherin and mast cell tryptase were assessed using fluorescence microscopy and processed using a digital imaging system (Carl Zeiss, Oberkochen, Germany). The distribution and fluorescence intensity of ZO-1 was evaluated using a confocal microscope (Carl Zeiss). The expression of E-cadherin and ZO-1 in the urothelium was quantified using the Image J software, which was developed by the National Institutes of Health [8]. The number of tryptase-positive mast cells was calculated in 5 consecutive high-power fields (×400) in the area with the highest infiltrate density, and the percentage was computed as the number of tryptase-positive mast cells/total cells per area unit (4 μm²)×100. Three tissue sections per sample from ESRD/CKD

bladder samples and controls were quantified, and the mean values were used in the subsequent statistical analyses [7].

Apoptosis was evaluated through staining with a terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay kit. The tissue sections were incubated with 100 μL of 20 μg/mL proteinase K (Calbiochem, Darmstadt, Germany) at room temperature for 10 minutes and then washed with PBS. The samples were covered with TdT equilibration buffer (Calbiochem) and incubated at room temperature for 30 minutes. After carefully blotting the 1×TdT equilibration buffer from the specimens, we applied the TdT labeling reaction mixture (Calbiochem) onto the specimens and incubated them for 90 minutes at 37°C. After washing the specimens with Tris-buffered saline, the specimens were mounted using fluorescein-FragEL mounting medium (Calbiochem). The total cell population was visualized using a 330- to 380-nm filter for DAPI. The fluorescein-labeled nuclei were visualized using the standard fluorescein filter (465–495 nm) of the Axiovert 200 Inverted Microscope (Zeiss, Thornwood, NY, USA). This procedure was described in our previous study [5].

Statistical Analysis

The urodynamic variables analyzed in this study included the first sensation of filling, full sensation (FS), urge sensation (US), cystometric bladder capacity (CBC), bladder compliance, Pdet, Qmax, and PVR. Descriptive statistics are expressed as mean ± standard deviation or percentages. Differences in the expression of E-cadherin, ZO-1, TUNEL, and mast cell activation (tryptase) between the ESRD and CKD subgroups and the control group were analyzed using the Kruskal-Wallis test (nonparametric analysis of variance). *Post hoc* tests based on the Mann-Whitney U-test with the Bonferroni correction to control for overall type I error were performed to compare the differences between 2 groups. Correlation coefficients were calculated between the urothelial dysfunction parameters and the corresponding urodynamic variables. P-values less than 0.05 were considered to indicate statistical significance. All calculations were performed using SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 27 patients with ESRD (n=13; mean age, 52.5±13.7 years) or CKD (n=14; mean age, 65.3±13.9 years), and 20 control subjects (mean age, 57.9±11.7 years) were enrolled in the

Table 1. Urodynamic parameters and urothelial dysfunction markers in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD)

Variable	Controls (n=20)	ESRD/CKD		
		Total (n=27)	With DU (n=8)	With BO (n=19)
Age (yr)	57.9±11.7	59.1±15.0	54.8±11.1	61.0±16.3
FSF (mL)	180.1±65.8	140.2±94.2	63.5±31.8*	154.2±95.6
FS (mL)	322.1±81.7	178.3±136.1*	66.3±49.6*	206.3±137.3*,#
US (mL)	403.5±104.0	195±133.9*	79.3±62.4*	223.9±132.4*
CBC (mL)	404.8±113	204.5±149.1*	79.3±62.4*	235.8±149.2*
Pdet (cm H₂O)	24.4±15.7	26.9±20.0	10.5±9.19	29.7±20.2
Qmax (mL/sec)	18.2±11.6	11.7±11.3	0	13.7±11.1#
PVR (mL)	51.8±84.0	104.8±164.5	95±77.8	106.4±177.3
Volume (mL)	363.9±175.1	145.7±130.1*	3.33±5.77*	181.3±120.9*,#
Pves (cm H₂O)	32.4±17.1	32.8±20.8	28.5±16.3	33.5±22.0

Values are presented as mean±standard deviation.
DU, underactivity; BO, bladder oversensitivity; FSF, first sensation of filling; FS, full sensation; US, urge sensation; CBC, cystometric bladder capacity; Pdet, detrusor pressure; Qmax, maximum flow rate; PVR, postvoid residual; Pves, intravesical pressure.
*$P<0.05$ compared with the controls. #$P<0.05$ compared between ESRD/CKD with DU and BO.

Table 2. Urothelial dysfunction markers in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) with detrusor underactivity (DU) or bladder oversensitivity (BO)

Variable	Controls (n=20)	ESRD/CKD		
		Total (n=27)	With DU (n=8)	With BO (n=19)
E-cadherin	38.4±19.2	27.1±26.0	14.7±25.5*	32.3±25.1
Mast cells	3.0±2.83	8.45±6.88*	8.52±7.71*	8.42±6.73*
TUNEL	0.49±0.99	1.96±1.86*	0.96±1.23	2.38±1.95*
ZO-1	8.23±4.99	4.73±3.01*	3.77±2.04*	5.14±3.29*

Values are presented as mean±standard deviation.
TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling; ZO-1, zonula occludens-1.
*$P<0.05$ compared with the controls.

final statistical analysis. Among the ESRD/CKD patients, 8 were anuric, and 19 were not anuric; 8 had a history of bladder UCC, and 19 were UCC-free. Urodynamically, 8 patients experienced DU, and 19 experienced BO. Seventeen patients had clinical symptoms of bladder pain, while the remaining 10 did not.

Increased bladder sensation and small voided volume were noted in the overall sample of ESRD/CKD patients. Patients with ESRD had a significantly smaller voided volume (110± 150.0 mL vs. 176.2±109.4 mL) and Qmax (6.6±7.1 mL/sec vs. 11.7±10.6 mL/sec) than CKD patients. Table 1 shows the differences in urodynamic variables between the control subjects and the ESRD/CKD patients. Overall, the ESRD/CKD patients had significantly lower FS, US, and CBC than did the controls. Patients with ESRD/CKD with DU had significantly lower FS,

CBC, Qmax, and voided volume than ESRD/DU patients with BO. Pdet showed no significant difference between the controls and patients with ESRD/CKD.

The bladders from patients with ESRD/CKD showed significantly higher mast cell counts, more apoptotic cells, and less ZO-1 expression than the bladders of control subjects. Patients with ESRD/CKD and DU had significantly less expression of E-cadherin and ZO-1, as well as a higher mast cell count. Patients with ESRD/CKD and BO had significantly higher mast cell counts, more apoptotic cells as indicated by TUNEL, and less ZO-1 expression than the controls (Table 2).

When we compared the urothelial dysfunction parameters according to the clinical characteristics of patients with ESRD and CKD, the expression of E-cadherin was lower in patients

Table 3. Differences in urothelial dysfunction markers in ESRD/CKD patients with different clinical characteristics

Clinical characteristic	E-cadherin	Mast cell activity	TUNEL	ZO-1
Normal (n = 20)	38.4 ± 19.2	3.0 ± 2.83	0.49 ± 0.99	8.23 ± 4.99
ESRD/CKD				
Male (n = 14)	24.7 ± 25.8	7.29 ± 7.91	2.17 ± 2.03*	5.67 ± 3.12
Female (n = 13)	26.3 ± 25.2	9.03 ± 5.85*	1.73 ± 1.76*	3.72 ± 2.77*
CKD (n = 14)	35.0 ± 28.23	7.94 ± 7.2*	1.96 ± 1.92*	5.31 ± 3.07*
ESRD (n = 13)	18.5 ± 21.2*	9.0 ± 6.77*	1.96 ± 1.88*	4.11 ± 2.93*
Anuria (n = 9)	6.12 ± 7.01*	7.46 ± 6.91	1.67 ± 1.87	3.67 ± 1.89*
Nonanuria (n = 18)	35.9 ± 26.1	8.87 ± 7.01*	2.08 ± 1.90*	5.18 ± 3.31
UTI (n = 11)	13.9 ± 16.7	10.3 ± 7.04*	2.04 ± 2.19*	4.06 ± 2.75*
Non-UTI (n = 16)	36.1 ± 27.8	7.16 ± 6.68	1.90 ± 1.68*	5.19 ± 3.17
TCC (n = 8)	25.3 ± 30.2	8.26 ± 6.54	1.31 ± 1.66	4.19 ± 1.37*
Non-TCC (n = 19)	27.8 ± 24.9	8.53 ± 7.19*	2.23 ± 1.92*	4.96 ± 3.48*
Pain (n = 17)	31.0 ± 27.1	8.5 ± 6.97*	2.19 ± 1.98*	5.29 ± 3.44
Nonpain (n = 10)	20.3 ± 23.8*	8.24 ± 7.1*	1.57 ± 1.68*	3.78 ± 1.86*

ESRD, end-stage renal disease; CKD, chronic kidney disease; ZO-1, zonula occludens-1; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling; UTI, urinary tract infection; TCC, transitional cell carcinoma.

with ESRD, anuria, and previous recurrent UTIs, as well as in the nonpain subgroup, than in the controls. Mast cell activity was significantly higher in female patients and the non-anuria, non-UCC, and all ESRD/CKD subgroups than in the controls. Apoptosis was significantly more common in the samples from patients of both genders with CKD and ESRD, with and without recurrent UTIs, and with and without pain, but this difference was only significant in the patients in the non-anuria and non-UCC subgroups. ZO-1 expression was significantly lower in both CKD and ESRD patients, and in both the UCC and non-UCC subgroups, but this difference was only significant in patients with anuria, females, and those who did not experience pain (Table 3).

Fig. 1 shows the urothelial barrier deficits and chronic inflammation in the bladders of the control subjects and the patients with ESRD/CKD with DU and those with BO. The expression of E-cadherin was significantly lower in ESRD/CKD patients with DU, but patients with ESRD/CKD with BO had similar E-cadherin expression levels to the controls. TUNEL staining was prominent in ESRD/CKD patients with BO, whereas ZO-1 expression was lower in the bladders of all ESRD/CKD patients than in the samples from the control subjects.

Among the urothelial dysfunction parameters, the expression of E-cadherin was significantly correlated with that of ZO-1 ($r^2 = 0.325$, P = 0.026) in patients with ESRD/CKD. Increased

mast cell activity was significantly correlated with the results of TUNEL staining ($r^2 = 0.341$, P = 0.019). In the patients with ESRD/CKD, but not in the controls, increased bladder sensation was significantly correlated with low expression of E-cadherin and ZO-1 (Table 4).

DISCUSSION

To our knowledge, this is the first study to investigate urothelial dysfunction in bladders from patients with ESRD and CKD. The results of this study show that chronic inflammation, increased apoptosis, and elevated defective barrier function protein levels were present to a remarkable extent in the bladder urothelium of patients with ESRD/CKD. Urothelial dysfunction parameters, such as E-cadherin and ZO-1, were also associated with increased bladder sensation and a small bladder capacity in patients with ESRD/CKD.

Patients with ESRD usually have a reduced bladder capacity, increased bladder sensation, and lower bladder compliance [3,4]. The risk of urinary tract UCC in patients with ESRD is also increased [2]. Patients with ESRD might also have DO and voiding dysfunction, such as frequency, urgency, and bladder outlet obstruction [9]. Urodynamic studies play a significant role in establishing a definitive diagnosis of lower urinary tract dysfunction prior to kidney transplantation [10]. Although
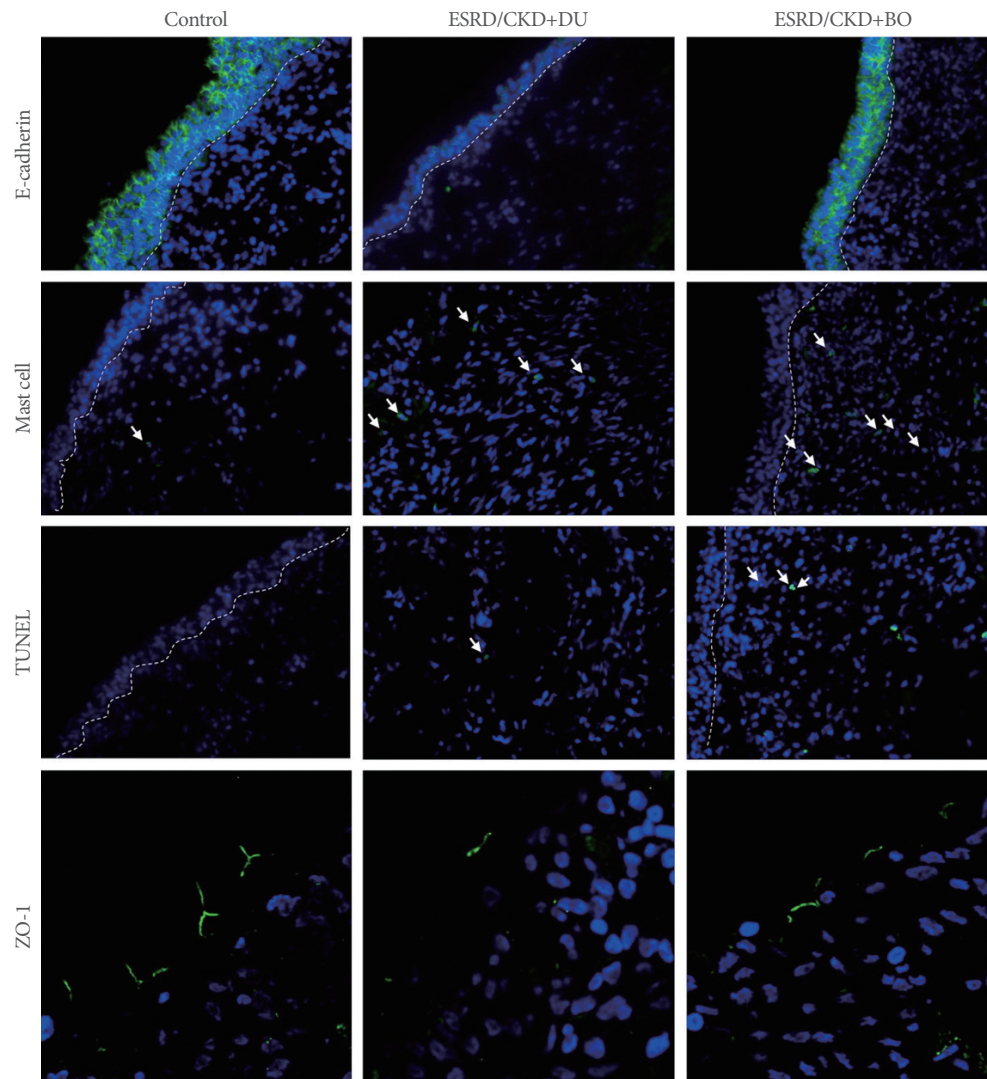
Fig. 1. Urothelial dysfunction and chronic inflammation in the bladders of patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) with urodynamically established detrusor underactivity (DU) or bladder oversensitivity (BO). The expression of E-cadherin was lower in the ESRD and CKD patients with DU, but was similar in those with ESRD/CKD and BO and the control subjects. Mast cell activity was higher in all patients with ESRD or CKD. Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) staining was prominent in ESRD/CKD patients with BO, while zonula occludens-1 (ZO-1) expression was lower in the bladders of all ESRD/CKD patients than in the samples from control subjects.

these bladder dysfunctions in patients with ESRD might not be associated with the development of UTIs, UTIs remain an important complication in patients with ESRD after kidney transplantation [11]. Our study also showed increased mast cell activity, increased urothelial apoptosis, and decreased expression of the barrier protein ZO-1 and the adhesive protein E-cadherin in the bladders of patients with ESRD who had experienced previous recurrent UTIs. These results suggest that defective urothelial barrier function might be the cause of UTIs in

ESRD patients. Our previous studies on urinary tract stones in women with recurrent UTIs also demonstrated similar patterns of urothelial dysfunction in the bladder epithelium [12,13]. These abnormal urothelial changes are related to the bladder dysfunction commonly experienced by patients with ESRD/CKD.

The bladder urothelium is considered not only to act as a barrier, but also to transmit signals of bladder stretching and noxious stimuli [14]. A previous study showed that the antipro-

Table 4. Correlations between urothelial dysfunction markers and urodynamic parameters in the control subjects and patients with ESRD/CKD

Variable	FSF (mL)	FS (mL)	US (mL)	CBC (mL)	Qmax (mL/sec)	Pdet (cm H$_2$O)	PVR (mL)	Vol (mL)	Compliance
Control subject (n=20)									
E-cadherin	0.26	-0.12	-0.19	-.014	-0.13	-0.10	0.05	0.16	-0.11
Mast cells	0.13	-0.16	-0.23	-0.19	0.04	0.03	0.08	0.29	0.34
TUNEL	-0.10	-0.12	0.46	-0.06	-0.10	-0.18	0.35	-0.00	-0.18
ZO-1	0.21	0.06	-0.44	0.06	-0.62*	-0.22	0.42	-0.28	0.23
With ESRD/CKD (n=27)									
E-cadherin	0.490	0.525*	0.554*	0.547*	0.742*	0.011	0.07	0.662*	0.456
Mast cells	0.522	0.513	0.482	0.500	-0.141	-0.425	0.650*	-0.167	0.177
TUNEL	0.006	0.171	0.149	0.146	0.328	0.099	-0.237	0.452	-0.105
ZO-1	0.709*	0.562*	0.578*	0.494	0.184	-0.328	0.456	0.033	-0.075

FSF, first sensation of filling; FS, full sensation; US, urge sensation; CBC, cystometric bladder capacity; Qmax, maximum flow rate; Pdet, detrusor pressure; PVR, postvoid residual; Vol, voided volume; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling; ZO-1, zonula occludens-1.
*P<0.05.

liferative factor presented by the urothelium induced increased membrane permeability in cell cultures; regulated the expression of cytokines, which are linked to enhanced purinergic signaling; and mediated increased bladder sensation [15]. Another study revealed that apoptosis was present in the urothelium of patients with IC and showed that it was possibly regulated by inflammatory pathways. Apoptotic signaling molecules were more common in the bladder tissues of IC patients [16]. The increased apoptosis in the bladder urothelium of IC patients could be due to the upregulation of inflammatory signals [16]. In this study, we observed the same patterns of inflammation, urothelial apoptosis, and barrier deficits in bladder samples from ESRD/CKD patients, suggesting that chronic inflammation might be a fundamental form of pathophysiology in the bladders of these patients.

In this study, the expression of E-cadherin was significantly reduced in patients with ESRD/CKD and DU, but not in patients with ESRD/CKD and BO. The role of E-cadherin in the pathophysiology of bladder dysfunction is still unclear. Some authors have suggested that IC involves abnormal differentiation in the bladder urothelium, with a loss of E-cadherin [17], but another study found increased expression of E-cadherin in IC [18]. In a previous study, we demonstrated a significant association between lower levels of E-cadherin expression and increased pain sensitivity or other clinical symptoms in IC patients [5]. Patients with recurrent UTIs also had lower levels of E-cadherin expression in the urothelium, suggesting that defective adhesive function is associated with impaired barrier function [13]. A molecular connection between E-cadherin and the

transient receptor potential vanilloid receptor subfamily 4 was also found, indicating that E-cadherin is related to bladder sensation through its stretching and barrier function [19]. In this study, all patients with DU had ESRD and anuria, and the bladder had not been filled with urine for a long time. We found that ESRD/CKD patients with DU and anuria who did not experience pain had lower levels of E-cadherin expression, suggesting that E-cadherin mediates bladder sensation and that the presence of urine might affect E-cadherin expression.

ZO-1 is a tight junction protein that provides the highly resistant urothelial barrier [20,21]. A previous study of patients with IC showed deficits of ZO-1 in the urothelium of patients with IC, but not in those with overactive bladder [7]. In this study, the reduced expression of ZO-1 and E-cadherin was remarkable in all ESRD/CKD patients, and less ZO-1 and E-cadherin expression was significantly associated with increased bladder sensation and decreased CBC. In addition, the expression levels of E-cadherin and ZO-1 were significantly correlated, suggesting that the urothelial expression of these proteins is decreased in ESRD/CKD, especially in patients with DU, and that defective barrier function leads to increased bladder sensation.

In current study, we noted that urothelial inflammation, increased apoptosis, and defective ZO-1 expression were similar in patients with ESRD and CKD. Patients with anuria had significantly lower levels of E-cadherin and ZO-1 expression than their nonanuric counterparts. These results indicate that the urothelial dysfunction might not be due to urine-derived factors, but could instead be due to other serological factors related to impaired renal function and increased systemic inflamma-

tion. Our results also corroborate those of a previous study investigating the expression of urothelial differentiation-associated antigens [22]. Patients with ESRD were not deprived of urothelial protein expression due to anuria. Interestingly, we found that the expression levels of E-cadherin and ZO-1 were significantly decreased in patients without pain symptoms. This result might have been due to severe bladder disuse and atrophy of the urothelium in ESRD patients with long-term anuria and the loss of barrier proteins.

Bladder mast cell activation has been reported as a representative pathological finding in a subset of IC patients [23]. Normal basal cell proliferation could be inhibited by chronic inflammation, which might affect apical urothelial function. In this study, the results of TUNEL staining were correlated with those of tryptase staining, indicating that chronic inflammation of the suburothelium was significantly associated with higher levels of urothelial apoptosis in the bladders of patients with ESRD/CKD. These associations demonstrate that inflammation caused increased apoptosis and affected urothelial sensory function in ESRD/CKD patients.

Bladder pain and irritative symptoms are common complaints of patients with ESRD [1,3]. Small bladder capacity and low bladder compliance result in high Pves with very small urine volumes [3,9,10]. In this study, we did not find a significant correlation between bladder compliance and urodynamic parameters in patients with ESRD/CKD. The expression of E-cadherin and ZO-1 likewise was not significantly lower in ESRD/CKD patients with bladder pain. BO and bladder pain symptoms are likely to occur due to a smaller bladder capacity rather than as a result of defective urothelial barrier function. Moreover, dilute and low-osmolarity urine, as well as changes in the components of the urine of ESRD/CKD patients, may cause bladder mucosal irritation. In the context of IC/painful bladder syndrome, chronic inflammation and deficits in the urothelium may lead to acute afferent nerve excitation and long-term plasticity that decreases the threshold for nociceptive and mechanoceptor afferent fibers [24,25]. We suggest that chronic pain syndrome in the bladders of patients with ESRD/CKD could be related to central nervous system sensitization and sustained abnormalities in or activation of the sensory afferent nerves in the urinary bladder. Analysis of the functional proteins in the urothelium might help explain the underlying pathophysiology.

The limitations of this study are the small number of cases, the lack of CKD grading, and the unknown duration of ESRD.

Nevertheless, chronic inflammation and defective urothelial barrier proteins were present in both CKD and ESRD patients, resulting in LUTS. Another limitation is the fact that the control group only included female patients. Male patients >50 years of age generally had prostatic problems potentially related to LUTS, which made it more complicated to identify a suitable control group. Normal bladder specimens from male patients <50 years of age are difficult to obtain. The aim of this study was to investigate urothelial dysfunction in ESRD/CKD patients, so female stress urinary incontinence patients without LUTS were used as controls with normal bladder function.

In conclusion, increased urothelial inflammation and apoptosis and decreased ZO-1 expression were found in patients with ESRD/CKD. Decreased E-cadherin expression was found only in patients with ESRD and DU, but not CKD and BO. These urothelial dysfunction markers were significantly associated with increased bladder sensation and reduced bladder capacity.

AUTHOR CONTRIBUTION STATEMENT

· Full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis: *Sheng-Fu Cheng*
· Study concept and design: *Hann-Chorng Kuo*
· Acquisition of data: *Sheng-Fu Cheng*
· Analysis and interpretation of data: *Sheng-Fu Cheng*
· Drafting of the manuscript: *Sheng-Fu Cheng*
· Critical revision of the manuscript for important intellectual content: *Yuan-Hong Jiang*
· Statistical analysis: *Sheng-Fu Cheng*
· Obtained funding: *Hann-Chorng Kuo*
· Administrative, technical, or material support: *Hann-Chorng Kuo*
· Study supervision: *Yuan-Hong Jiang*

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