Original Research Paper

Volume - 12 | Issue - 10 | October - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

IMMUNOHISTOCHEMICAL EXPRESSION OF C-KIT AND S-100 MARKERS IN URETEROPELVIC JUNCTION OBSTRUCTION AND ITS CORRELATION WITH HISTOPATHOLOGY AND MASSON'S TRICHROME STAINING

Dr. Chaitra. D*	Senior Resident, Department of Pathology, Osmania Medical college, Hyderabad. *Corresponding Author
Dr. G. J. Vani Padmaja	Professor &HOD, Department of Pathology, Osmania Medical college, Hyderabad.
Dr. Kayla Geetha	Associate Professor, Department of Pathology, Osmania Medical college, Hyderabad.
Dr. Yeshwanth Vedire	Tutor, Osmania Medical college, Hyderabad.

ABSTRACT **Background:** Congenital obstructive nephropathy is the principal cause of end stage renal disease (ESRD) in children (Benfieldet al. 2003). Obstructive nephropathy leads to Hydronephrosis, defined clinically by an enlargement of the kidney as a result of urine accumulation in the renal pelvis or calyces. [1,2] Ureteropelvic junction (UPJ) obstruction is the most common cause of hydronephrosis with an estimated incidence of 1 in 1000–1500 newborns (Changet al. 2004). [2,3] **Aims:** To evaluate the pathogenesis of congenital ureteropelvic junction obstruction (UPJO) by histopathology, IHC markers CD117 & S100 and special stain Masson's Trichrome. **Methods And Materials:** The study group consists of 50 Pediatric cases presenting with intrinsic ureteropelvic junction obstruction and 15 patients with Wilms tumor with no UPJO formed the control group. All the cases and controls were subjected to histopathological examination, immunohistochemical staining with markers CD117 & S100 and special stain Masson's Trichrome. Mann – Whitney U test and Pearson's chi-square test were used for statistical analysis. **Results:** The cases had reduced CD117 and S100 expression, Irregularity of muscle fibers, increased wall thickness & muscle thickness and submucosal collagen deposition compared to controls. **Conclusions:** Decreased ICC interpreted by decreased CD117, decreased neural innervation interpreted by decreased S100, structural changes - Irregularity of muscle fibers arrangement, increased submucosal collagen deposition and increased smooth muscle thickness possibly cause disruption of the mobility of UPJ and lead functional obstruction.

KEYWORDS : UPJO, CD117, S-100, Masson's Trichrome

INTRODUCTION:

Congenital obstructive nephropathy is the principal cause of end stage renal disease (ESRD) in children (Benfield ***et al.*** 2003).This contrasts with adult ESRD that for the larger half originates from ageing and type II diabetes. Obstructive nephropathy leads to Hydronephrosis, defined clinically by an enlargement of the kidney as a result of urine accumulation in the renal pelvis or calyces. [1,2] Ureteropelvic junction (UPJ) obstruction is the most common cause of hydronephrosis with an estimated incidence of 1 in 1000–1500 newborns (Chang ***et al.*** 2004). [2,3]

The mechanism of congenital ureteropelvic junction obstruction has not been known well. It is currently believed that the pathogenesis of the disease is complex and involves multiple factors. Decrease in the number of smooth muscle cells, interstitial Cajal-like cells and nerve fibers in this region together with abnormal arrangement of smooth muscle cells and increase in collagen deposition are some of the suggested mechanisms.

The current study is about the evaluation of expression of C- kit and S-100 and correlation of findings in Masson's trichrome in congenital UPJO specimens

METHODS:

The study group consists of 50 Pediatric cases presenting with intrinsic ureteropelvic junction obstruction and 15 patients with Wilms tumor and normal UPJ formed the control group. All the cases and controls were subjected to histopathological examination, immunohistochemical staining with markers CD117 & S100 and special stain Masson's Trichrome. Mann – Whitney U test and Pearson's chi-square test were used for statistical analysis**.** The cases associated with Vesico ureteric reflux, posterior urethral valve, Vesico ureteric junction obstruction & duplication and extrinsic ureteropelvic junction obstruction were excluded from the study. The institutional ethical committee approval was taken.

Scoring And Evaluation:

Interstitial Cajal-like cells were counted in the randomly-selected 5 HPF(s) in each group. Mast cells are also immunoreactive for c- kit and act as internal control. Both were differentiated based on the morphology and location. ICCs were identified in the inner border of the circular muscle layer in parallel orientation with muscle fibers. ICCs had a fusiform cell body with a thin cytoplasm, a large oval nucleus. Mast cells were found with a round central nucleus. Mast cells were found in the submucosa, muscularis mucosa and mucosa.

Nerve fibers were immunoreactive for S-100 and were counted in the randomly-selected 5 HPF(s) too. The nerve fibers are found in adventitia and within muscle layers.

Sections stained with Masson's trichrome staining were used for evaluating irregular arrangement of muscle fibers and morphometry. Collagen deposition has blue colour in trichrome-masson staining and Muscular component is red. Nuclei stain black.

Arrangement of muscle fibers was compared qualitatively between the two groups.

Using Procam app in the computer connected to microscope and camera, the thickness of the UPJ wall , submucosal collagen and muscular wall thickness were measured in pixels. Using conversion factor 4X= 1.3672 micro meter/ Pixel, the value in pixels were converted to micro meter.

RESULTS:

Among 50 cases of UPJO, the lowest age was 12 days and highest age was 12 years. (Range 12days – 12 Years) The mean age is 32.3523 months (2.7 years). Most of the cases presented within 1 year of age. Among 1 year of age, highest cases were noted in 2 months of age.

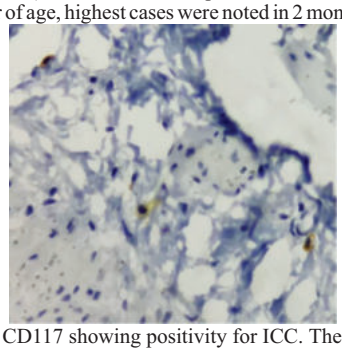

Figure 1 IHC CD117 showing positivity for ICC. The cell present in the centre is mast cell and other 2 fusiform shaped cells are ICC.

41(82%) male children and 9(18%) female children presented with UPJO. Males were most commonly affected than females. Male: Female ratio is 4.6: 1. There were 31(62%) cases of UPJO affecting Left side and 19(38%) cases on Right side. Left side UPJO was most common. The children with UPJO presented with chief complaints of fever, pain abdomen, vomiting, abdominal distension and haematuria. Few cases were diagnosed antenatally.

Number of ICC per 5 HPFs(Mean+ SD) cases is 15.9 +4.1020 /5HPF and controls is 34.86667+3.9617 /5HPF. P value is <.00001

Expression of S100 in UPJO: The mean + SD for cases and controls were 6.3 + 1.9416/5 HPF and 9.866667 +2.7047/5 HPF respectively. The p value was significant (p< .00001)

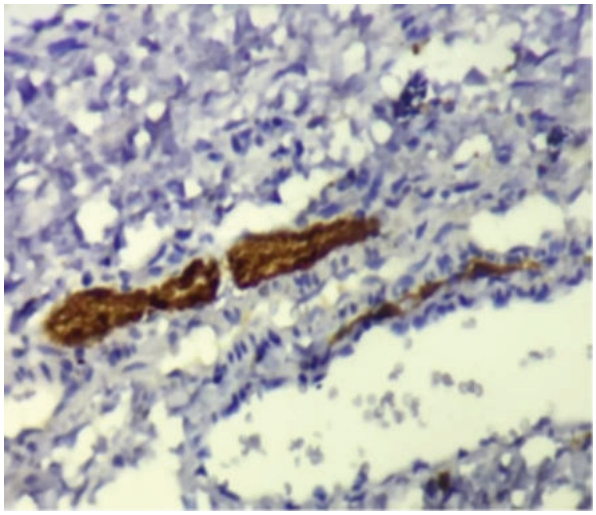

Figure2 S100 shows thickened nerve fibres

Irregular arrangement of muscle fibres was found in 35 (70%) cases and 2 (13.3%) controls. The difference was significant between the 2 groups with p value- 0.0001

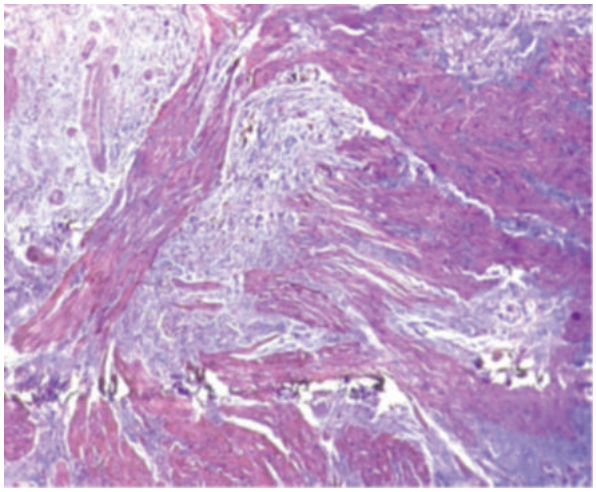

Figure3: MTS(Masson's Trichrome Stain) images showing irregular arrangement of muscle fibres(collagen in blue and smooth muscles in red)

Morphometry:

The mean values of the wall thickness, submucosal collagen thickness and muscle wall thickness were 1412.1289 µm, 414.9229 µm, 755.5099 µm (in cases) and 764.6130 µm, 220.0775 µm, 299.5334 µm (in controls) respectively with p values <.00001 for wall thickness, 0.0001 for subepithelial collagen thickness and <.00001 for muscle thickness

DISCUSSION:

Ureteropelvic junction obstruction (UPJO), or pelviureteric junction obstruction, is defined as a blockage or obstruction of urine flow from the kidney into the proximal upper ureter. UPJO has a ratio of 2:1 in boys compared with girls, and the left side is affected in approximately two-thirds of cases. The condition can occur bilaterally also.[4]

Extensive use of antenatal ultrasonography (US) and modern imaging technique help in earlier diagnosis of hydronephrosis. Before the advent of US, congenital hydronephrosis presented throughout childhood and even adulthood with various symptoms such as abdominal or flank pain, recurrent urinary tract infections (UTIs), abdominal mass, renal stones, and hematuria.[5] Delay in diagnosis or presentation leads to increased chances of renal damage and loss. Earliest detection and prompt treatment prevents such untoward effects.

UPJO may be congenital or acquired. The causes can be intrinsic or extrinsic. Congenital UPJO usually results from intrinsic disease due to abnormality in the lamina muscularis, increase in collagen between the muscle bundles and elastin in the adventitia [6-9], valves produced by infoldings of ureteral mucosa [6,10], abnormalities of ureteral innervations [11,12] Abnormalities of microstructure as well as functional disorders impeding the urine outflow from the renal pelvis are regarded as intrinsic. [13]

Extrinsic causes of UPJO include a crossing lower pole renal vessel (aberrant, accessory, or early branching) causing an impingement on the ureter, obstructing flow and hinders its development. Anterior crossing vessels are more common than posterior ones. [4,14] Congenital abnormalities of the kidney, such as horseshoe kidneys or duplex kidneys, Scar formation secondary to ureteric manipulation by surgery and Fibroepithelial polyps (a rare cause of UPJO) [4]

The histopathological findings of UPJO include urothelial lining - intact and in few cases it was ulcerated, subepithelial fibrosis with increased collagen fibres, smooth muscle hypertrophy and chronic inflammatory cell infiltrate

ICC were described for the first time in the gastrointestinal tract by Ramon Y. Cajal in 1893. [13,15-18] Interstitial cells of Cajal (ICC) were first described as “neuron-like cells” at the motor neuron endings in the gastrointestinal system. Thuneberg suggested that these cells had pacemaker activity in the intestine. [19,20] Recently, research has been concerned with interstitial cells of Cajal (ICCs) at the UPJ.

ICC have gap junctions with smooth muscle cells which give way to nerve terminals; these cells are called pacemaker cells. Although the embryologic origin is unclear, it has been shown that interstitial cells originate from the mesenchymal cells.[19,21] ICC express c-kit (CD117), a tyrosine kinase, which lead to identification of ICCs in the human urinary tract. This in turn made it possible to label ICCs with antibodies to the proto-oncogene c-kit . In order to distinguish between ICCs in the urinary tract and ICCs in the gastrointestinal tract, few authors call the former as interstitial cells of Cajal-like cells (ICC-LCs).[13]

In the study UPJO cases show less ICC cells compared to controls with significant p value. This leads to decreased motility and functional obstruction. Similar results were found in studies by Mehrazma et al[22] and Pande et al[23]. Pande et al in their study concluded that ICC are significantly low at UPJ in cases of congenital UPJO when compared to controls without any obstruction. Inugala et al found out that the cases which had no ICC at the lower margin of the resected specimen and at the UPJ had bad outcome.[19] suggesting the importance of ICC in motility.

The nerve fibres in cases are less than in controls. The nerve fibres aid in motility along with ICC cells. Decreased nerve fibres leads to obstruction. Similar study results were found in studies by Kuvel et al[24] and Kaya et al[25]

Increased wall thickness, subepithelial collagen deposition, increased muscle thickness and irregular arrangement of muscle fibres are seen in UPJO cases compared to controls. Mehrazma et al and Pan et al[26] studies have similar result. Pan et al opined that circular enhanced musculature may cause a sphincter-like activity with holding up of urine.

CONCLUSION:

The pathogenesis of UPJO is multifactorial. The possibilities according to the present study are decreased ICC interpreted by decreased CD117(leads to failure of transmission of peristaltic waves across the UPJ) decreased neural innervation interpreted by decreased S100, irregularity of muscle fibre arrangement, increased submucosal collagen deposition and increased smooth muscle thickness These changes may cause disruption of the mobility of UPJ and lead to

functional obstruction.

In congenital UPJO cases, using CD117 and S100 gives the surgeon idea about adequate clearance of obstructed segment. Only by complete excision of obstructed segment, post-operative outcome will be better without recurrence. Studies have indicated that cases with recurrence have low ICC cells at the resected margins.

REFERENCES:

1. Benfield M.R., McDonald R.A., Bartosh S., Ho P.L., Harmon W. (2003) Changing trends in pediatric transplantation: 2001 Annual Report of the North American Pediatric Renal Transplant CooperativeStudy. Pediatr. Transplant. 7, 321–335.
2. Klein, J., Gonzalez, J., Miravete, M., Caubet, C., Chaaya, R., Decramer, S., Bandin, F., Bascands, J. L., Buffin-Meyer, B., & Schanstra, J. P. (2011). Congenital ureteropelvic junction obstruction: human disease and animal models. *International journal of experimental pathology*, *92*(3), 168–192. https://doi.org/10.1111/j.1365-2613.2010.00727.x
3. Chang C.P., McDill B.W., Neilson J.R. et al. (2004) Calcineurin is required in urinary tract mesenchyme for the development of the pyeloureteral peristaltic machinery. J. Clin. Invest. 113, 1051–1058.
4. Hashim Hashim, Christoper R. J. Woodhouse EAU(2012) Ureteropelvic Junction Obstruction European Urology Supplements 11 (2012) 25-32 https://doi.org/10.1016/j.eursup.2012.01.004
5. Carr MC, El-Ghoneimi A (2007) Anomalies and surgery of the ureteropelvic junction. In: Campbell-Walsh urology,Section XVII: pediatric urology, 9th edn. Saunders Elsevier,pp 3359–3382
6. Murnaghan GF (1958) The physiology of hydronephrosis. Postgrad Med J 34(389):143–148
7. Notley RG (1968) Electron microscopy of the upper ureter and the pelvi-ureteric junction. Br J Urol 40(1):37–52
8. Hanna MK, Jeffs RD, Sturgess JH et al (1976) Ureteral structure and ultrastructure. PartI: the normal human ureter. Urol 116:718–724
9. Hanna MK, Jeffs RD, Sturgess JH et al (1976) Ureteral structure and ultrastructure: part 11: congenital hydronephrosis and primary obstructive megaureter. J Urol 116:725–729
10. Maizels M, Stephens FD (1980) Valves of the ureter as a cause of primary obstruction of the ureter: anatomic, embryologic and clinical aspects. J Urol 123:742–747
11. Krakos M, Andrzejewska E (2004) Abnormalities of ureteral innervation as the base of etiopathogenesis of congenital hydronephrosis in children. Przegl Pediatr 34(2):94–97
12. Wang Y, Puri P, Hassan J et al (1995) Abnormal innervations and altered nerve growth factor messenger ribonucleic acid expression in ureteropelvic junctionobstruction. J Urol 154:679–683
13. Kolęda, Piotr & Apoznanski, Wojciech & Wozniak, Zdzislaw & Rusiecki, Lesław & Szydelko, Tomasz & Pilecki, Witold & Polok, Marcin & Kałka, Dariusz & Pupka, Artur. (2011). Changes in interstitial cell of Cajal-like cells density in congenital ureteropelvic junction obstruction. International Urology and Nephrology. 44. 7-12. 10.1007/s11255-011-9970-5.
14. Kiil F (2002) Analysis of myogenic mechanisms in renal autoregulation. Acta Physiol Scand 174(4):347–355
15. Wang Y, Puri P, Hassan J et al (1995) Abnormal innervations and altered nerve growth factor messenger ribonucleic acid expression in ureteropelvic junction obstruction. J Urol 154:679–683
16. Solari V, Piaseczna-Piotrowska A, Puri P (2003) Altered expression of interstitial cells of Cajal in congenital ureteropelvic junction obstruction. J Urol 170:2420–2422
17. Metzger R, Schuster T, Till H et al (2005) Cajal-like cells in the upper urinary tract: comparative study in various species. Pediatr Surg Int 21:169–174
18. Metzger R, Schuster T, Till H et al (2004) Cajal-like cells in the human upper urinary tract. J Urol 172(2):769–772
19. Inugala, Anusiri & Reddy, RameshKota & Rao, BhuvaneshwarNadipalli & Reddy, SreenivasP & Othuluru, Radhika & Kanniyan, Lavanya & Kumbha, Nagarjuna & Srirampur, Srinivas. (2017). Immunohistochemistry in Ureteropelvic Junction Obstruction and Its Correlation to Postoperative Outcome. Journal of Indian Association of Pediatric Surgeons. 22. 129. 10.4103/jiaps.JIAPS_254_16.
20. Thuneberg L. Interstitial cells of Cajal: intestinal pacemaker cells? Adv Anat Embryol Cell Biol 1982;71:1-130
21. Young HM. Embryological origin of interstitial cells of Cajal. Microsc Res Tech 1999;47:303-8.
22. Mehrazma, Mitra & Tanzifi, Parin & Rakhshani, Naser. (2014). Changes in Structure, Interstitial Cajal-like Cells and Apoptosis of Smooth Muscle Cells in Congenital Ureteropelvic Junction Obstruction. Iranian journal of pediatrics. 24. 105-10.
23. Pande T, Dey SK, Chand K, Kinra P. Influence of interstitial cells of cajal in congenital ureteropelvic junction obstruction. J Indian Assoc Pediatr Surg [serial 76 online] 2020 [cited 2020 Nov 2];25:231-5. Available from: https://www.jiaps.com/text.asp?2020/25/4/231/287647
24. Kuvel M, Canguven O, Murtazaoglu M, Albayrak S. Distribution of Cajal like cells and innervation in intrinsic ureteropelvic junction obstruction. Arch Ital Di Urol Androl Organo Uff [Di] Soc Ital Di Ecogr Urol e Nefrol 2011; 83: 128e32
25. Kaya C, Bogaert G, de Ridder D, Schwentner C, Fritsch H, Oswald J, et al. Extracellular matrix degradation and reduced neural density in children with intrinsic ureteropelvic junction obstruction. *Urology.* 2010;76:185–189. [PubMed] [Google Scholar]
26. Pan, Pradyumna & Sachdeva, Neeraj. (2019). Immunohistochemistry and morphometric analysis of pelviureteric junction complexes in children with hydronephrosis. Indian Journal of Pathology and Microbiology. 62. 49. 10.4103/IJPM.IJPM_535_17.