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FUNCTIONAL UROLOGY

Does diabetes affect the distribution and number of interstitial cells and neuronal tissue in the ureter, bladder, prostate, and urethra of humans?

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Introduction The aim of this study was to investigate and compare the distribution and number of interstitial cells (ICs) and neuronal tissue in the ureter, bladder, prostate, and urethra of human patients with and without diabetes.

Material and methods Human tissue was obtained from patients who had undergone radical cystectomy for bladder cancer (10 diabetic and 11 non–diabetic males). Interstitial cells were stained immunohistochemically with anti–human CD117 (c–kit) rabbit polyclonal antibody, Vimentin, and Connexin–43. Neural tissue was stained with synaptophysin. The number of ICs and neurons was evaluated and compared between the groups (diabetic *versus* non–diabetic).

Results The mean number of c–kit (+) ICs in bladder lamina propria was significantly decreased in diabetics (32.40 ±12.96 *versus* 57.18 ±25.37, p = 0.036). The mean number of ICs in the detrusor muscle was significantly decreased in diabetics (40.50 ±16.79 *versus* 64.55 ±22.08, p = 0.013). Between the groups, no significant differences were detected regarding the number of ICs at the level of the ureter, urethra, and prostate. No significant differences were detected regarding the number of nerves in the ureter, bladder, prostate, and urethra of both groups.

Conclusions The number of ICs may be decreased in the lamina propria and detrusor muscle of the human bladder in diabetes. This can be an underlying cause of lower urinary tract (LUT) dysfunction in diabetics. Research into the development of drugs targeting or stimulating IC function in order to prevent diabetic LUT dysfunction is warranted.

Key Words: bladder ‹› diabetes ‹› human ‹› interstitial cells ‹› neurons ‹› prostate ‹› ureter ‹› urethra

INTRODUCTION

Diabetes is a serious metabolic disease that affects many organs. Almost onequarter of patients admitted to the hospital are reported to have diabetes [1]. In the urinary tract, diabetes may lead to urinary bladder dysfunction such as acontractile bladder [2] and may also lead to functional and anatomical disturbances of the external urethral sphincter resulting in voiding dysfunction and urinary incontinence [3, 4, 5].

Previous research has demonstrated that diabetes leads to nerve function disturbances, loss of innervation of the neuromuscular nerve terminals, and altered sympathetic/parasympathetic innervation [2, 6, 7]. Therefore, it is thought that the main underlying mechanism of diabetic organ dysfunction is neurological in origin and can involve autonomic nerves, peripheral nerves, or both [2].

Cells having similar morphological characteristics to the interstitial cells of Cajal of the gastrointestinal tract have also been demonstrated in the hu-

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man urinary tract [8]. Interstitial cells (ICs) in the urinary tract have been suggested to play important functional roles including acting as stretch or chemical sensors which trigger detrusor contractions in the bladder [8, 9, 10] and maintain urethral tone by modulating the frequency of tonic contractions of the urethral smooth muscle [11]. The functional importance of prostatic ICs is currently not known. In human tissues, ICs and nerves were demonstrated to be closely located to each other, suggesting that these cellular components work together [8, 9, 10].

We have recently demonstrated that the amount of both ICs and neural tissue was significantly decreased in the bladder of rabbits with diabetes compared to that of the control group [12]. No significant differences were found regarding the same cells at the level of the urethra and prostate [12]. Therefore, it has been suggested that ICs and neurons may be adversely affected by diabetes in the human urinary tract and thus regarded as a new mechanism for the development of diabetic lower urinary dysfunction [13].

It is known that c–kit is a specific marker for ICs [4, 8, 12]. Additionally, antibodies targeting connexin 43 [8, 14] and vimentin [8, 15] molecules were also used to stain ICs in the human urinary tract. Therefore, we used antibodies targeting c–kit, connexin 43 and vimentin in order to stain and detect ICs in the human urinary tract tissues.

Synaptophysin is a major protein of membrane neurotransmitter–containing vesicles and it is used as a neurofilament marker of neural tissue [12, 16, 17, 18]. Therefore, we used synaptophysin in order to detect the neural tissue in the urinary tract in our study.

To the best of our knowledge, the impact of diabetes on the amount of ICs and neural tissue in the human urinary tract has not yet been investigated. In an effort to further investigate our hypothesis in humans, in the present study we compared the distribution and number of ICs and neuronal tissue in the ureter, bladder, prostate, and urethra of humans with and without diabetes.

MATERIAL AND METHODS

Human tissue was obtained from patients who had undergone radical cystectomy for bladder cancer (10 diabetic, 11 non–diabetic males). All of the diabetic patients had type–2 diabetes. The mean patient age was 65.7 ±9.2 (range, 51–78) in the diabetic group and 57.1 ±13.2 (range, 40–75) in the non–diabetic group ($p > 0.05$). Patients without previous intravesical therapy and systemic preoperative neoadjuvant chemotherapy history were chosen and included in our study. The characteristics of diabetic and non–diabetic patients who underwent radical cystoprostatectomy for bladder cancer are shown in Table 1.

In order to standardize sampling and limiting factors that might influence the number of ICs and nerves, particularly in the urinary bladder, we selected patients who had not undergone previous intravesical chemotherapy or immunotherapy and systemic chemotherapy for bladder cancer. Tissues for evaluation were obtained from the lateral bladder walls. Additionally, all the microscopic evaluations were performed by experienced uro–pathologists and tissue sections without any microscopic tumors or inflammation that represented the normal urinary tract tissue layers were selected.

In addition to the ICs, antibodies targeting vimentin also extensively stained fibrocytes, lipocytes, smooth muscle cells, vascular endothelial cells, and peripheral nerve cells. Therefore, although we were able to demonstrate ICs with vimentin immunohistochemically, we did not perform statistical analysis between the groups by using vimentin immunohistochemical staining.

Histopathological evaluation and immunohistochemistry

Immunohistochemical studies including c–kit (CD117), connexin 43, vimentin and synaptophysin molecules were performed on all tissues. First unstained whole sections cut at 5–μm thickness were prepared from blocks for immunostaining. Immunohistochemical staining was performed by the standard streptavidin–biotin complex method with antibodies raised against c–kit (rabbit polyclonal, A4502 Dako,1:400); connexin 43 (rabbit polyclonal 71–0700, Invitrogen, 1:100); vimentin (Dako, Clone V9, M0725, 1:100) and synaptophysin (clone SY38,M0776 Dako, 1:20). Secondly, sections were deparaffinized and rehydrated, and endogenous peroxidase activity was blocked with a 0.3% solution of hydrogen peroxidase in phosphate–buffered saline (0.01 mol/L, pH 7.5) at room temperature for 10 minutes. The sections were treated with 0.01 mol/L sodium citrate buffer (pH 6.0) in a pressure cooker for 10 minutes. Then, primary antibodies were allowed to react at room temperature for 30 minutes for all four stains. After washing the samples/slides in phosphate–buffered saline, a secondary antibody was applied for 10 minutes, followed by streptavidin–peroxidase complex (ScyTek Laboratories, Logan, UT). Peroxidase was visualized by diaminobenzidine tetrahydrochloride containing 0.3% H_2O_2. After rinsing the samples/slides in deionized water and counterstaining with

Table 1. *Characteristics of diabetic and non–diabetic patients who underwent radical cystectoprostatectomy for bladder cancer*

	Age	Surgery type	Previous intravesical chemotx or immunotx	Neoadj. CTx	Duration of DM (years)	Tx of DM	Bladder pathology	Prostate pathology	Right ureter pathology	Left ureter pathology	Urethra pathology
Diabetic patients (n=10)											
1.	78	Open	None	None	5	OAD + Diet	UCC (pT2, high grade)	Tumor (–) (Chronic active prostatitis,BPH)	Tumor (–)	Tumor (–) (Chronic inflammation)	Tumor (–)
2.	54	Open	None	None	3	Insulin + Diet	UCC (pT2, high grade)	PCa, BPH	Tumor (–)	Tumor (–)	Tumor (–)
3.	67	Open	None	None	7	Diet	UCC (pT2, high grade)	HPIN, BPH	Tumor (–)	Tumor (–)	Tumor (–)
4.	73	Open	None	None	2	OAD + Diet	UCC (pT2, high grade)	HPIN, BPH	Tumor (–)	Tumor (–)	Tumor (–)
5.	75	Open	None	None	9	OAD + Diet	UCC (pT2, high grade)	BPH	Tumor (–)	Tumor (–)	Tumor (–)
6.	66	Robotic	None	None	2	OAD + Diet	CIS and granulation tissue	BPH	Dysplasia	Tumor (–)	Tumor (–)
7.	69	Robotic	None	None	10	Insulin + Diet	UCC (pT2, high grade)	BPH	Tumor (–)	Tumor (–)	Tumor (–)
8.	68	Open	None	None	10	Insulin + Diet	UCC (pT2, high grade)	BPH	Severe dysplasia	Tumor (–) (Chronic inflammation)	Tumor (–)
9.	56	Open	None	None	7	Insulin + Diet	No residual tumor in the bladder	PCa, BPH	Tumor (–)	Tumor (–)	Tumor (–)
10.	51	Open	None	None	7	Insulin + Diet	UCC (pT2, high grade)	BPH	Tumor (–)	Tumor (–)	Tumor (–)
Non–diabetic patients (n=11)											
1.	40	Open	None	None	–	–	UCC (pT2, high grade)	PCa, BPH	Tumor (–)	Tumor (–)	Tumor (–)
2.	46	Open	None	None	–	–	UCC (pT2, high grade)	Granulamatous prostatitis,BPH	Tumor (–)	Dysplasia	Tumor (–)
3.	72	Open	None	None	–	–	UCC (pT2, high grade)	PCa, BPH	Tumor (–)	Tumor (–)	Tumor (–)
4.	75	Open	None	None	–	–	UCC (pT2, high grade)	HPIN, BPH	Tumor (–)	Tumor (–)	Tumor (–)
5.	55	Robotic	None	None	–	–	UCC (pT2, high grade)	BPH	Dysplasia	Tumor (–)	Tumor (–)
6.	70	Robotic	None	None	–	–	UCC (pT2, high grade)	PCa, BPH	Tumor (–)	Tumor (–)	Tumor (–)
7.	70	Robotic	None	None	–	–	UCC (pT2, high grade)	BPH	Tumor (–)	Tumor (–)	Tumor (–)
8.	39	Open	None	None	–	–	UCC (pT2, high grade)	BPH	Tumor (–)	Tumor (–)	Tumor (–)
9.	59	Open	None	None	–	–	UCC (pT2, high grade)	BPH	Tumor (–)	Tumor (–)	Tumor (–)
10.	47	Robotic	None	None	–	–	UCC (pT2, high grade)	BPH	Tumor (–)	Tumor (–)	Tumor (–)
11.	55	Open	None	None	–	–	UCC (pT2, high grade)	HPIN, BPH	Tumor (–)	Tumor (–)	Tumor (–)

Chemotx: Chemotherapy, Immunotx: Immunotherapy, Neoadj CTx: Neoadjuvant chemotherapy, DM: Diabetes Mellitus, Tx: Treatment, PCa: prostate cancer, HPIN: High–grade prostatic intraepithelial neoplasia, BPH: benign prostate hyperplasia, OAD: Oral anti–diabetic drugs, UCC: Urothelial cell carcinoma, CIS: Carcinoma in situ.

Harris hematoxylin, the slides were dehydrated and mounted. Gastrointestinal stromal tumor, islet cells in pancreas, heart tissue and a leiomyoma were used as control tissues for c–kit, synaptophysin, connexin 43 and vimentin, respectively.

Evaluation of immunostaining

Positive staining of ICs and neural tissue in the lamina propria of bladder, detrusor muscle, prostate, ureter, and urethra was evaluated separately. C–kit positive ICs were counted in 10 consecutive high–power fields under a light microscope (Nikon 80i, Japan). A similar counting process was performed along the entire urinary tract for connexin 43 positive ICs and synaptophysin positive neural tissue. The statistical comparison between groups was based on the total number of ICs and neural tissue counted in 10 high–power fields.

Statistical analysis

Statistical analysis of the c–kit, connexin 43, and synaptophysin immunostaining between the groups was performed with the Mann Whitney U test with the use of the commercially available software Scientific Package for Social Sciences (SPSS 16). $P < 0.05$ was considered statistically significant.

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RESULTS

We were able to successfully stain and demonstrate ICs with c–kit and connexin 43 antibodies in our study. Similarly, neural tissue was successfully stained and demonstrated with synaptophysin immunohistochemistry. Statistical analysis was performed between the groups by using c–kit, connexin 43 and synaptophysin immunostaining.

The number of c–kit positive and connexin 43 positive ICs was detected to be significantly decreased at the level of the bladder lamina propria and detrusor muscle in diabetic patients compared to non–diabetics, respectively ($p < 0.05$ for both) (Tables 2 and 3). However, no significant differences were detected at the level of ureter, urethra and prostate between the two groups by using c–kit and connexin 43 staining, respectively (> 0.05 for both) (Tables 2 and 3). No significant differences were detected in terms of the number of nerves between diabetic and non–diabetic groups at the level of bladder lamina propria, detrusor muscle, ureter, urethra, and prostate ($p > 0.05$) (Table 4).

Table 2. *Comparison of number of interstitial cells in diabetic and non–diabetic human urinary tract tissues by immunostaining with c–kit*

Interstitial cells	DM (n=10)	non–DM (n=11)	p
Ureter	39.30 ±19.09 (range, 17–78)	48.82 ±17.63 (range, 18–88)	0.197
Urethra	51.90 ±22.89 (range, 21–88)	55.27 ±15.13 (range, 25–76)	0.705
Prostate	64.10 ±12.31 (range, 49–84)	58.82 ±16.60 (range, 32–87)	0.605
Bladder lamina propria	32.40 ±12.96 (range, 17–58)	57.18 ±25.37 (range, 25–91)	0.036
Bladder detrusor	40.50 ±16.79 (range, 21–82)	64.55 ±22.08 (range, 18–97)	0.013

Table 3. *Comparison of number of interstitial cells in diabetic and non–diabetic human urinary tract tissues by immunostaining with connexin 43*

Interstitial cells	DM (n=10)	non–DM (n=11)	p
Ureter	36.70 ±13.26 (range, 16–55)	46.00 ±14.96 (range, 20–77)	0.197
Urethra	42.90 ±16.63 (range, 24–75)	52.45 ±13.16 (range, 26–70)	0.173
Prostate	61.80 ±12.31 (range, 50–80)	59.00 ±13.00 (range, 40–80)	0.654
Bladder lamina propria	30.50 ±14.74 (range, 16–65)	50.91 ±19.18 (range, 22–75)	0.020
Bladder detrusor	36.80 ±17.05 (range, 20–80)	64.55 ±17.46 (range, 22–80)	0.006

Table 4. *Comparison of number of nerves in diabetic and non–diabetic human urinary tract tissues by immunostaining with synaptophysin*

Nerves	DM (n=10)	non–DM (n=11)	p
Ureter	1.50 ±1.84 (range, 0–5)	1.45 ±1.37 (range, 0–4)	0.173
Urethra	0.80 ±0.92 (range, 0–3)	1.82 ±1.94 (range, 0–6)	0.809
Prostate	5.20 ± 4.26 (range, 2–16)	4.45 ±1.92 (range, 2–8)	0.809
Bladder lamina propria	1.40 ±0.70 (range, 1–3)	2.55 ±2.30 (range, 2–8)	0.197
Bladder detrusor	3.30 ±1.49 (range, 2–6)	4.82 ±2.89 (range, 1–12)	0.061

C–kit positive ICs were detected in the bladder lamina propria layers of both diabetic and non–diabetic patients (Figure 1). Interestingly, ICs were also demonstrated in the urothelial layer of the urinary blad-

der. In some histopathological sections, c–kit positive ICs were located around small vessels in the lamina propria of the urinary bladder. In addition, c–kit positive ICs were demonstrated among detrusor smooth muscle bundles of the urinary bladder (Figure 2). In the ureter and urethra, c–kit positive ICs were also present in both groups. Prostate tissues of diabetic and non–diabetic patients also contained c–kit positive ICs. Interstitial cells were additionally demonstrated in the urinary tract of both diabetic and non–diabetic patients by connexin 43 immunostaining (Figures 4 and 5).

The appearance of c–kit positive interstitial cells, as seen on light microscopy, in the lamina propria and detrusor muscle of the urinary bladder of non–diabetic patients is shown in Figures 6 and 7. We detected ICs in the urothelial layer of the urinary bladder that might suggest a sensorial role. The location of c–kit positive ICs around small vessels in the lamina propria of the urinary bladder might suggest that these cells secrete into the blood some mediators which regulate urinary tract function. In the urinary bladder detrusor muscle, c–kit positive ICs were demonstrated both in the connective

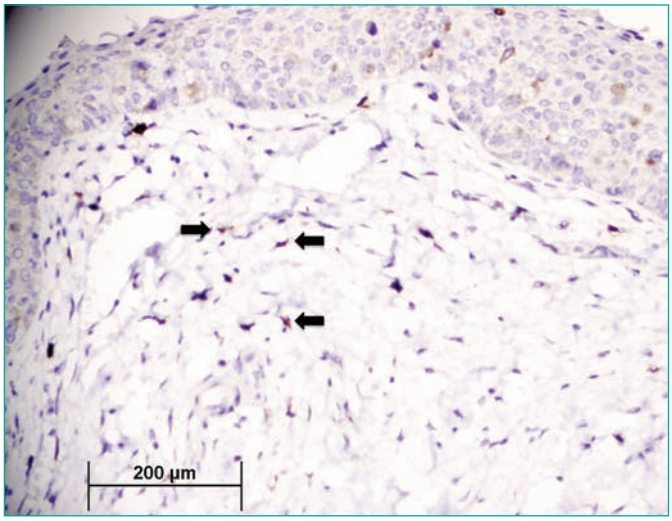

Figure 1. *Light microscopic appearance of c–kit positive interstitial cells in the bladder lamina propria of a diabetic patient (x400). Arrows indicate some of the interstitial cells.*

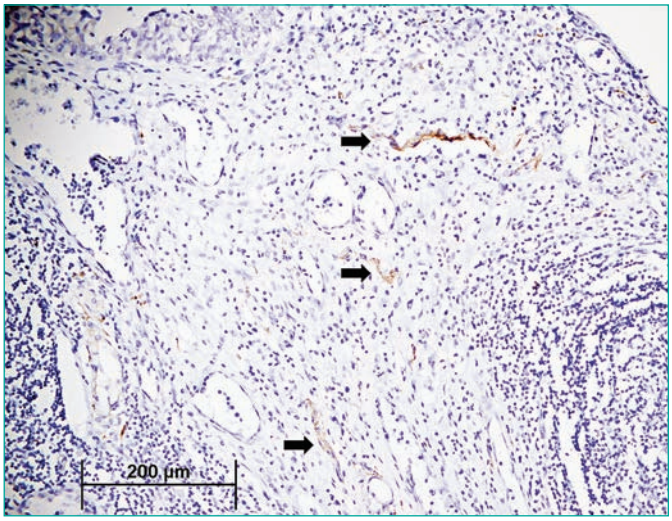

Figure 3. *Light microscopic appearance of nerves stained with synaptophysin in the urinary bladder of a non–diabetic patient (x400). Arrows indicate some of the nerve fibers.*

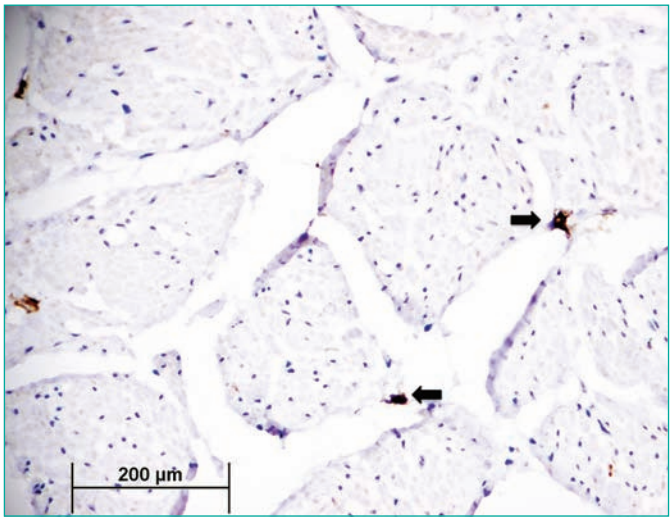

Figure 2. *Light microscopic appearance of c–kit positive interstitial cells among detrusor smooth muscle bundles of the urinary bladder of diabetic patients (x400). Arrows indicate some of the interstitial cells.*

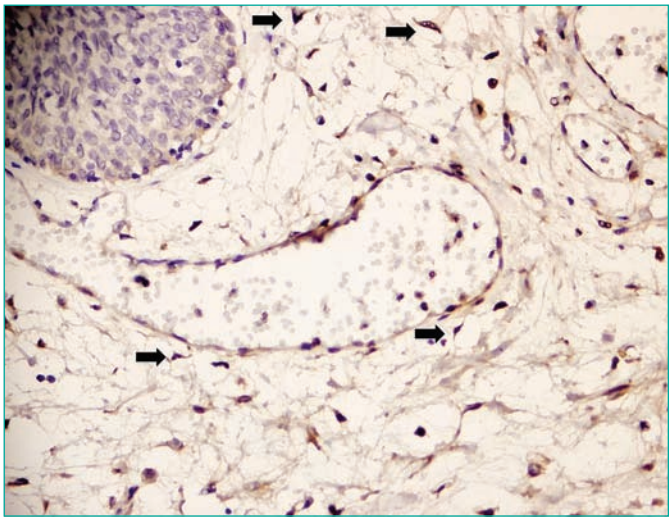

Figure 4. *Light microscopic appearance of connexin (+) interstitial cells in the lamina propria of the urinary bladder of a non–diabetic patient (x400). Arrows indicate some of the interstitial cells located around vessels.*

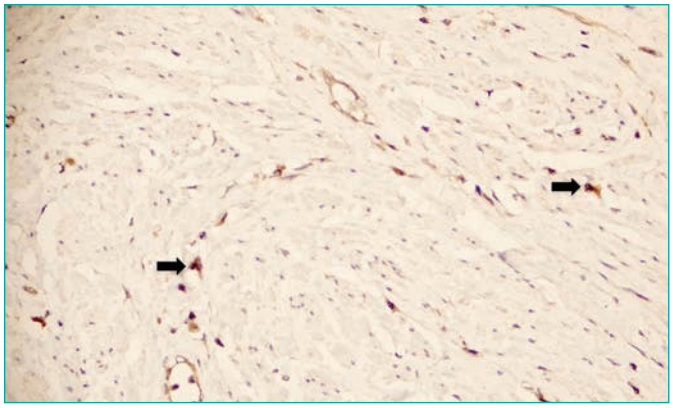

Figure 5. *Light microscopic appearance of connexin (+) interstitial cells in the detrusor of the urinary bladder of a non–diabetic patient (x400). Arrows indicate some of the interstitial cells.*

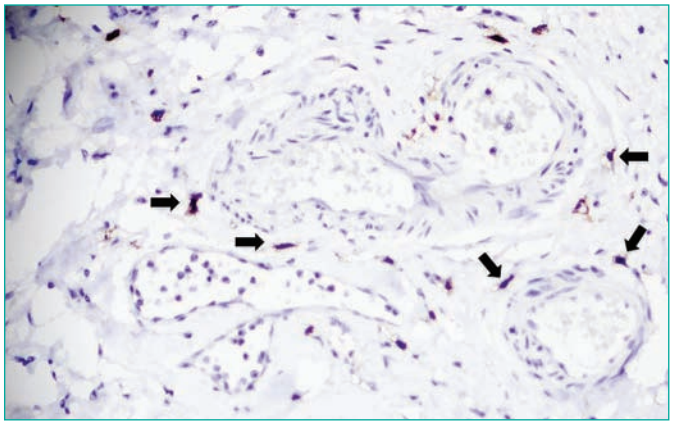

Figure 6. *Light microscopic appearance of c–kit (+) interstitial cells in the lamina propria of the urinary bladder of a non–diabetic patient (x400). Arrows indicate some of the interstitial cells located around blood vessels.*

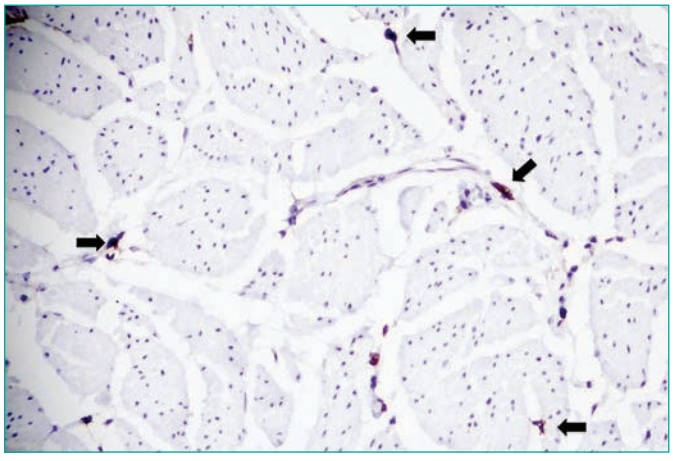

Figure 7. *Light microscopic appearance of c–kit (+) interstitial cells in the detrusor of the urinary bladder of a non–diabetic patient (x400). Arrows indicate some of the interstitial cells.*

tissue areas that are present among the individual detrusor smooth muscle fibers and around the muscle bundles. Sometimes these cells were located very closely, as if attached to, individual detrusor muscle cells but not in the smooth muscle cells themselves. This might suggest a regulatory role in contraction and relaxation of the detrusor smooth muscle and thus the bladder itself.

Nerves detected immunohistochemically by synaptophysin staining were detected microscopically by antibodies targeting synaptophysin both in diabetic and non–diabetic urinary tract tissues (Figure 3).

DISCUSSION

The aim of the present study was to compare the distribution and number of ICs and neuronal tissue in the ureter, bladder, prostate, and urethra of humans with and without diabetes. In our study, the mean number of c–kit positive ICs in the bladder lamina propria and detrusor muscle was significantly decreased in diabetics. Conversely, no significant differences between the groups were detected regarding the number of ICs at the level of the ureter, urethra, and prostate. The number of nerves was also similar along the whole urinary tracts of both groups.

It is known that pacemaker cells located in the renal pelvis initiate spontaneous peristaltic activity [19, 20, 21]. Electrical activity in the ureter is propagated to the distal parts as a contraction wave propelling urine in boluses distally from the renal pelvis into the urinary bladder [21, 22]. In our study, c–kit positive ICs were demonstrated in the lower ureter in both groups, suggesting a possible role in ureteric peristalsis. Other investigators also showed the presence of ICs in the human ureter [8]. Although we did not evaluate upper ureteral segments and uretero–pelvic junctions in our study, ureteral ICs may play an important functional and regulatory role in ureteral functioning. In a recent population based, case–control study by Chung et al. it has been demonstrated that patients who were diagnosed with urinary calculi are at an increased risk of diabetes mellitus at 5–year follow–up [23]. More research is required to show if diabetes affects the peristaltic function of the ureters via interfering with ureteral IC and nerve function resulting in upper urinary tract dysfunction and urolithiasis formation. If ureteral IC function is adversely affected in diabetes, agents that stimulate IC function might be administered to patients with diabetes in order to prevent urinary calculi formation or to facilitate spontaneous stone passage.

In the present study, c–kit positive ICs were also demonstrated in the urethra and prostate tissues of both groups. Nguyen DT et al. demonstrated and suggest-

ed that a specialized group of c–kit immunoreactive prostatic ICs, located between glandular epithelium and smooth muscle stroma, play a similar role to the ICs of Cajal of the gastrointestinal system [24]. Similarly, Van der Aa F et al. also suggested that prostatic ICs might have an important role in the regulation of spontaneous electrical activity, maintaining prostatic contractions, and overall prostatic tone in humans [25]. Although no significant differences were detected concerning the mean number of prostatic and urethral ICs between diabetic and non–diabetic groups in our study, it would be interesting to know if diabetes has an impact on prostatic IC function such as generation of pacemaker signals and slow wave activities and triggering prostatic smooth muscle contractions.

Interstitial cells in the urinary tract may play important roles in clinical conditions such as overactive bladder, detrusor–sphincter dyssynergia, acontractile bladder, and neurogenic bladder. As an example, Vahabi et at recently investigated the role of c–kit positive ICs in mediating muscarinic receptor–induced phasic contractions of isolated bladder strips from streptozotocin (STZ) – induced diabetic rats (n = 5) and to confirm the expression and location of ICs in the rat bladder. Their data showed the presence of c–kit–positive ICs in rat urinary bladder and their importance in mediating muscarinic receptor–induced phasic contractions of bladder strips from control (n = 5) and diabetic (n = 5) rats. Although the role of these ICs did not seem to be significantly altered by the diabetic state, bladder strips from 1–week diabetic rats showed carbachol–induced phasic contractions, which were greater in amplitude, but had lower frequency, than the controls [26]. Recently, Kanai et al. suggested that IC mediated activity in the bladder was initiated in the lamina propria by responding to urothelial factors. In addition, they stated that ICs may act syncytially through gap junction coupling and modulate detrusor activity through unknown mechanisms. Therefore, ICs seem to play a critical role in lower urinary tract function and physiology [27].

Some investigators evaluated urinary continence outcomes in diabetic and non–diabetic patients. As an example, Teber et al. recently demonstrated that patients with diabetes required longer time to recover urinary continence in the postoperative period compared to non–diabetic patients after laparoscopic radical prostatectomy (LRP) [28]. The duration of diabetes was also suggested to have a significant impact on post–prostatectomy incontinence occurrence [28]. Although the mean duration of diabetes was relatively short (3.8 years) in our study, the mean number of ICs in the urinary bladder was detected to be significantly lower in patients with diabetes. Longer diabetes duration periods might further affect the amount of ICs and perhaps also the amount of nerves in the urinary tract; this needs to be evaluated in future studies. Compared to the published animal research related to diabetes and amount of urinary tract ICs that included between 5 to 8 animal subjects in the experiments and evaluations [12, 29], we included the tissue samples of 10 diabetic and 11 control human subjects. In a very recent review on ICs by Juszczak et al., it was concluded that there is increasing evidence suggesting that ICs may play a role in the development of urinary tract dysfunction including detrusor overactivity, primary obstructive megaureter, and congenital ureteropelvic junction obstruction. In addition, disturbances of spontaneous contractility caused by altered signal transduction of ICs located between nerves and detrusor muscle cells and altered signal transduction between urothelium and afferent nerve endings via suburothelial ICs were suggested as novel pathomechanisms for development of detrusor overactivity. Lastly, ICs were suggested as a novel target for treating detrusor overactivity [30]. Karoli et al. investigated the prevalence of bladder dysfunction and its relation to other chronic complications of diabetes in women with type 2 diabetes. Lower urinary tracts symptoms (LUTS) (expand abbreviation) related to bladder dysfunction were/was reported in 67% and prevalence of overactive bladder (OAB) was 53%. Urodynamic evaluation proved stress urinary incontinence in 48%, detrusor overactivity in 23% and detrusor underactivity/insufficiency(?) in 11%. Peripheral neuropathy, nephropathy, and the presence of metabolic syndrome were found to be significantly associated with moderate to severe LUTS and OAB [31]. Hill et al. reported that diabetic cystopathy, particularly in long–standing diabetes, has a significant impact on the quality of life of the patients along with other significant individual health risks [32]. Therefore, diabetes seems to have a significant impact on the lower urinary tract voiding function of the patients.

Chen et al. explored the role of stem cell factor (SCF) on the loss of ICs in the bladder of diabetic rats (n = 8). Their findings suggested that the loss of ICs in the bladder tissue of diabetic rats could be attributed to a deficiency in endogenous SCF. They suggested that the beneficial effect of exogenous SCF on diabetic depletion of ICs could provide a rationale for the use of SCF as a potential therapeutic drug in treating patients with diabetes–related voiding dysfunction [29]. This study further supports a potential link between bladder overactivity and diabetes.

Our study has some limitations. A major concern

of using human cystectomy specimens obtained from patients with bladder cancer might be that specific markers of ICs could also be expressed by other cell types. As an example, Corteggio et al. stated that alterations in connexin expression could be associated with oncogenesis [33]. They evaluated biochemical and immunohistochemical expression of connexin 43 in samples of normal (n = 2), dysplastic (n = 3) and neoplastic (n = 23) bovine urothelium. Their study found that normal and dysplastic urothelium had membrane expression of connexin 43. On the other hand, expression of connexin 43 was detected to be reduced in carcinoma in situ samples. Papillary urothelial carcinomas showed moderate expression whereas invasive carcinoma showed loss of connexin 43 expression [33]. Connexin gap junction proteins have been suggested to act as tumor suppressors due to their main function of cell coupling through gap junctions [34]. In a study that included rat bladder carcinoma BC31 cell lines with dominant negative mutants of connexin 43, the effect of impaired communication on the tumorigenicity of cancer cells was suggested to depend on the subcellular location of connexin [34]. As in our study, others also used tissue samples obtained from macroscopically and microscopically normal areas of radical cystectomy specimens because it is extremely difficult, for ethical reasons, to obtain these tissue samples from healthy subjects [35]. Another limitation might be related to the presence of mast cells in the lower urinary tract which may also stain positively with c–kit. However, they can be easily distinguished from ICs by their round cell body, round nucleus, and cellular appearance [12]. In addition, ICs have fusiform cell bodies, a large oval nucleus, and bipolar dendritic processes [12]. Although patients with bladder cancer are not the ideal model for this kind of study; our tissue samples constitute a very selective group of patients who did not undergo any preoperative intravesical or systemic chemo and/or immunotherapy and/or radiotherapy that might possibly have an impact on the evaluation. Additionally, tissue sections without any microscopic tumors were selected for histopathologic evaluation. Studies on human cadavers without bladder cancer (diabetic and non–diabetic) could be carried out for further investigation.

Diabetes may decrease the number of ICs at the level of the lamina propria and detrusor muscle of the human bladder which may be one of the underlying mechanisms of diabetic LUT dysfunction. Longer diabetes duration periods might further affect ICs and perhaps nerves in the urinary tract that needs to be evaluated by future studies. Although patients with bladder cancer are not the ideal model for this kind of study, our tissue samples constitute a very selective group of patients who did not undergo any preoperative intravesical or systemic chemo and/or immunotherapy and/or radiotherapy that might possibly have an impact on the evaluation. Besides, tissue sections without any microscopic tumor were selected for histopathologic evaluation. Studies on human cadavers without bladder cancer (diabetic and non–diabetic) could be carried out for further investigation. Diabetes may also affect the peristaltic function of the ureters via interfering with ureteral IC and nerve function which may lead to upper urinary tract dysfunction and urolithiasis formation. If ureteral IC function is adversely affected in diabetes, agents that stimulate IC function might be administered to patients with diabetes in order to prevent urinary calculi formation or to facilitate spontaneous stone passage. Therefore, different tissue states and diseases such as diabetes might have an impact on the amount of ICs and their function. Due to the existing information suggesting that ICs in the urinary tract play important functional roles including acting as stretch or chemical sensors triggering detrusor contractions in the bladder [8, 9, 10], it can be deduced that ICs must be metabolically active in order to carry out these functions. Thus, as diabetes is primarily a metabolic disease, it can adversely affect the function and number of ICs. More research needs to be done to explain the detailed mechanisms involved in these processes.

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