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Morphological Aspects and Distribution of Interstitial Cells of Cajal in the Human Upper Urinary Tract

Üst Üriner Sistemde İnterstisyel Cajal Hücrelerinin Morfolojik Özellikleri ve Dağılımları

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ABSTRACT

Objective: The mechanism by which the ureter propels urine towards the bladder has a myogenic origin, through peristaltic contractions. This pyeloureteral autorhythmicity is generated by specialized, electrically active cells, the interstitial cells of Cajal, located in the proximal regions of the upper urinary tract. The aim of this study was to describe the exact location and the distribution of interstitial Cajal cells in the human upper urinary tract and to analyze their normal number and morphology. This is a preliminary study, which will allow the study of these cells in different urinary tract pathologies.

Material and Method: Urinary tract fragments were sampled at different levels, from 13 autopsy cases. Cases with clinical evidence of renal disease, and with histological changes in the kidney or in the urinary tract tissue samples, visible in hematoxylin-eosin staining, were excluded. The interstitial Cajal cells were highlighted with anti-CD117 antibody, immunohistochemically.

Results: Cajal cells were indirectly highlighted by the presence of a finely granulated cytoplasm indicating immunoreactivity. These cells were spindle-shaped or stellate, with cytoplasmic extensions at one or both poles of the cell and large oval nucleus. We found that interstitial Cajal cells were located at all upper urinary tract levels, with a higher predominance in the calyces and pyelon. Interstitial Cajal cells were observed mostly between the two layers of the muscularis, but also between the muscle bundles. Most often, these cells were parallel to the muscle fibers.

Conclusion: Our study describes the method of detection of interstitial Cajal cells in normal human urinary tract. These results can be used to analyze the number, morphology and the location of these cells in different congenital pathologies, such as vesicoureteral reflux, pyeloureteral junction obstruction or primary obstructive megaureter.

Key Words: Interstitial cells of Cajal, Urinary tract, Immunohistochemistry, CD117 antigen

ÖZ

Amaç: İdrarın üreterden mesaneye doğru, peristaltik hareketler aracılığıyla iletilme mekanizmasının miyojenik bir kökeni vardır. Bu üreteropelvik otoritmisite, üst üriner kanalın proksimal kısımlarında yer alan, özelleşmiş, elektriksel olarak aktif, interstisyel Cajal hücreleri tarafından oluşturulur. Bu çalışmanın amacı; üst üriner kanalda interstisyel Cajal hücrelerinin yerleşimini ve dağılımlarını açıklamak ve bunların normal sayı ve morfolojilerinin analizini yapmaktır. Bu bir ön çalışmadır ve bu çalışma sayesinde ileride diğer üriner sistem patolojilerinde Cajal hücrelerinin yeri araştırılabilir.

Gereç ve Yöntem: 13 otopsi olgusunun üriner kanalının farklı seviyelerinden örnekler alınmıştır. Klinik olarak kanıtlanmış böbrek hastalığı olan ve hematoksilen-eozin boyamayla gösterilebilen, böbrek veya üriner kanalda histolojik değişikliklerin olduğu örnekler çalışma dışı bırakılmıştır. İnterstisyel Cajal hücreleri CD117 antikoru ile immünohistokimyasal olarak gösterilmiştir.

Bulgular: İnterstisyel Cajal hücreleri, immünoreaktiviteye işaret eden ince granüllü sitoplazmaları sayesinde belirlenmişlerdir. Bu hücreler iğsi veya yıldızsı şekilli olup hücrenin bir veya her iki kutbunda sitoplazmik uzantıları ve büyük oval bir çekirdekleri vardır. İnterstisyel Cajal hücrelerinin, kaliksler ve pelvis renaliste daha baskın olmakla birlikte tüm üst üriner kanal seviyelerinde yer aldığı gözlenmiştir. İnterstisyel Cajal hücreleri en çok müsküler tabakanın iki katmanı arasında ve kas demetlerinin arasında görülmüştür. Çoğunlukla bu hücreler kas liflerine paralel yerleşim göstermektedir.

Sonuç: Çalışmamız, normal insan üriner kanalında interstisyel Cajal hücrelerinin tespit edilme yöntemini göstermektedir. Bu çalışmanın sonuçları, bu hücrelerin vesikoüreteral reflü, üreteropelvik bileşke darlıkları ya da obstrüktif megaüreter gibi farklı konjenital patolojilerde, sayı, morfoloji ve lokalizasyonlarının belirlenmesine katkıda bulunabilir.

Anahtar Sözcükler: İnterstisyel Cajal hücreleri, Üriner kanal, İmmünohistokimya, CD117 antijeni

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INTRODUCTION

The mechanisms by which the ureter propels urine towards the bladder are generally accepted to have myogenic origin, with peristaltic contractions propagating toward the bladder without being influenced by nerve (1-3). As a consequence, the presence of specialized electrically active cells in the proximal regions of the upper urinary tract, which generate pyeloureteral autorhythmicity, has been theorized. Initially, the most likely candidates for these pacemaker cells were considered "atypical" smooth muscle cells (SMC), in which several morphological and electrical characteristics similar to cardiac sino-atrial node cells were observed. Consistent with the presence of a proximal pyelocalyceal pacemaker, atypical smooth muscle cells were found to be more numerous in the proximal regions of the pelvis, their number decreasing with the distance from the renal fornix. These cells were not highlighted in the ureter. However, considerable evidence in humans and pigs show that, after kidney transplantation or pyeloureteral/ureteral surgery, the ureter, once disconnected from the renal pelvis, is spontaneously active, and develops rudimentary peristaltic waves. More recently, similarities were found between "atypical" SMC and cells with similar morphological and electrical features to the intestinal pacemaker cells (4,5), recognized as interstitial cells of Cajal (ICC). They have been highlighted in the upper urinary tract in humans and several mammals, and their important role in producing and coordinating pyeloureteral peristalsis was settled (6).

Based on these observations, the aim of this study is to describe the location of ICC cells and their morphology, and quantitatively analyze their distribution along the human upper urinary tract.

MATERIAL and METHODS

13 cases from autopsies performed at the Pathology Department of the County Emergency Hospital Mureş, were included in this study. They were selected from patients with ages varying between 7 months and 83 years, who had no medical documentation of renal, urinary voiding system diseases.

The tissue samples were collected from several levels of the upper urinary tract, as follows: 1st level: Kidney; 2nd level: Calyces; 3rd level: Pyelon; 4th level: Pyeloureteral junction; 5th level: Proximal ureter; 6th level: Middle ureter; 7th level: Distal ureter - intramural part.

In the kidney a sagittal section was performed, and the samples containing both cortical and medullar regions were collected. Patients with histological changes of the kidney, highlighted in hematoxylin-eosin (H&E) staining were excluded from study.

From all the levels of the upper urinary tract, the samples were collected by cross sections through their long axis, performed at 3-5 mm. In the ureter, prior sectioning, to prevent the collapse during processing, a catheterization with a suitable silicone tube was performed. The samples were fixed in 10% formaldehyde solution. Paraffin embedding was performed according to the standard protocol. The best samples, containing a morphologically intact tissue, were selected by microscopic examination in H&E staining.

For immunohistochemistry (IHC), the DAKO protocol was used. After incubation at 56°C, the samples were dewaxed and rehydrated. Antigen unmasking was done with EDTA buffer in 1:10 concentration, moist heat (98-99°C) and cooling to room temperature. To block endogenous peroxidase, Large Volume Hydrogen Peroxide Block was used. Blocking non-specific reactions was made with the Large Volume Ultra V Block. CD117 primary antibody was used at a concentration of 1:600. After visualizing by 3,3-diaminobenzidine (DAB), a hematoxylin counterstain was performed. All samples were processed in the same time and normal breast epithelium was used as external positive control. An optical microscope Olympus BX 46 (Olympus America, Center Valley, PA, USA) with a digital camera was used to analyze the specimens, at different magnifications. Randomized high-power fields of each level were analyzed at a magnification of 400x by three distinct examiners. Three different fields per level were analyzed by each examiner. ICCs were morphologically studied and counted. Data obtained from counting being ordinal variables, the median and the range were calculated.

RESULTS

C-kit positive cells were indirectly highlighted by the presence of a finely granulated cytoplasm, which indicates a strong immunoreactivity to CD117 (Figure 1). These cells were observed at the junction between the internal and external smooth muscle layers, and also between muscle bundles (Figure 2). Most often, the arrangement of the cells was parallel to the muscle fibers. Differentiation from other CD117 positive cells, such as mast cells or macrophages, was based on morphological features, as ICC are spindle-shaped or stellate, with cytoplasmic extensions at one or both cell poles and a large oval nucleus, whilst mast cells are round with round nucleus, and no dendritic processes (Figure 1). Another differentiation criterion was the location of the cells, ICCs, unlike mast cells and macrophages, are not found in the lamina propria or submucosa.

The ICCs were present at all upper urinary tract levels (levels 2-7), being more numerous in the calyces and pyelon (Figure 3). Only scattered cells were present in the middle and distal ureter. The ICCs count showed a decrease from the proximal to distal part of the upper urinary tract. This can be seen from the data obtained by each examiner. The resulting median and range are highlighted in Table I.

No correlation between patients' age, sex and the number of interstitial cells of Cajal was found.

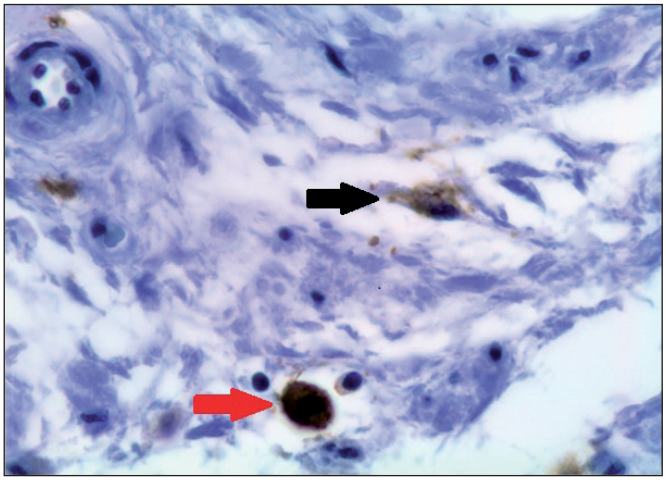

Figure 1: Interstitial cells of Cajal (ICC) and a mast cell in renal calyces (black arrow: ICC, red arrow: mast cell). The ICC presents a finely granulated cytoplasm, which indicates a strong immunoreactivity to CD 117. ICC is spindle-shaped, with cytoplasmic extensions at both cell poles and a large oval nucleus.

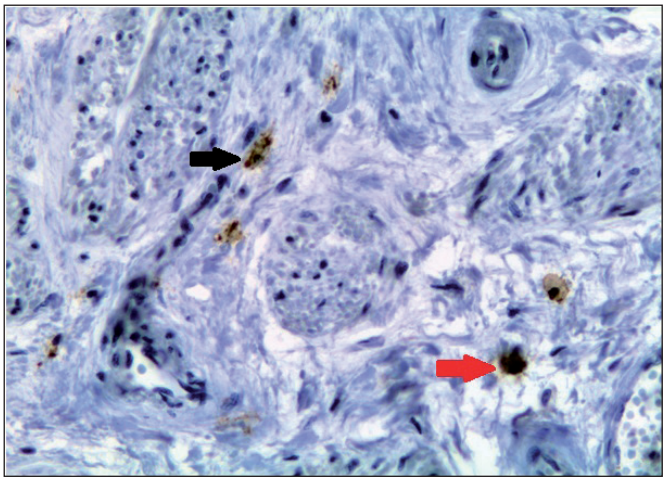

Figure 2: Interstitial cells of Cajal (ICC) and mast cells in upper urinary tract muscle layers (black arrow: ICC, red arrow: mast cell). Spindle shaped ICC (black arrow) highlighted by CD117 immunoreactivity, parallel to the smooth muscle fibers. The round cell (red arrow) is a mast cell.

Table I: Processed data indicating the reduction in number of interstitial cells of Cajal from proximal toward the distal part of the urinary tract

Level		Median (min-max)
2nd	**Total**	**6 (4-9)**
	Examiner 1	6 **(4-8)**
	Examiner 2	6 **(4-9)**
	Examiner 3	7 **(4-8)**
3rd	**Total**	**5 (2-8)**
	Examiner 1	5 **(2-8)**
	Examiner 2	5 **(2-7)**
	Examiner 3	5 **(3-7)**
4th	**Total**	**4 (2-7)**
	Examiner 1	4 **(2-6)**
	Examiner 2	4 **(2-6)**
	Examiner 3	5 **(2-7)**
5th	**Total**	**3 (1-6)**
	Examiner 1	3 **(1-6)**
	Examiner 2	3 **(1-5)**
	Examiner 3	3 **(1-5)**
6th	**Total**	**2 (1-5)**
	Examiner 1	2 **(1-4)**
	Examiner 2	3 **(1-5)**
	Examiner 3	3 **(1-5)**
7th	**Total**	**2 (0-5)**
	Examiner 1	2 **(1-5)**
	Examiner 2	2 **(1-4)**
	Examiner 3	2 **(0-4)**

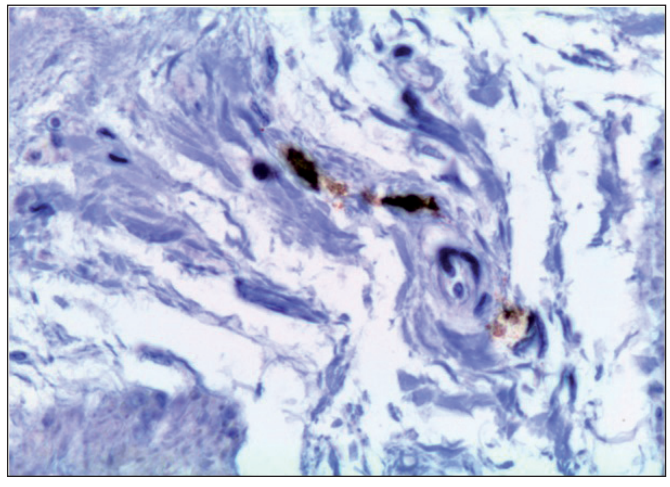

Figure 3: A couple of interstitial cells of Cajal in the renal pelvis.

DISCUSSION

Interstitial cells of Cajal (ICC) were first described by Ramon y Cajal as a characteristic interstitial cell network (7,8). In 1982, Thuneberg proposed the hypothesis that ICC could act as pacemaker cells and as a conductor of impulses in muscle layers of the intestine, generating the peristaltism in an analogue manner with the cardiac pacemaker cells.

Since then, many researchers, in physiological and morphological studies, have provided evidence, strengthening Thuneberg's hypothesis and concluding that ICC are distinct mesenchymal cells (9) with pacemaker or neuromediator function in the muscle layer of the gastrointestinal tract (4,5).

In the digestive tract, ICC were found around the muscle layer of the esophagus, stomach, small and large intestine. They form two- and three-dimensional networks and bundles of cells that provide close contacts between them or with nerve plexus and smooth muscle cells (10-12).

In the urinary tract, these cells were described in various manners, sometimes leading to contradictory results. Lang et al. have published in 1999 the first paper describing a population of electrically active cells in the upper urinary tract of guinea pigs (13). In electron microscopy, these cells appeared stellate, with an oval nucleus, numerous mitochondria occupying 4% of the section area of the cell, a well developed Golgi apparatus, but without contractile filaments or immunoreactivity to α smooth muscle actin. Plasma membrane showed a discontinuous basal lamina. These interstitial cells had tight and adherent junctions between each other (80%) or between them and smooth muscle fibers (13). Further studies were extended to rats (14).

Another study, carried out by Metzger et al. on the human upper urinary tract described a population of c-kit positive spindle cells in the internal and external smooth muscle layers and also in the lamina propria (15). In a later study on different species of mammals, it was concluded that these cells had the same distribution in several species (16). In addition, Solari et al. have described c-kit positive cells in the human pyeloureteral junction as spindle cells, with two specific dendrites (17), while Pezzone et al., who studied the upper urinary tract in mice, described Cajal cells as having a stellate form (18). These cells were described as forming networks, located adjacent to the internal muscle layer and between the internal and external layers of SMC (18).

Our study reports the presence and describes the morphological characteristics of the ICCs in the upper urinary tract. We found spindle-shaped or stellate ICCs, with cytoplasmic extensions at one or both cell poles and a large oval nucleus, morphological features already described in other studies (15-17). The location of these cells at the junction between the internal and external smooth muscle layers, and also between muscle bundles has been emphasized also by other authors (6,10,15,19). Unlike Metzger et al (15) we did not found ICCs in the lamina propria. Regarding the shape of these cells, in our study, ICCs were more often spindle-shaped while other authors describe them as stellate cells (18). They formed a histologically well-defined network placed in the muscle layer of the urinary voiding system. Showing the same morphology, ultrastucture and immunophenotype as the ICCs of the gastrointestinal tract, ICCs of the urinary tract are thought to have similar functions: pacemaker cells, inducing smooth muscle contraction (18). They also seem to act as intermediaries in transmitting nerve signals to smooth muscle fibers (20,21). When evaluating the number of ICCs, we noticed that it gradually decreases from the proximal toward the distal urinary tract system. This result would lead us to conclude that in the upper urinary tract, the large number of cells could be related to the initiation of the peristaltic wave. Once initiated, a lower number of cells is necessary for its propagation.

To properly identify ICCs in IHC, they should be differentiated from mast cells, macrophages and other hematopoietic cells, which also express CD117.

Out of ICCs, our study has revealed the ubiquity of mast cells in the muscle layer and lamina propria of the human upper urinary system. The presence of these cells may suggest important functional properties (16,22). There is a clear evidence that mast cells contribute to smooth muscle stimulation and neuronal activity by releasing a variety of cytokines with pro-and anti-inflammatory properties that modulate function, lifespan and proliferation of other cells (22,23). Structural and functional relationships between ICCs and mast cells have been documented in the gastrointestinal tract too, where intramuscular ICCs are surrounded mostly by mast cells (24). This evidence deserve further research which can clarify the functionality of this cellular complex.

An increasing number of pathologies related to ureteral peristalsis, and the abnormal urine flow are reported in the recent literature. ICCs could be involved in these pathologies, and hence the importance of the studding them in normal and pathological conditions. Koleda et al. described an ICC increased number in pyeloureteral junction obstructive disease, and explained the phenomenon as a compensatory mechanism to the loss of the ability to conduct the urine

from the pyelon toward the ureter (25). Kang incriminates the low number of ICCs in the ureter along with smooth muscle apoptosis (19) as a possible mechanism in the development of congenital vesicoureteral reflux and obstructive megaureter (26).

These studies can be a starting point for further investigations, on human surgical samples or on animal models that can provide more information about the involvement of ICCs in different pathological conditions and the modulation of their function.

In conclusion; The information provided by our study demonstrates the ubiquitary distribution of interstitial cells of Cajal in the upper urinary tract. The ultra-specialized behavior of these cells can be better understood through morphological and functional studies in pathological conditions of the urinary tract. Understanding ICC abnormalities in the urinary tract can provide many explanations concerning congenital pathological conditions such as vesicoureteral reflux, pyeloureteral junction obstruction or primary obstructive megaureter.

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