Original Research Article

Assessment of distribution of Interstitial cell of Cajal like cell across upper urinary tract region in children with pelvic ureteric junction obstruction

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Abstract

Aims and objectives: To study the distribution of Interstitial cell of Cajal like cell (ICC-LC) across upper urinary tract region in children presenting with pelvic ureteric junction obstruction (PUJO) and its association with renal functional and sonological parameters of patients. **Material and method:** A prospective study was done on twenty children (excluding neonates) with primary PUJO undergoing dismembered pyeloplasty. All subject underwent renal sonography [anteroposterior diameter of pelvis (APPD), pelvi-calyceal ratio (P/C ratio), mid-polar renal parenchymal thickness (MPPD)] and functional imaging scan.Three specimens were taken intra-operatively from above, at and below PUJ and were examined immunohistochemically using CD-117 to count ICC-LC using standard criteria. ICC-LC distribution was correlated with above stated parameters. **Result:** The number of ICC-LC showed a continuous decreasing trend craniocaudally across PUJ. P/C ratio and APPD showed parallel trend with ICC-LC distribution across PUJ, while split renal function (SRF) showed inverse relationship with expression of ICC-LC. Group with lesser severity of obstruction (APPD<30 mm, SRF≥40%) showed uniform decreasing trend in number of ICC-LC craniocaudally across PUJ. More severely obstructed patients (APPD≥30 mm, SRF<40%) had decreasing trend up to the level of PUJO followed by a sudden relatively increased expression of ICC-LC below PUJ. **Conclusion:** The expression of ICC-LC shows a uniformly decreasing trend across PUJ when the severity of obstruction is less. Resurgence in the number of ICC-LC below PUJ in subjects with severe obstruction hints at emergence of new pacemaker area below severely blocked PUJ akin to that seen in complete heart-block patients and deserve early attention.

Key words: Pelviureteric obstruction, Children, Interstitial cell of Cajal

Introduction

Pelvic ureteric junction obstruction (PUJO) is the most common cause of congenital obstruction of urinary system with the prevalence of 1 in 1000 to 2000 neonates.[1] The cause of this obstruction of propulsion of urine across pelvic ureteric junction (PUJ) is not yet been known well though it is considered to be multi-factorial.[2] It is accepted that the mechanism by which urine gets propelled towards the bladder is myogenic in nature (that is without being influenced by nerves),[2-4] most likely due to presence of pacemaker cells in urinary tract. The latter were considered to be "atypical" smooth muscle cells (SMC), bearing several morphological and electrical characteristics similar to cardiac sino-atrial node cells. These were found to be more numerous in the proximal regions of the pelvis, with their number decreasing with the distance from the renal fornix. As per considerable evidences, these are present even in ureter as depicted by spontaneous peristaltic wave generation in ureter after disconnection.

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Recently, similarities were also found between these "atypical" SMC present in urinary tract and the intestinal pacemaker cells [5,6] recognized as interstitial cells of Cajal (ICC). Hence, these were also called Interstitial cells of Cajal like cells (ICC-LC). Akin to gut motility, the distribution and expression of ICC-LC have also been linked topyelo-ureteral peristalsis. ICC-LC are known to possess c-kit receptor, similar to the mast cell having similar receptor. However, mast cell can be differentiated from ICC-LC based on their morphology and distribution within the tissues.

Though few workers had found significant decrease in number of ICC-LC in upper urinary tract in UPJ region in subjects with PUJO, other reports have contradictorily shown either increase in density [7] or no change in density [8] of these cells in this region. Furthermore, there is experimental evidence to suggest that the number of ICC-LC could be related to severity of PUJO; but none of the available studies have touched this aspect.

In view of what has been stated above a need of a study was felt wherein the distribution of ICC-LC across the upper urinary tract could be studied in subjects with PUJO in relation with sonological findings to fill in the lacuna of knowledge in this aspect.

Methods and materials

It was a prospective analytical study done in the Department of Paediatric Surgery at a tertiary referral center over 21 months (November 2016 to July 2018) including twenty consecutive children of PUJO (up to 12 years age) undergoing dismembered (Anderson-Hynes) pyeloplasty based on standard indications. Neonates,

syndromic patients, patients having secondary PUJO, vesicoureteric reflux (VUR), previous surgery on kidneys and extrinsic causes of PUJO were excluded from the study. All subjects underwent renal sonological scan for antero-posterior diameter of pelvis (APPD), SFU grading, Renal parenchymal thickness in mm at mid-polar level (MPPT).Functional imaging using DTPA/ LLEC was done for all subjects as renal dynamic scan (RDS) for pattern of execration curve and split renal function (SRF).

Three specimens of 5 mm each were taken from the redundant excised pelvis above PUJO (sample 1), at the site of PUJ obstruction (sample 2), and just below PUJ (sample 3). Layers of lamina propria and muscularis mucosa were examined by immune-histologically using CD117. In each specimen, 10 neighbouring well-stained, oriented high-power fields (HPF) of 0.136 mm^2 were evaluated, and the number of c-kit positive ICC-LCs/HPF were counted.

The distribution of ICC-LCs/10HPF was computed for each subject at, above and below PUJO subjects. Further number of ICC-LC at three locations were correlated with APPD, SFU grading, MPPT, SRF, APPD/ MPPT ratio (P/C ratio) following classifying subjects based on severity of obstruction.

Result

The striking observation was that in composite group of subjects, the number of ICC-LC showed continuous decreasing trend across PUJ from above downwards. The mean number of ICC in samples 1, 2 and 3 were 22.9±10, 17.4±7 and 15±3.1 respectively (**figure 1**). Overall there was fall in number of ICC by 15% between sample 1 and 2 and 10% between sample 2 and 3, though neither of these differences was statistically significant.

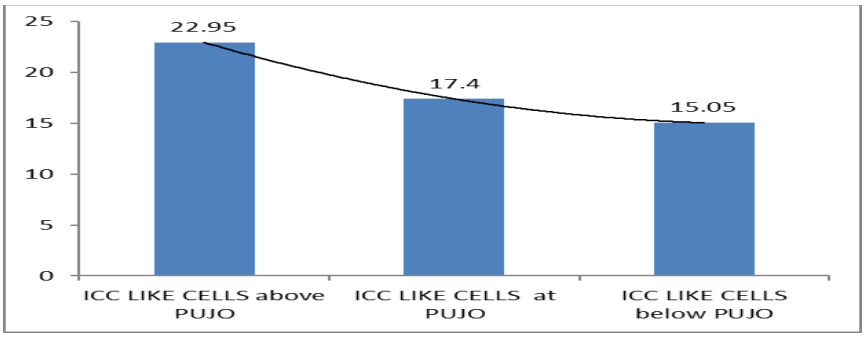

Fig 1:median number of ICC-LC across PUJO

Another interesting observation was that each of parameters P/C ratio and APPD showed variable relation with ICC-LC distribution across PUJO. Both these parameters had positive correlation with ICC-LC number both above and below PUJO, but negative correlation at level of PUJO (**figure 2**). Another noteworthy fact was a reverse relation between ICC density and SRF.

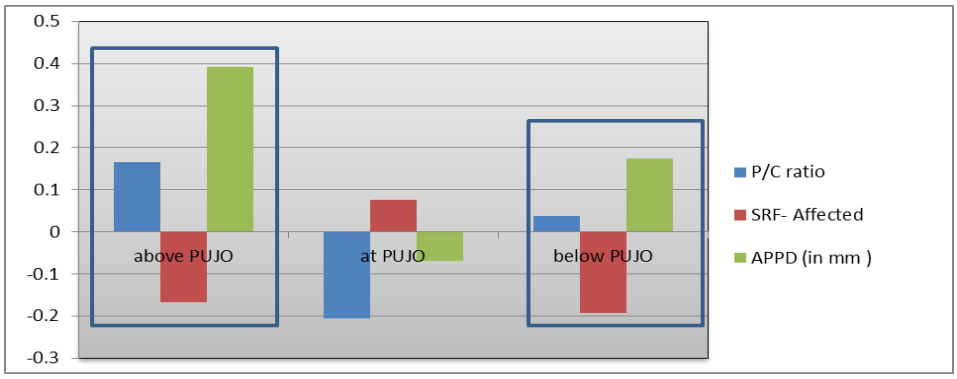

Fig 2: Association of SRF , APPD and P/C ratio at three levels

These observations led us to delve further to analyze how the distribution of ICC-LC could differ between those with lesser and severe degree of obstruction. This was done by dividing the data into two subgroups by using the median values of the group i.e. APPD (30 mm), SRF (40%), MPPT (5mm), P/C ratio (5.1). Those with less favorable parameters were grouped as comparatively more obstructed. The distribution of ICC-LC was studied in relation to each of these parameters.

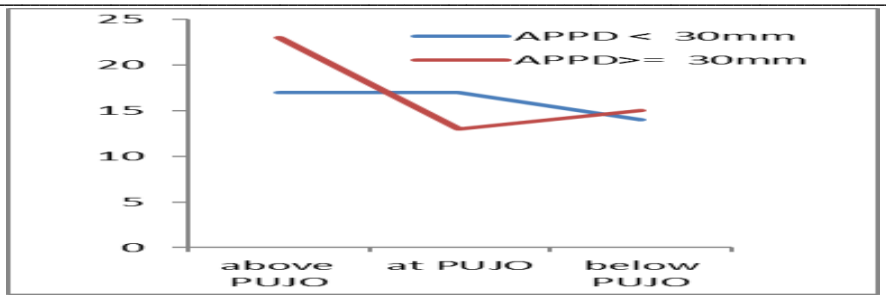

Fig 3:median number of ICC-LC across PUJ in association with APPD

Subjects with APPD≤30 mm showed uniform cranio-caudal decreasing trend of median value of ICC-LC count from above downwards. On the other hand, children with APPD >31.5mm initially showed a more severe degree of fall (**Figure 3**). This was followed by unexpected 16% increase in their number from level of PUJ to below it. So, subjects with higher APPD (≥30 mm) not only showed different trend of distribution of ICC-LC from those with lesser severe degree of obstruction but in them, there was significant correlation between percent fall of ICC-LC between sample 1 and 2 with APPD (**table 1**).

Table 1:percentage median fall in number of ICC-LC

	Percent median fall in ICC-LC between above and at the level of PUJ	Percent median fall in ICC-LC between above and at the level of PUJ
APPD<30mm	10.5	13.3
APPD>30mm	40	-16
significance	P<0.05	p>0.05

In renal units with SRF<40% (poorer function), there was 33.3% decrease in the expression of ICC-LC from above PUJ (sample 1) to the level of PUJ (sample 2), which was statistically not significant (**figure 4**).

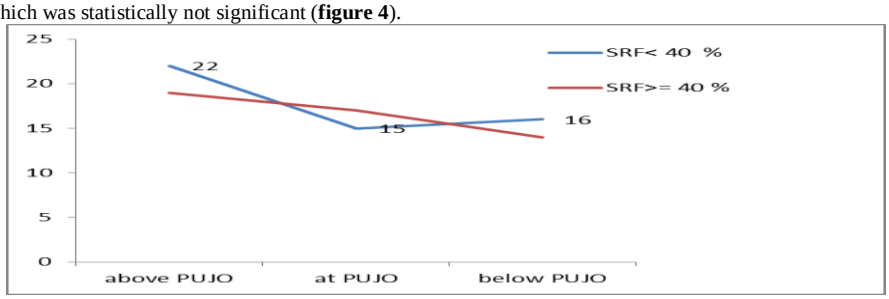

Fig 4:median number of ICC-LC across PUJ in association with SRF

Thereafter, there was 16.6% median increase in the expression of ICC-LC from sample 2 to sample 3 (PUJ downwards). The patients with SRF≥40% (better functioning) paralleled the trend of lesser APPD <30 mm i.e.14% fall between sample 1 and 2and 12% fall between sample 2 and 3 in (**table 2**), none of these being significant.

Table 2: percentage median fall in number of ICC-LC in relation to SRF

	Percent median fall in ICC-LC between above and at the level of PUJ	Percent median fall in ICC-LC between above and at the level of PUJ
SRF<40%	33.3	-16.6
SRF>40%	14	11.7
Significance	P>0.05	p>0.05

Similar trend of difference in ICC-LC distribution was noted for better and worse functioning renal units using MPPT<5mm or P/C ratio≥5.1also, but no difference between drop in number of ICC-LC between sample 1 and 2 was significant.

Discussion

Although ICC was first described in 1982 by Cajal [9] in GIT, it was Lang et al. who in the year 1999 first described a population of electrically active ICC-LC in upper urinary tract of guinea pig, [10] However, work on the human urinary system in this context was very sparse. The earliest study in this regard on human being was first done by Solaris et al.in 2003 [11]. They concluded that the density of ICCs was markedly decreased in the obstructed PUJ specimens at the level of obstruction [11]. However, some contradictory observations were made by Koleda et al. in 2012. They described an increase in ICC-LC at the level of PUJ in pyelo-ureteral junction obstructive disease, and explained the phenomenon as a compensatory mechanism for the loss of the ability to conduct the urine from the pelvis towards the ureter [7] due to increased fibrosis and loss of smooth muscle cells (SMC), which are primary pacemaker cells. Compensatory hyperplasia of the ICC-LC (responsible for the secondary peristalsis) occurred to overcome this obstruction.In our study, the number of ICC-LC were higher above PUJ in comparison to the number of ICC-LC at the level of PUJ and below PUJ. Also, there was less steep decline in expression of ICC-LC from the level of PUJO to below it than what was noted between above the level of PUJ to PUJ. The mean number of ICC-LC above PUJ, at PUJ and below PUJ are 22.9±10.9, 17.4±7, 15±3.1) respectively. Also, the number of ICC-LC decreased from cranially to caudally across PUJ.The main fact that emerges out in our study is that the number of ICC-LC decreases at the level of PUJ in patient with PUJO. It may be due to fibrosis and replacement of ICC-LC at level of obstruction. Few of the previous studies, have shown paucity or absence of ICC-LC in the urinary tract, associated with increased density of fibrotic tissue [11-13].The only earlier work in English literature which had studied the c-kit +ve cells above and lower to the PUJ was done by Kuvel et al [14]. In their study, they examined the obstructed segment of 32 cases with intrinsic PUJO; the segment of PUJ with chronic obstruction (15 cases with nephrectomy due to chronic obstruction associated with lithiasis, tumor and reflux) and normal PUJ segment (30 patients where nephrectomy was done due to renal tumor or trauma). They observed that the number of ICC-

LC was significantly decreased at the level of PUJ in subjects with intrinsic PUJO. However, within 5 mm proximity above and below PUJO, the number of ICC-LC were higher. Also the number of ICC-LC proximal to obstructed PUJ in children with intrinsic PUJO was higher than what was found in controls.Most of the studies concluded that the number of ICC-LC were fewer in pyeloureteric region with loss of motility and increased fibrosis. This justifies our observation of decreased number of ICC-LC at PUJO due to intrinsic fibrosis. The study also showed that the degree of severity and/or duration of obstruction based on P/C ratio, APPD and parenchymal thickness could have affected the distribution of ICC-LC across PUJ.An interesting observation was that P/C ratio and APPD showed direct relationship with ICC-LC distribution across PUJO whereas SRF of affected side showed inverse relationship with ICC-LC distribution across PUJO. While P/C ratio and APPD showed positive correlation with ICC-LC expression above the PUJO and below the PUJO but it showed negative correlation with ICC-LC at the level of PUJO; SRF expectedly showed just the opposite trend. This may reflect a higher degree of PUJO obstruction in this set of subjects, which led to an increased workload to overcome the obstruction at PUJO causing an increase the expression of ICC-LC above PUJO and increased secondary peristalsis below the PUJO leading to resurgence in their number. We found that while there was uniformly decreasing trend of ICC numbers craniocaudally across PUJO in children with less severe PUJO; those with severe obstruction had an initial steep decrease in the expression ICC-LC between above PUJ to the level of PUJ followed by a relative **increase** in the ICC-LC number between PUJ and below it. This relative increase in number of secondary peristaltic cells below PUJO probably occurred due to their secondary hyperplasia of these cells below obstructed PUJ to propel the urine by secondary peristalsis. There was significant correlation ($p<0.05$) between the percentage fall of ICC-LC between sample 1 and 2 in both the groups. In severe degree of obstruction, resurgence in number of ICC-LC below PUJ is indicative emergence of secondary peristalsis below PUJ. This bears resemblance of phenomenon of electrical activity in complete heart block where once the primary pace maker fails there is activation and resurgence of secondary lower pacemaker cells to takes up its role. The ICC-LC shows structural and physiological similarity with pacemaker cell of the heart and nerve cells**.** The hypothesis of ICC as nerve cells by Cajal was based on the observation that ICC associated with the myenteric plexus, stained with methylene blue and silver impregnation according to the Golgi method, similar to neural tissues. Keith saw structural similarities with sinoatrial node cells and hypothesized them to be pacemaker cells [15]. Electrophysiologically, intracellular calcium handling plays a key role in the gut pacemaker responsible for spontaneous rhythmicity, as well as in the cardiac pacemaker responsible for spontaneous beating.[16]

Conclusion

The expression of ICC-LC shows a uniformly decreasing trend across PUJ when the severity of obstruction is less. In severe obstruction there is sharp decrease in the number of ICC-LC at the level of PUJ with resurgence in the number of ICC-LC below PUJ. Resurgence in the number of ICC-LC below PUJ in subjects with severe obstruction and in young infants hints at emergence of new pacemaker area below severely blocked PUJ akin to that seen in complete heart-block patients and deserves early attention. This ICC-LC can be use in the future for labeling biopsy and proper functional anastomosis between pelvis and ureter in case of pelvicureteric junction obstruction similar to the Hirschsprung disease. In future ICC-LC guided anastomosis between pelvis and ureter will be of great help especially in redo-pyeloplasty.

Conflict of Interest: Nil
Source of support: Nil

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