ONLINE FIRST

ISSN: 0015-5659

e-ISSN: 1644-3284

Telocytes and Cajal cells distribution in renal pelvis, ureteropelvic junction (UPJ), and proximal ureter in normal upper urinary tract and UPJ obstruction: reappraisal of the etiology of UPJ obstruction

Authors: M. Wishahi, A. A Mehena, H. Elganzoury, M. H. Badawy, E. Hafiz, T. El-Leithy

DOI: 10.5603/FM.a2020.0119

Article type: ORIGINAL ARTICLES

Submitted: 2020-08-17

Accepted: 2020-09-23

Published online: 2020-09-23

Articles in "Folia Morphologica" are listed in PubMed.

Telocytes and Cajal cells distribution in renal pelvis, ureteropelvic junction (UPJ), and proximal ureter in normal upper urinary tract and UPJ obstruction: reappraisal of the etiology of UPJ obstruction

Running head: Telocytes and Cajal cells in upper urinary tract

M. Wishahi[1], A.A Mehena[1], H. Elganzoury[1], M.H. Badawy[1], E. Hafiz[2], T. El-Leithy[1]

[1]Department of Urology, Theodor Bilharz Research Institute, Cairo, Egypt

[2]Department of Pathology and Electron Microscopy, Theodor Bilharz Research Institute, Cairo, Egypt

Address for correspondence: Mohamed Wishahi, Department of Urology, Theodor Bilharz Research Institute, El Nile Street, Embaba Giza-12411, P.O. Box 30, Cairo, Egypt, tel: +2 012 222 47437, e-mail: moh.weshahy@gmail.com

Abstract

Background: Telocytes and Cajal cells have been described in human urinary tract and reproductive system in women and men. Telocytes and Cajal cells have been differentiated from other interstitial cells and were described to be an element in smooth muscle conductivity. Previous studies examined the ureteropelvic junction (UPJ) segment in patients with UPJ obstruction (UPJO) and attributed the etiology of UPJO to the low density or absence Cajal cells and telocytes. The present work aimed at the demonstration of the presence and the density of telocytes and Cajal cells in the upper urinary tract (UUT) in cases with normal UUT and UPJO. It included UPJ segment, renal pelvis, and proximal ureter. The morphological pattern of distribution of collagen in relation to smooth muscle was investigated in normal and obstructed UUT.

Materials and methods: The study was carried out on 12 surgical specimens, 5 of them represented the normal UUT and underwent nephrectomy for oncological reasons. Seven patients

underwent dismembered pyeloplasty for UPJO. Surgical specimens included renal pelvis, UPJ segment, and proximal ureter. They were subjected to standard hematoxylin and eosin stain, Gomori's trichrome stain, immunohistochemistry (IHC) with c-kit, and transmission electron microscopy (TEM).

Results: Telocytes and Cajal cells were demonstrated in the muscular layer of both normal UUT and UPJO with high density in the proximal ureter in normal UUT as well as in UPJO. The UPJ segment in normal UUT had moderate density of Cajal cells and telocytes while in UPJO the cells were scanty or absent. Renal pelvis in normal UUT showed excess density of cells while obstructed renal pelvis showed scanty Cajal cells and telocytes. Ultrastructural study showed the presence of Cajal cells, telocytes, stem cells, fibroblasts, smooth muscle cells, and collagen in different densities and distribution in normal and obstructed UUT.

Conclusions: Examination of the UPJ segment of UPJO revealed that Cajal cells and telocytes were scanty or absent, collagen to muscle ratio was high. The low density of Cajal cells and telocytes in the renal pelvis of the obstructed UUT, compared to the normal, points out to the role of the renal pelvis in the pathogenesis of UPJ obstruction.

Key words: Cajal cells, telocytes, ureteropelvic junction, urinary tract, interstitial cells

INTRODUCTION

The upper urinary tract is anatomically divided into renal pelvis, ureteropelvic junction (UPJ) and proximal ureter. The three parts have several cellular varieties: smooth muscle cells, blood vessels, and the interstitium of the connective tissues containing several types of interstitial cells: fibroblasts, mast cells, resident macrophages, stem cells, plasma cells, lymphocytes, granulocytes and monocytes, and, in addition, two unique types of interstitial cells: the interstitial like-cells of Cajal (Cajal cells), and telocytes. Telocytes and Cajal cells have been described to be responsible for the transmission of electric activity to smooth muscle cells and for their rhythmic contraction and relaxation. Cajal cells and telocytes were described in the urinary tract and their function is initiation of urine transport. Cajal in 1910 was the first to describe a distinct cell in the gut which was named on him later [1]**.** Cajal cells were described to be responsible for gut motility. Evidence suggests that Cajal cells' function is the generation of the electrical activities. They are unique cells that either have intrinsic pacemaker activity or are

able to perform stimulus-induced pace-making, they act as networks for inhibitory and excitatory functions [9]. Cajal-cells were identified with immunohistochemistry (IHC) as c-kit positive-stained cells and were demonstrated in the human urinary tract [16] ,upper urinary tract [6,8], ureter [3], urinary bladder [5], and urethra [14]. Interstitial cells of Cajal were demonstrated by transmission electron microscopy (TEM) studies. They are distinct from smooth muscle cells, fibroblasts, and monocytes, the surface membrane has abundant invaginations and prominent endoplasmic reticulum, they are characterized by being uninucleate with thin cytoplasm and numerous mitochondria [4,5,7,8,19,11,20]. Low density or altered function of Cajal cells were attributes to the etiology of UPJ obstruction [3,4,7,15,21]. Elken et al [4] studied UPJ specimens from patients with UPJ obstruction and normal control, examining them with TEM, light microscopy, and immunohistochemical stain. They found out that the c-kit positive-stained cells were located near the circular muscle layer and were encountered more frequently in the control group. The ratio of areas with no cells in the UPJ obstruction group was significantly higher when compared to the control group. The working group of Popescu and co-workers in Romania in the year 2010 described cells which were semi-similar to Cajal cells and named them "Telocytes" [11,12]. Telocytes were detected in the urinary system [22], kidney cortex [13], and urinary bladder [17]. Examination of tissue specimens with TEM would determine the presence of telocytes. The ultrastructural characteristics of the telocytes are their long cytoplasmic processes which are called Telopodes. The telopode is characterized by the alternance of thin tracts with dilations. The thin segments are called podomers and the dilated regions podoms. Podoms have a functional unit consisting of mitochondria, endoplasmic reticulum, and caveolae. The bodies of Telocytes vary in shape: fusiform, pyriform, or triangular. The nucleus contains condensed heterochromatin; the surrounding cytoplasm is scarce and encompasses few organelles [2,10,17,19]. Ultrastructural features characterizing and differentiating telocytes and Cajal cells were described in detail by Vannuchi et al [17,18,19]. Telocytes were attributed to the etiology of UPJ obstruction [21]. We investigate the distribution of Cajal, telocytes, interstitial cells, and morphometric pattern of collagen to smooth muscles in normal UUT and UPJ obstruction by using hematoxylin and eosin stain, Gomori's modified Masson's trichrome stain for light microscopy, IHC and TEM.

MATERIAL AND METHODS

Patients

Twelve patients were included in the study. Of this total number, 5 patients, who had undergone nephrectomy for oncological reasons, represented the normal upper urinary tract without obstruction. Mean age was 52±7 years (3 males, and 2 females; age range was 44-57 years). They had renal cell carcinoma >7cm located in the periphery of the kidney, preoperative imaging studies showed normal pelvicalyceal system without obstruction. Seven patients, who had undergone Anderson-Hynes dismembered pyeloplasty for unilateral UPJ obstruction without crossing vessels, represented the UPJ obstruction. Mean age was 28 ± 10 years (5 male, 2 females; age range was 18-35. Imaging studies showed dilated pelvicalyceal system, obstructive pattern of the clearance curve and T1/2 greater than 20 minutes when evaluated with diuretic renogram, split renal function <30%. The surgical specimens were composed of part of the dilated renal pelvis, the UPJ segment, and portion of the proximal ureter. The 12 surgical specimens were processed for histological, IHC, TEM studies. The institutional review board (IRB) approved the present study (Research Ethics Committee: TBRI- Protocol No: PT 536). The study was conducted in compliance with the relevant laws and regulations, good clinical practice, and ethical principles as described in the World Medical Association's Declaration of Helsinki. Requiring patients to provide informed consents was waived by the IRB because of the retrospective feature of the present study.

Histopathology

Excised specimens from ureter, pelviureteric junction and renal pelvis were immediately fixed in a 10% formalin solution, processed in a tissue processor (Thermo Scientific STP 120 Spin, UK) and embedded in paraffin. Sections, 4 microns thick, were then stained with hematoxylin and eosin (H&E) stain to examine histopathologic changes. Gomori's modified Masson's trichrome stain was used to assess the fibrosis. The examined parts were the proximal ureter, UPJ segment, and renal pelvis. Standard hematoxylin and eosin were primarily done to define the area in the segment to be compared in every studied parts. The examination with Gomori's modified Masson's trichrome stain showed the distribution of collagen fibers in different segments of UUT in normal and obstructed cases.

Immunohistochemistry

Immunohistochemistry was performed on 5 microns thick sections of formalin-fixed and paraffin-embedded tissue samples. Sections were picked on charged glass slides and deparaffinized, hydrated, then treated for antigen retrieval at a high pH (pH 8) using an automated immunostainer (Dako, Denmark). Rabbit polyclonal anti-CD117 (c-kit) antibodies (CD 117, Cat. No. A4502, Dako, Denmark) at dilution 1:200 were used to label the Cajal cells. Goat anti-rabbit biotinylated immunoglobulins/HRP (Cat. No. P0448, Dako, Denmark) were used at dilution 1:300. Streptavidin–biotin–peroxidase complex and peroxidase-DAB (3,3′diaminobenzidine) (Dako, Denmark) detection method was preformed according to the manufacturer's instructions. Sections were counterstained with Mayer's hematoxylin. Positive and negative control slides were included in each run. As a negative control, a tissue section was processed as described but with the primary antibody omitted.

Transmission electron microscopy

Immediately following surgical excision, samples from the three (blinded) specimens were cut into 1x1 mm pieces, fixed in 2.5% glutaraldehyde in cacodylate buffer for 2 h at 4^{o}C. The tissues were then washed in cacodylate-sucrose buffer and post-fixed for 1 h at 4^{o}C in 2% osmium tetroxide. After dehydration in graded ethanol, the samples were impregnated in Epon 812 substitute (EMBed-812 Kit, Electron Microscopy Science, USA) at room temperature, and polymerized at 60 °C for 48 h. Semi-thin sections were cut, stained with methylene blue-azure II, and examined by light microscopy to choose the region of interest for ultrathin sectioning. The ultrathin sections were then prepared using an Ultra cut R ultramicrotome (Leica, Vienna, Austria), double stained with uranyl acetate and lead citrate. Cellular ultrastructural morphological characterization was examined at 80 kV with a Philips EM 208 S electron microscope (Philips Optics, Eindhoven, The Netherlands). Identification and differentiation of telocytes and Cajal was done according to the description of different authors.

Results

Distribution of Cajal cells in normal upper urinary tract and in ureteropelvic junction obstruction: The study was done on 5 normal upper urinary tracts and 7 cases with ureteropelvic junction obstruction (UPJO) by IHC. Immunostaining with c-kit to demonstrate the distribution of Cajal cells showed that the distribution was different and specific to each segment in both

normal and obstructed UUT. Cajal cells were demonstrated in the muscular layer of UUT in normal and UPJ obstruction. In normal UUT, high density was demonstrated in the proximal ureter, excess in renal pelvis, and moderate in the UPJ segment. In obstructed UUT, high density of Cajal cells was demonstrated in the proximal ureter, moderate density in renal pelvis, but in the UPJ segment the cells were scant or absent. (Fig.1).

Collagen to smooth muscle ratio in normal upper urinary tract and ureteropelvic junction obstruction: The study by light microscopy using H&E and eosin stain as well as Gomori's modified Masson's trichrome stain showed that in normal UUT the collagen/muscle ratio was 2.3 for the proximal ureter, 1.5 for the UPJ segment, and 1 for the renal pelvis. The collagen/muscle ratio in UPJ obstruction showed that, compared to normal UUT, there was increased collagen in the UPJ segment, moderate collagen distribution in the renal pelvis, while the proximal ureter had an identical ratio (Fig.1).

Telocytes, cajal cells, smooth muscle cells, fibroblasts, collagen, and stem cells detection by TEM: Transmission electron microscopy examination demonstrated the presence of telocytes (Fig. 2), and Cajal cells (Fig. 3). Stem cells, fibroblasts, collagen fibers, and myoblasts were demonstrated in all specimens of normal UUT and UPJO (Fig. 4). In the normal UUT, they were in excess in proximal ureter and renal pelvis, but moderately expressed in the UPJ segment. In UPJO, these interstitial cells were few in the renal pelvis, rare in the UPJ segment but in excess in the proximal ureter.

Correlation between density of Cajal cells and collagen/muscle ratio in normal UUT and UPJO: Distribution of interstitial cells in proximal ureter and renal pelvis in obstructed cases was similar to normal. Distribution of density of Cajal cells was correlated to the ratio of collagen to muscle fibers: the more muscle fibers in relation to collagen, the higher the density of Cajal cells. This finding was obvious in proximal ureter and renal pelvis in normal UUT. Diminished distribution of smooth muscle cells in the UPJ segment was associated with relative low density of Cajal cells and telocytes.

DISCUSSION

Cajal cells which are c-kit positive were demonstrated in this study in the muscular layer of normal UUT and in UPJO. Identification and characterization of these cells and their

differentiation from other interstitial cells were in accordance with descriptions by previous studies on the upper urinary tract using IHC and TEM [3,4,5,6,7,8,9,11,20]. In our study, we showed that, in the normal UUT, Cajal cells were densely present in the proximal ureter, excessively in the renal pelvis, and moderately dense in the UPJ segment. In the UPJO, we demonstrated the presence of Cajal cells in the UPJ segment in few numbers, in moderate numbers in the renal pelvis, and in high numbers in the proximal ureter. Our results differ from previous studies [3,4 ,7,15,21] where only the UPJ segment was examined and changes in proximal ureter and renal pelvis were not considered. Elken et al [4] searching for Cajal cells in the UPJ segment in UPJO in pediatric patients, found out that the Cajal cells were either rare or absent. Our study confirmed their presence in the UPJ segment in UPJO, although not in the same density as in the normal UPJ segment. The difference in findings would be attributed to different age groups, as Elken et al studied the pediatric age group, while our study was applied to adults.

Examining the whole UUT in both normal and obstructed cases, we found that the three anatomical divisions express the same cells in different density, i.e. on cellular basis they are one unit. While in the past their division was based on shape and configuration, our concept is that the UUT is one entity and each part would play a role in the pathogenesis of UPJO.

Our study showed that the relationship between muscle density and collagen differed from one segment to the other. In both normal and obstructed UUT, the muscle fibers were of higher concentration than the collagen in proximal ureter, renal pelvis, and UPJ segment, respectively.

Transmission electron microscopy of the normal UUT and UPJ obstruction showed the presence of telocytes in the proximal ureter, renal pelvis, and UPJ segment. Our work examined the three parts of UUT and confirmed the presence of telocytes. Previous reports by Zheng et al [22], Qi et al [13] , Vannucchi et al [17,18], and Gevaert et al[6] supported our findings. In our study on different parts of the UUT by TEM, we demonstrated the presence of stem cells, in addition to fibroblasts, myoblasts, telocytes, and Cajal cells. Previous studies on the interstitial cells of the UUT concentrated on one cell type, our study investigated all cell types and demonstrated their existence in normal and obstructed UUT.

CONCLUSIONS

Telocytes and Cajal cells were demonstrated with different density in the muscular layers of the ureteropelvic junction(UPJ) segment, the renal pelvis, and the proximal ureter in normal and obstructed upper urinary tract secondary to UPJ obstruction. The low density of Cajal cells and increased collagen fibrils in the UPJ segment, in addition to the low density of Cajal cells in renal pelvis of UPJ obstruction,would be attributed to the etiology of UPJ obstruction.

Abbreviations UPJ: Ureteropelvic junction; UPJO: ureteropelvic junction obstruction; UUT: upper urinary tract; H&E: hematoxylin and eosin; IHC: immunohistochemistry; TEM: transmission electron microscopy.

REFERENCES

1. Cajal SR. Histologie du système nerveux de l'homme et des vertébrés. Maloine (Paris). 1909-1911; 2:891-942, doi.org/10.5962/bhl.title.48637
2. Cretoiu S, Popescu lM. Telocytes revisited .BioMol Concepts 2014; 5(5): 353–369, doi. 10.1515/bmc-2014-0029, indexed in PubMed: 25367617

3. David SG, Cebrian C, Vaughan ED Jr, et al. c-kit and ureteral peristalsis. *J Urol*. 2005;173(1):292-295. doi:10.1097/01.ju.0000141594.99139.3d, indexed in PubMed: 15592099

4. Eken A, Erdogan FS, Kuyucu Y, Seydaoglu G, Polat S, Satar N. Immunohistochemical and electron microscopic examination of Cajal cells in ureteropelvic junction obstruction. Can Urol Assoc J. 2013;7(5-6):e311-316, doi.org/10.5489/cuaj.11293, indexed in PubMed: 3668404

5. Gevaert T, De Vos R, Everaerts W, et al. Characterization of upper lamina propria interstitial cells in bladders from patients with neurogenic detrusor overactivity and bladder pain syndrome. *J Cell Mol Med*. 2011;15(12):2586-2593. doi:10.1111/j.1582-4934.2011.01262.x, indexed in PubMed: 4373427

6. Gevaert T, De Vos R, Van Der Aa F, et al. Identification of telocytes in the upper lamina propria of the human urinary tract. J Cell Mol Med. 2012;16:2085–2093, doi: 10.1111/j.1582-4934.2011.01504.x., indexed in PubMed: 3822978

7. Koleda P, Apoznanski W, Wozniak Z, et al. Changes in interstitial cell of Cajal-like cells density in congenital ureteropelvic junction obstruction. Int Urol Nephrol. 2012;44:7-12, doi.org/10.1007/ s11255-011-9970-5, indexed in PubMed: 3253995

8. Lang RJ, KLemm MF. Interstitial cell of Cajal-like cells in the upper urinary tract. J Cell Mol Med. 2005; 9:543-556, doi.org/10.1111/j.1582-4934.2005.tb00487.x, indexed in PubMed: 6741340

9. Maeda H, Yamagata A, Nishikawa S, et al. Requirement of c-kit for development of intestinal pacemaker system. Development 1992; 116: 369-375, indexed in PubMed: 1283735

10. Petre N, Rusu M. C, Jianu A M. Telocytes of the mammary gland stroma. Folia Morphol *2016;75(2):224-231*, DOI: 10.5603/FM.a2015.0123, indexed in PubMed: 26711648

11. Popescu LM, Ciontea SM, Cretoiu D. Interstitial Cajal-like cells in human uterus and fallopian tube. Ann NY Acad Sci. 2007; 1101: 139–165, doi:10.1196/annals.1389.022, indexed in PubMed: 17360808
12. Popescu, L. M, Faussone-Pellegrini M.-S. TELOCYTES - a case of serendipity: the winding way from Interstitial Cells of Cajal (ICC), via Interstitial Cajal-Like Cells (ICLC) to TELOCYTES. J. Cell. Mol. Med.2010; 14, 729–740, doi: 10.1111/j.1582-4934.2010.01059.x., ndexed in PubMed: 3823108

13. Qi G, Lin M, Xu M, et al. Telocytes in the human kidney cortex. J Cell Mol Med. 2012;16:3116–3122, doi: 10.1111/j.1582-4934.2012.01582.x, indexed in PubMed: 4393739

14. Sergeant GP, Thornbury KD, McHale NG, et al. Interstitial cells of Cajal in the urethra. J Cell Mol Med. 2006;10:280–291, doi: 10.1111/j.1582-4934.2006.tb00399.x., indexed in PubMed: 3933121

15. Solari V, Piotrowska AP, Puri P. Altered expression of interstitial cells of Cajal in congenital ureteropelvic junction obstruction. J Urol, 2003, 170: 2420-2422
DOI: 10.1097/01.ju.0000097401.03293.f0, indexed in PubMed:14634443
16. Van der Aa F, Rosa's T, Blyweert W, et al. Identification of kit positive cells in the human urinary tract. J Urol. 2004; 171:2492-2496, doi.org/10.1097/01.ju.0000125097.25475.17, indexed in PubMed:6710050

17. Vannucchi M-G, Traini C, Guasti D, et al. Telocytes subtypes in human urinary bladder. J Cell Mol Med. 2014;18:2000–2008. doi: 10.1111/jcmm.12375, indexed in PubMed: 4244015

18. Vannucchi MG, Traini C. Interstitial cells of Cajal and telocytes in the gut: twins, related or simply neighbor cells? BioMol Concepts. 2016; 7(2): 93–102, doi. 10.1515/bmc-2015-0034, indexed in PubMed: 26992201

19. Vannucchi MG. The Telocytes: Ten Years after Their Introduction in the Scientific Literature. An Update on Their Morphology, Distribution, and Potential Roles in the Gut. Int. J. Mol. Sci. 2020, 21(12), 4478, doi:10.3390/ijms21124478, indexed in PubMed: 7352570

20. Yamataka A, Ohshiro K, Kobayashi H, et al. Abnormal distribution of intestinal pacemaker (C-KIT-positive) cells in an infant with chronic idiopathic intestinal pseudoobstruction. J Pediatr Surg. 1998;33:859-862, doi.org/10.1016/S0022-3468(98)90660-1, indexed in PubMed:9660215

21. Yang X, Zhang Y, Hu J. The expression of Cajal cells at the obstruction site of congenital pelviureteric junction obstruction and quantitative image analysis. J Pediatr Surg. 2009;44:2339-2342, doi: 10.1016/j.jpedsurg.2009.07.061, indexed in PubMed: 20006022

22. Zheng Y, Zhu T, Lin M, et al. Telocytes in the urinary system. J Transl Med 10, 188 (2012). https://doi.org/10.1186/1479-5876-10-, indexed in PubMed: 3527325.

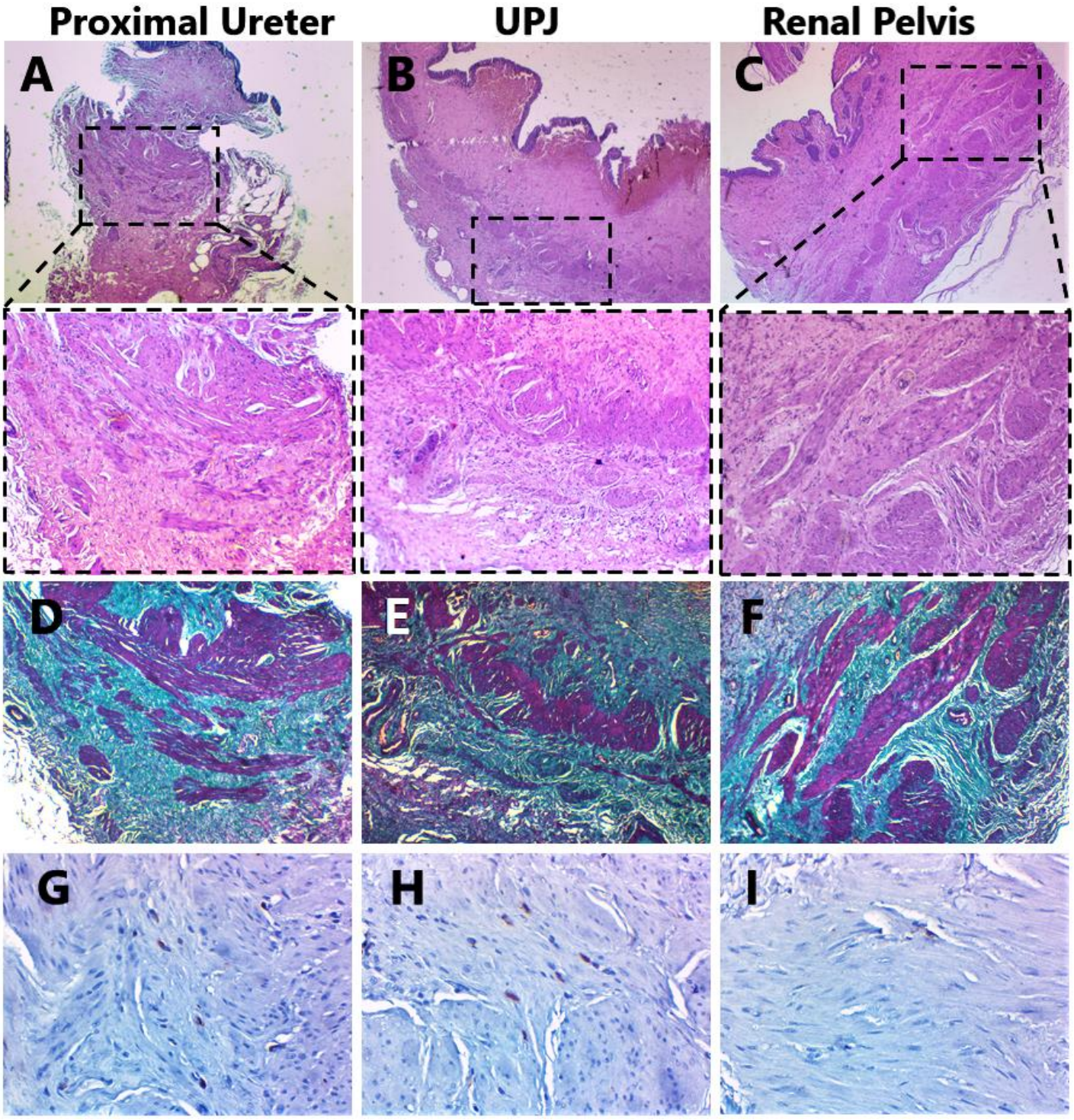

Figure 1. Morphological pattern of collagen to smooth muscles distribution in normal upper urinary tract, sections were stained with hematoxylin and eosin stain and Gomori's stain (A-F). Pattern of distribution of Cajal cells in normal Upper urinary tract demonstrated with c-kit immunohistochemistry (G,H,I).

Left column represents the proximal ureter***, middle column*** represents ureteropelvic junction (UPJ) segment***, right column*** represents renal pelvis. ***Upper row:*** Hematoxylin and eosin stained sections from muscularis propria with high insert box; proximal ureter **(A),** ureteropelvic junction segment **(B)** and renal pelvis **(C).** (Scale bar=2mm). ***Middle row****:* Gomori's trichrome stained sections showing smooth muscle bundles of the muscularis propria with collagen fibers. (collagen/muscle ratio of 2.3 for the proximal ureter **(D)** 1.5 for UPJ segment **(E),** and 1 for the renal pelvis **(F),** (Scale bar= 2mm). ***Lower row*****:** Immunohistochemical staining with c-kit (CD 117) showing distribution of interstitial cells of Cajal in the upper urinary tract. The proximal ureter has the highest condensation of Cajal cells **(G),** the UPJ has a considerable density of Cajal cells **(H),** few cells are in the renal pelvis **(I);** (Scale bar= 500μm).

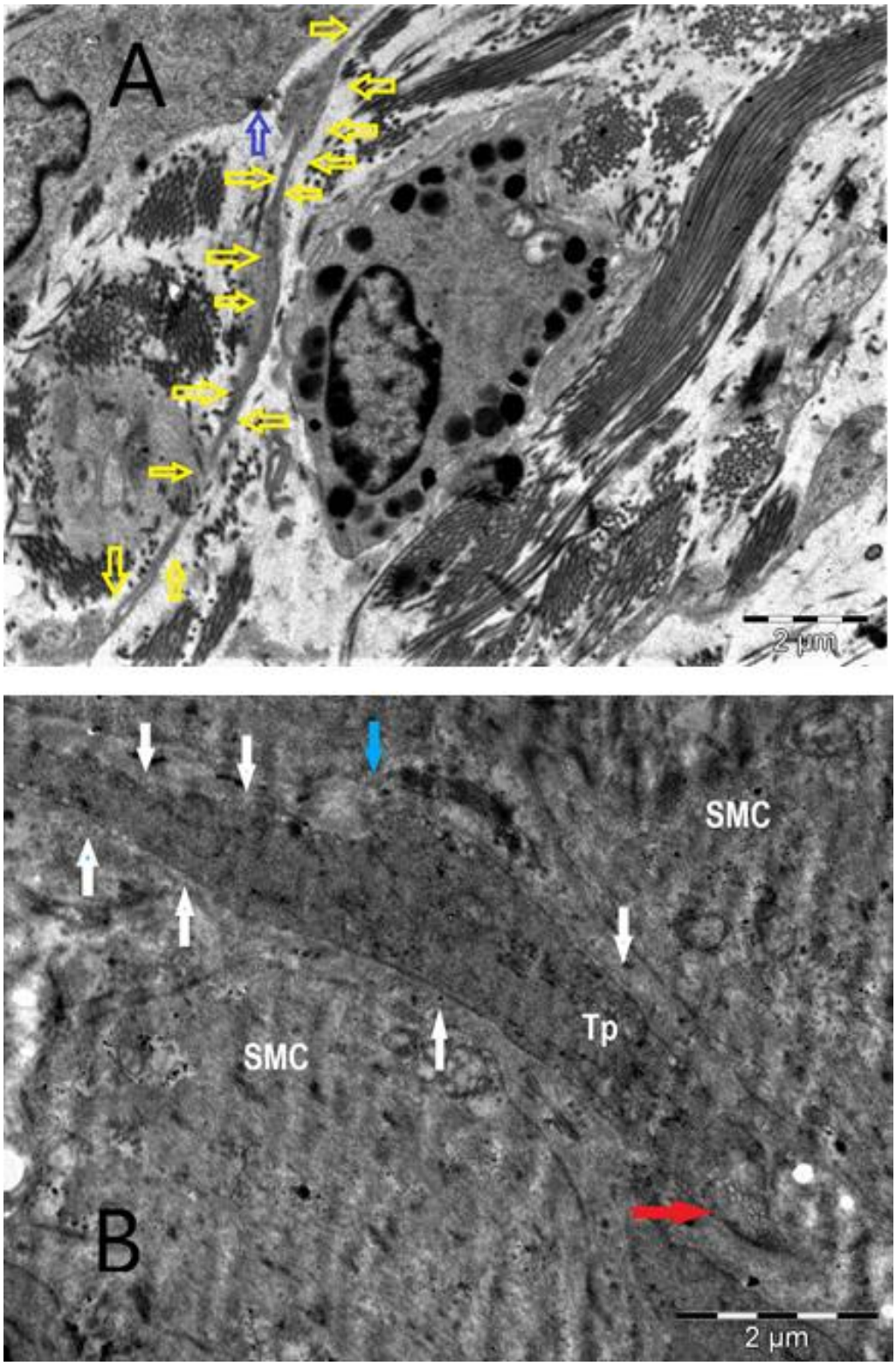

Figure 2. Transmission electron microscopy (TEM) of the muscle layer of the ureteropelvic junction in normal upper urinary tract. **(A)** Telocytes cytoplasmic process (telopode) (indicated with yellow arrows). The blue arrow indicates a contact between a telocyte and a myofibroblast

(red arrow). The telepode is characterized by a long slender cytoplasmic elongation that gets into contact with interstitial and smooth muscle cells. **(B)** Telopode is intervening between two smooth muscle cells (yellow arrow).The blue arrow indicates a group of caveolae, the white arrow is an attachment site between the telepode and smooth muscle cell (yellow arrow) (Scale bar: A=2μm; B=2μm).

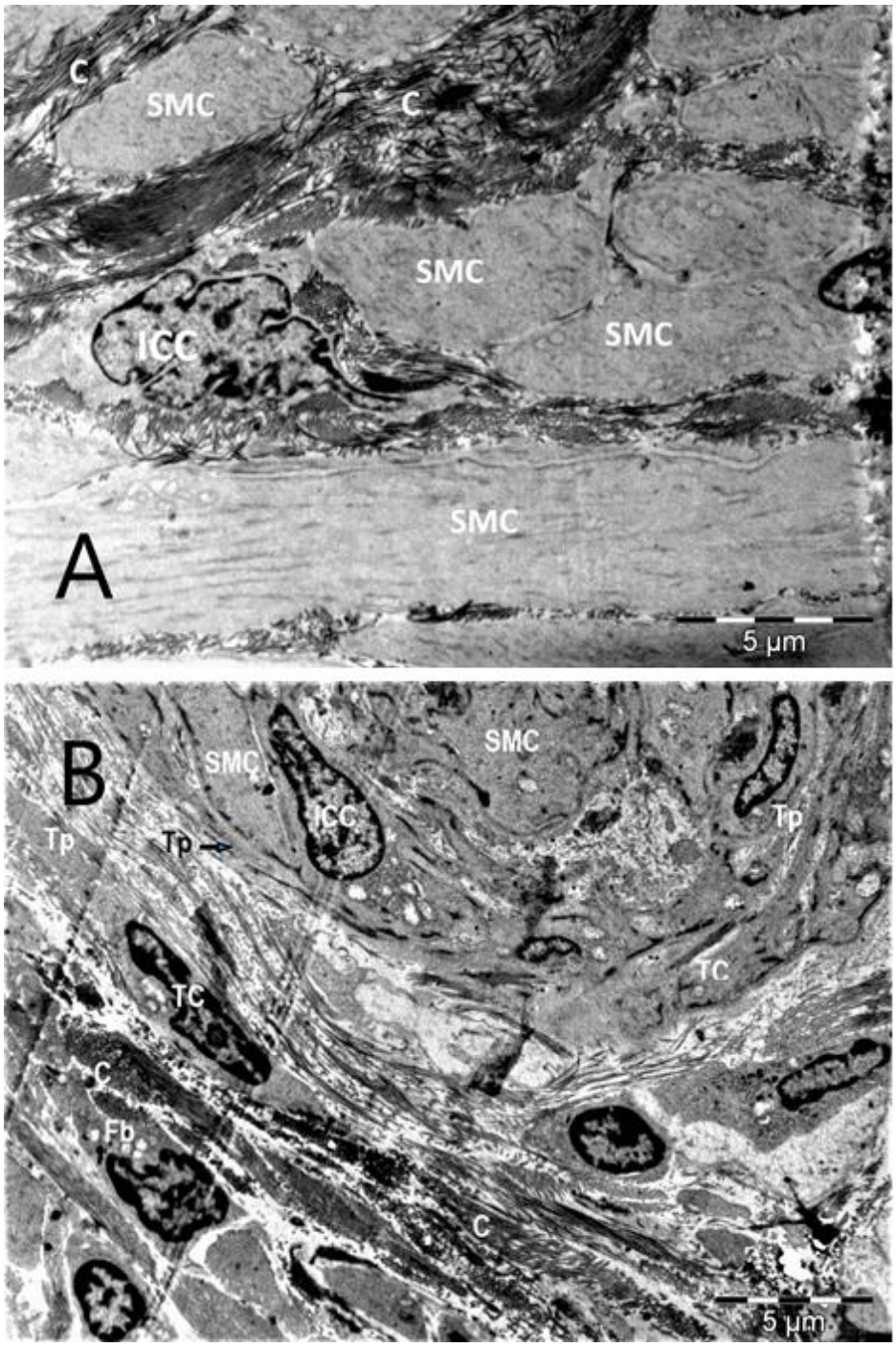

Figure 3. Transmission electron microscopy (TEM) of muscle layer of the ureteropelvic junction in normal upper urinary tract**.(A)** Interstitial cell of Cajal is located between longitudinal and transverse muscle layer, it is characterized by notched nucleus and scant cytoplasm with cytoplasmic extension intervening between the smooth muscle cells. The segment shows properly arranged smooth muscle cells (SMC), few collagen (C). **(B)** Telocytes (TC), telopodes(Tp), and interstitial cells of Cajal (ICC) are existing in-between smooth muscle cells (SMC), there are scattered collagen fibers (C). ICC: interstitial cell of Cajal; TC: telocytes; Tp. Telopode; SMC: smooth muscle cells; C: Collagen. (Scale bar: A=2µm; B=2µm).

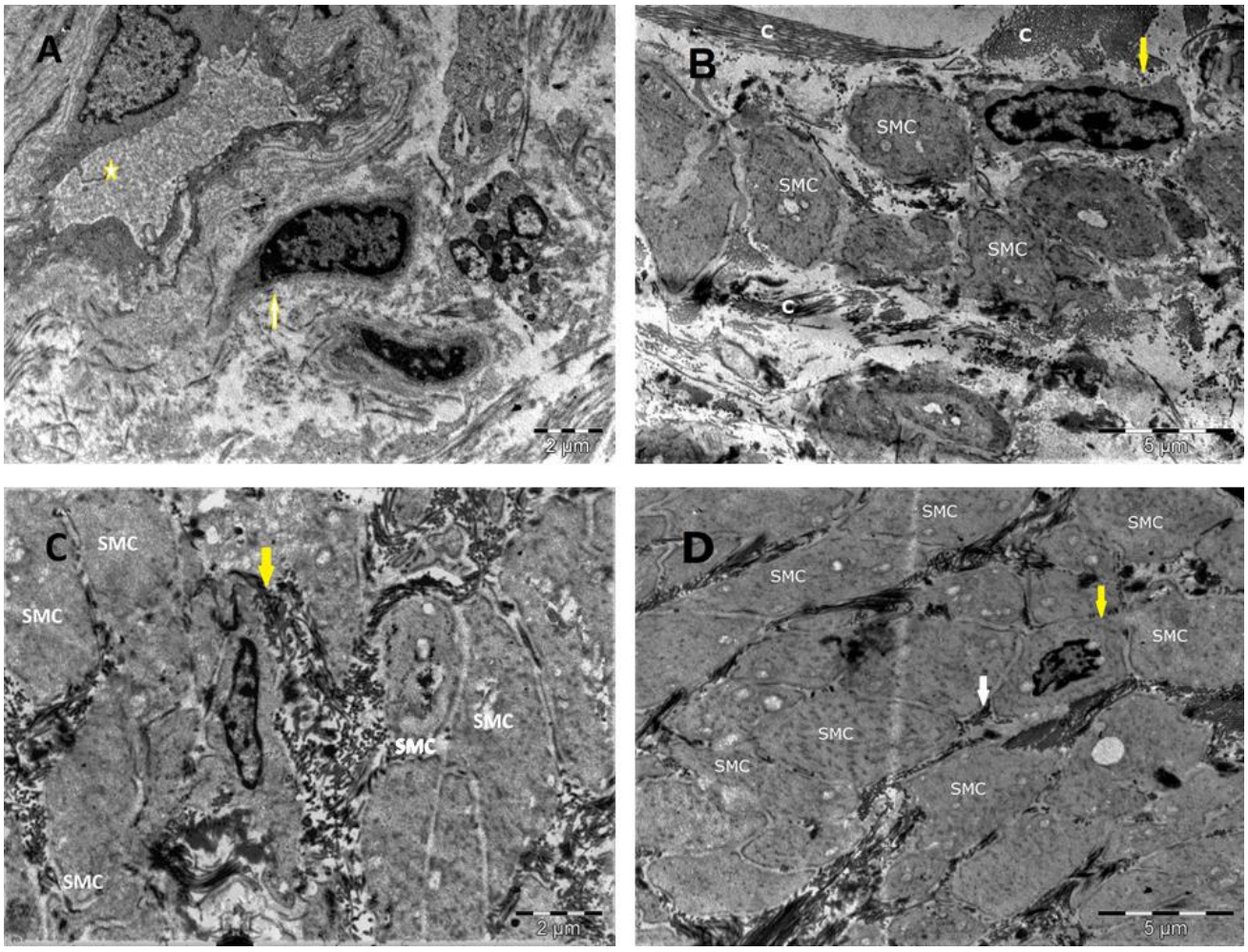

Figure 4 (**A).** Dormant stem cell resident by the nearby capillary (arrow). It shows notched nucleus as well as rough endoplasmic reticulum alongside the nucleus. It also lacks the elongated processes of the telocytes, capillary vessel (Asterix); bar = 2 µm; (**B).** Mesenchymal stem cells (yellow arrow), with scanty rim and obscured detailed cytoplasm, and polymorphic nuclei with deep notches. Smooth muscle cells (SMC); collagen(C);(Scale bar=5µm) ;(**C).** Myofibroblast (yellow arrow) showing peripheral myofilaments with focal densities, and a dense basement membrane-like material. They are surrounded by matrix collagen fibers, smooth muscle cells (SMC) are in close cell-to-cell adhesion and few collagen fibers intervening in the contact areas (Scale bar=2µm); (**D).** Myocyte (yellow arrow). The black dots in the cytoplasm are the dense bodies. The red arrow indicates collagen fibers of connective tissue, smooth muscles are in close contact to each other (SMC); (Scale bar=5 µm).