Interstitial cells of Cajal in the ureteropelvic junction: does its expression have a role in the pathogenesis of congenital ureteropelvic junction obstruction?

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Background
One of the most frequent urological abnormalities in pediatric urology and the most frequent cause of hydronephrosis in the pediatric age group is uteropelvic <ureteropelvic?> junction obstruction (UPJO). Although its etiology has been the subject of several investigations, it is still unknown.
Objective
Comparing the quantitative changes in the interstitial cells of Cajal (ICC) between specimens with UPJO and specimens without hydronephrosis using light microscopy and correlating these changes to the etiology of the disease.
Methods
Patients with UPJO and patients without obstruction were compared immunohistochemically with c-kit (CD117) between February 2020 and February 2022 at Ain Shams University Hospital to quantify the ICC, which is situated close to the circular muscle layer and parallel to the muscle cells.
Results
Light microscopic analysis revealed that the control group's Cajal cell density was much higher than the number of cells in the patients with UPJ blockage.
Conclusion
Our research found that the mean density of the ICC was lower in UPJO patients compared with controls, indicating that the absence of the ICC may contribute to the pathogenesis of UPJO.

Keywords:
hydronephrosis, immunohistochemistry, interstitial cells of Cajal, light microscopy, uteropelvic junction obstruction

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Introduction

One of the most prevalent pediatric genitourinary defects is congenital ureteropelvic junction obstruction (UPJO). The increasing decline in kidney function and probable loss of the renal unit are the main issues with this obstructive syndrome [1]. It has been hypothesized that conduction issues with the smooth muscle peristaltic wave are related to the specific etiology of ureteropelvic junction blockage [2].

Most of the time, ureteropelvic junction obstruction is thought to be a functional obstructive condition caused by abnormalities in the smooth muscle's gestational maturation and/or the innervation of the pyeloureteral transitional segment with subsequent defective peristaltic waves (aperistaltic segment) that will affect urine's ability to exit the renal pelvis and enter the ureter [3,4]. At this level, it has been demonstrated that there is excessive collagen deposition as well as smooth muscle discontinuity or disproportionate longitudinal smooth muscle fiber presence [5]. The electrical activity generated by a pacemaker positioned in the pyelocaliceal area might occasionally be defectively propagated as a result of an improper innervation [6]. Similar to pacemaker cells, Cajal's interstitial cells can produce spontaneous slow waves. Numerous writers have looked at the connection between ICC and cystourethral peristalsis after realizing their importance in the regulation and control of gastrointestinal motility) [7]. ICC may contribute to the pathophysiology of UPJO because they act as pacemaker cells, which can produce spontaneous slow waves to start smooth muscle peristalsis and serve as a bridge between neurons and smooth muscles [8]. Using light microscopy and c-kit (CD117), our study intends to identify differences in the density of ICC between specimens with UPJO and specimens without hydronephrosis, which may explain the cause of the UPJO.

Patients and methods

This cohort study was conducted in Ain Shams University Hospitals between February 2020 and February 2022 after approval of the Institutional Review Board.

Patients presented with an established diagnosis of UPJO with an indication of surgical repair were offered to participate in this study by performing a histopathological study of the UPJO after surgical correction with a c-kit antibody. It was performed to detect the expression of ICC in specimens.

Children with primary UPJO older than 6 months or weighted more than 6 kg were included. Patients with associated renal anomalies causing UPJO-like aberrant vessels, associated stones, or recurrent UPJO were excluded.

The following data were recorded preoperatively: gender, age at presentation, age at operation, antenatal diagnosis, symptoms, previous history of urinary tract infection, kidney functions, pelviabdominal ultrasound with kidney measurements including anteroposterior diameter of the pelvis, and diethylenetriamine penta-acetic acid (DTPA) scan.

Mean, standard deviation (±SD), and range were used for parametric numerical data, while median and interquartile range (IQR) were used for nonparametric numerical data. Frequency and percentage were used for nonnumerical data.

Informed consent was taken from the caregivers. The study was approved by the research ethics committee of Faculty of Medicine, Ain Shams University and General Surgery Department (IRB no.00006379).

Operative technique (Anderson-Hynes pyeloplasty)

This procedure was popularized and modified by Anderson and Hynes [9]. The patient was prepared, and the incision was done to access the renal hilum and the UPJ directly. The renal pelvis and obstructed PUJ were transected, leaving the traction suture in place at the most dependent portion of the inferior renal pelvis. The ureter was then incised for triangulated anastomoses to be done. A double J catheter was inserted. The ureter was anastomosed to the transected renal pelvis using 6–0 or 7–0 monofilament absorbable sutures.

Immunohistochemical staining

Tissues obtained from the cases of UPJO after surgery were about 2–3 cm in length containing the narrowed segment, while cases of the normal kidney were conned down with the continuation of the ureter, and a sample of about 1 cm was used.

Paraffin blocks were prepared and then slides were taken 4–5 μ in thickness from the paraffin-embedded tissues.

Slides were then prepared to be stained by an automated stainer (Ventana BenchMark) using Polyclonal Rabbit Anti-Human CD117, c-kit (code A4502 Dako Cytomation, Denmark), with a dilution of 1:200 (positive control using a positive c-kit in proto-oncogene activated in gastrointestinal stromal tumors (GIST)) [10] in the pathology department, Ain Shams University, Faculty of Medicine.

Light microscopy

An optical microscope Ray Wild (star 31) (Germany) was used in the evaluation of the c-kit-positive ICC in the immunohistochemically stained slides. We used the scoring method of Koleda *et al.* [11].

In each UPJO case, optical microscopy with a magnification of 200x and 400x was used to evaluate 11 well-stained magnification fields of 0.136 mm^2 each, and the density of c-kit-positive ICC was graded. The grades: sparse, moderate, and many indicated 0–1, 2–3, and 4–8 cell bodies per high-power field, respectively.

As two cell types showed c-kit immunoreactivity, ICC and mast cells, the former were identified in the inner border of the circular muscle layer in parallel orientation with muscle fibers. In addition, ICC had a fusiform cell body with a thin cytoplasm with a large oval nucleus. Mast cells also had oval nuclei but with a granular cytoplasm and spindle-like extensions and were found in the submucosa, muscularis mucosa, and mucosa.

Statistical evaluation

The collected data was revised, coded, tabulated, and introduced to a PC using the Statistical Package for the Social Sciences **(SPSS 25).** Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Results

In total, 19 patients were included in the study. Of this total number, all the specimens were examined by immunohistochemical methods: 17 patients with UPJ obstruction (median age 11 months) and 2 patients without obstruction (median age 20 months).

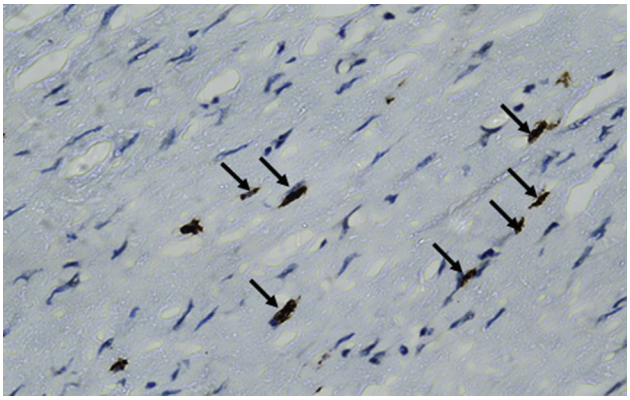
The control group as described in Table 1 consisted of two cases, one male and one female with ages of 12 and 28 months, respectively. In this group, total nephrectomy was conducted for pathologies other than UPJO (Wilms' tumor and mesoblastic nephroma). Both specimens were graded 9 in the mean score of the positivity of the ICC using a c-kit.

The study group as shown in Table 2 consisted of 17 cases of UPJO, 13 males and 4 females with a median age of 11 months. They suffered from hydronephrosis with a mean AP diameter of 42 mm and a decrease in the split function of the affected side ranging from 37% to 42.3%. Attacks of urinary tract infection were described in 52.9% of the cases. This group underwent Anderson-Hynes pyeloplasty in the Pediatric Surgery Department, Ain Shams University Hospitals.

Figure 1 shows a section of UPJ used as a positive control for the C-kit expression in ICC. Light microscopy with 400x magnification demonstrated 7 cells/HPF-positive C-kit ICC as compared with the section in Fig. 2, which showed only one cell/HPF in a case of UPJO indicated by black arrows. Red arrows indicate c-kit-positive mast cells.

The mean ICC score showed a significant decrease in ICC distribution in the UPJO study group versus the control group, ranging from 0 to 5 in the former as compared with 9 in the latter group. Furthermore,

Figure 1



Section in the normal UPJ with abundant ICC. c-kitx400.

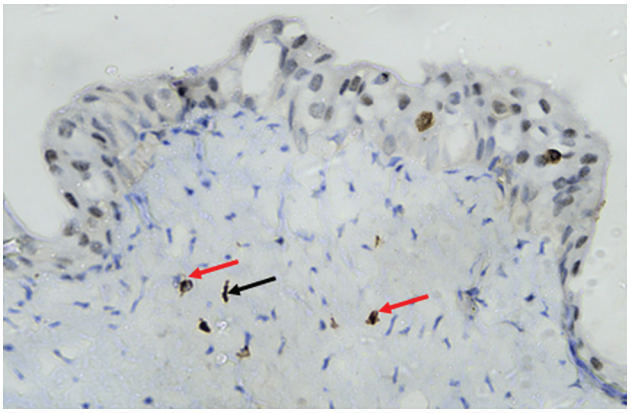
Table 1 Description analysis for the control group

Normal kidney group (*N*=2)	N/Mean	%/SD	Median (IQR)	Range
Sex				
Male	1	50.0%		
Female	1	50.0%		
Age in months	20.00	11.31	20 (12–28)	(12–28)
Mean of ICC	9.00	0.00	9 (9–9)	(9–9)
Result of mean score of normal group	9	2	100.0%	

Table 2 Description analysis for the UPJO study group

Abnormal diagnosis group (17)	N/Mean	%/SD	Median (IQR)	Range
Sex				
Male	13	76.5%		
Female	4	23.5%		
Age in months	16.26	15.48	11 (5–15)	(1.5–48)
AP diameter preoperative in mm	42.29	14.60	42 (31–52)	(21–75)
Serum creatinine (mg/dL)	0.70	0.29	0.6 (0.5–0.95)	(0.3–1.2)
DTPA split function of the affected side (%)	39.66	4.11	40.81 (37–42.3)	(30–44.5)
DTPA split function in the non-affected side (%)	58.93	4.15	58 (56–60)	(53–70)
Dysuria				
No	8	47.1%		
Yes	9	52.9%		
Result of mean score of the abnormal group	0	2	11.8%	
	1	3	17.6%	
	2	7	41.2%	
	3	2	11.8%	
	4	1	5.9%	
	5	2	11.8%	

Figure 2



Section in a case of UPJO showing marked deficiency in the number of ICC (black arrow) (one cell/HPF) in comparison to control. Red arrows indicate C-kit-positive mast cells. C-kitx400.

among the study group, 41.2% of the UPJO cases had a mean of 2.

Discussion

Postnatal hydronephrosis is a major clinical problem for infants with a prevalence of about 0.2–2% in all pregnancies [1], 44% of which are mainly due to UPJO [12]. Primary or congenital UPJO is still a matter of controversy with various mechanisms postulated to define its mechanism. No specific delineation was reached regarding the specific etiology of UPJO, yet many histological studies have been made [13].

Putting in mind the physical characteristics of the UPJO in which there was a decrease in the peristaltic motion of the UPJ and correlating it to the decrease in the density of the ICC might provide a potential explanation for the UPJO. However, congenital UPJO's pathogenesis is still up for debate. There are numerous factors, including muscular structural issues. The decline in the ability of the narrow segments to extend, which results in the functional discontinuity of ureteropelvic muscle contractions, can be explained by atrophy, or a reduction in the number of smooth muscle cells, a reduction in the number of ICC and nerve endings, and an increase in the collagen deposits between the muscular clusters [7]. According to some studies, the obstruction's fundamental cause is a problem with intrinsic function, and morphological alterations are only incidental [14].

Many researches concurred with our results like studies by Eken A *et al.* [15], Solari *et al.* [7], and Yang *et al.* [16] which found a decrease in the expression of ICC at the site of UPJO in comparison to the control group in which the positive c- kit ICC was predominantly detected in the muscle layers. This observation led to the same conclusion that we deducted in our study that the decreased expression of the ICC is an etiological factor in the UPJO.

Contrary to our results, Koleda *et al.* [11] found an increase in the mean density of the ICC in patients with UPJO arguing that the increase in the ICC is a compensatory mechanism. This may be a side effect of the blockage, which prevents urine from being expelled properly, increasing the pacemaker ICC cells, generating intrinsic muscle hypertrophy, and ultimately, UPJO. Therefore, it may be hypothesized that the variation in the severity or length of blockage caused a change in c-kit expression, which is why the results from Solari *et al.* and Yang *et al.* were different.

However, analysis by Apoznanski W *et al.* [17] comparing specimens from 20 patients with UPJO and 5 control patients concluded that there are no differences in the distribution of c-kit-positive ICCs between patients with UPJO and those in the control group, proving that UPJO is not related to changes in the ICC distribution. This study, like ours, evaluated 11 high-power fields from each specimen, each measuring 0.136 mm^2, at extreme magnification.

Kuzgunbay *et al.* created experimental obstruction in UPJ in a study conducted among 109 rats to evaluate acquired ureteral obstruction. The affected cases showed a sudden rise in the c–kit-positive ICC reaching the peak level after 14 days, which is higher than the mean of the control group, then gradually decreased to reach a plateau after 60 days. Thus, it was suggested that the expression of c-kit-positive ICCs was time related [18].

Up till now, all previous studies done including our study are observatory and speculative regarding the role of the ICC in the UPJO, yet it is noteworthy that the difference in the distribution of the ICC plays a role in the pathogenesis of the UPJO.

Other possible explanations have been adopted, such as those by Hunziker M. *et al.*, who found that altered SK3 channel expression was seen in PDGFR+ cells in UPJ obstruction, suggesting that the impairment of SK3 activity across the UPJ may perturb upper urinary tract peristalsis and result in UPJO. However, ICC might play a role or be a key step in the pathogenesis [19].

In addition, Müslim Y. *et al.* found that the UPJO group had considerably greater collagen thickness values than the control group, indicating that collagen deposition is an etiological component of the UPJO [20]. In addition, in UPJO instances, Mehramza M *et al.* discovered intrinsic smooth muscle abnormalities, muscle layer thinning with collagen deposition, and a reduction in the ICC distribution [21].

It is no doubt that UPJO is a complex pathology with interconnected etiological factors and more studies are needed to gather all the ends to draw a complete picture of the disease.

The major limitation in our study was the 2-year time frame and the COVID restrictions limiting the number of patients generally and the control group specifically. Also, the control group was limited by decreased incidence of nephrectomies in the absence of disruption to the histopathology of the UPJ.

Conclusion

In contrast to the control group, our study found that the mean density of the ICC was lower in instances of UPJO, indicating that the absence of the ICC may contribute to the pathogenesis of UPJO.

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Nil.

Conflicts of interest

All authors declare that they have no competing interests related to this study.

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