International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 6, June 2024 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_13.6.19

ORIGINAL RESEARCH

Immunohistochemical Evaluation Of Neuronal Dysfunction In Pelviureteric Junction Obstruction

[1]Dr. Sweta Asati, [2]Dr. Gaurav Gupta, [3]Dr. Swati Asati, [4]Dr. Rajnish Kalra, [5]Dr. K.N. Rattan

[1]Assistant Professor, Department of Pathology, KM Medical College, Mathura, U.P., India
[2]Assistant Professor, Department of Medicine, Autonomous State Medical College, Firozabad, U.P., India
[3]Assistant Professor, Department of Obstetrics and Gynecology, Vyas Medical College & Hospital, Jodhpur, Rajasthan, India
[4]Professor, Department of Pathology, Pt. B.D. Sharma, PGIMS, Rohtak, Haryana, India
[5]Senior Professor and Head, Department of Pediatric Surgery, Pt. B.D. Sharma, PGIMS, Rohtak, Haryana, India

Corresponding author
Dr. Rajnish Kalra
Professor, Department of Pathology, Pt. B.D. Sharma, PGIMS, Rohtak, Haryana, India

Received date: 14 April, 2024 Acceptance date: 20 May, 2024

ABSTRACT

Aim: To assess immunohistochemical evaluation of neuronal dysfunction in pelviureteric junction obstruction. **Material And Methods**: Present study was conducted in Department of Pathology in collaboration with Department of Pediatric Surgery, Pt. B D Sharma PGIMS, Rohtak (Haryana). Forty five specimens of resected portion of pelviureteric junction during pyeloplasty from cases of primary pelviureteric junction obstruction constituted the study group. Patients with pelviureteric junction obstruction other than primary obstruction such as obstruction secondary to stones, external pressure, previous surgery etc. were excluded. Eleven specimens of normal PUJ obtained at autopsy of age matched pediatric population constituted the control group. The specimens were fixed and examined grossly for any macroscopic abnormality. Specimens were processed from pelviureteric junction and upper and lower resected margins. An attempt was also made to process the whole specimen and sections included both the cut ends. Sections were processed by routine histological technique for paraffin embedding. **Results**: Significant transmural inflammation was seen in 2 cases while 2 other cases showed lymphoid follicles formation in the wall. Lamina propria showed significant fibrosis in 7 cases (15.6%) and was seen only in cases of PUJ obstruction so it was a highly significant finding ($p < 0.001$). The difference of mean positivity was highly significant (p value=0.0001) and presence of 3 or less ICC was significantly associated with PUJ obstruction (38 of 45 cases). Although not significant statistically (p value = 0.172), the mean number of Synaptophysin positive cells was less in PUJ obstruction cases (3.84±3.4/HPF) than controls (6.36±5.36/HPF). **Conclusion:** Neuronal markers showed consistent decreased expression of CD117 with a tendency for other neuronal markers (S-100 and Synaptophysin) to be expressed at level lower than normal indicating thereby neuronal dysfunction with reduced neuronal drive as primary defect in PUJ obstruction and the muscular changes being secondary as a compensatory mechanism.

Keywords: Immunohistochemical, Neuronal Dysfunction, Pelviureteric Junction Obstruction

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INTRODUCTION

Pelviureteric junction obstruction (PUJ) is most common cause of upper urinary tract obstruction in children. PUJ obstruction is characterized by impaired urine flow from the renal pelvis to proximal ureter followed by dilatation of collecting system and potential damage to renal parenchyma with renal insufficiency. Chronic obstruction results in greater injury to the immature kidney than to the adult kidney. PUJ obstruction can be primary (congenital) or secondary (acquired). The primary obstruction is broadly divided into two categories: those lesions that involve the PUJ intrinsically and those that are extrinsic. Congenital causes are more common than acquired conditions. It is more commonly reported in boys than girls. Left sided obstruction predominates in the neonates. Congenital PUJ obstruction is the most common anomaly of genitourinary tract pediatric urologist has to deal with.[1]

Obstruction at PUJ causes complex changes in pelviureteric wall like epithelial atrophy, ulceration, degeneration and metaplastic changes in urothelium, chronic inflammatory infiltrate in lamina propria, hypertrophy/hyperplasia/atrophy of myocytes with predominantly longitudinal arrangement, intra and interfascicular collagen proliferation and extensive

DOI: 10.69605/ijlbpr_13.6.19

fibrosis. Histological alteration in kidney like glomerulosclerosis, dilated tubules and dense chronic inflammatory infiltrate have also been noted in many studies.[2,3]

Transport of urine from kidney to bladder is an active process based on well coordinated one way peristalsis. It is believed that contraction creating peristaltic wave in the ureter is initiated by cells with pacemaker activity in proximal part of renal calyces. Both origin and conduction mechanism of peristalsis wave are not completely known .[4]

Increasing evidence indicates that ICC like cells displaying many of the features of intestinal ICC and immunoreactivity to antibodies against c-kit are present in PUJ.[5,6] ICC have been found in GIT, urinary bladder, prostate and PUJ of many mammals. Role of ICC has been proved in pathogenesis of Hirschsprung's disease, intestinal pseudo-obstruction syndrome and constipation.[3,7]

Neuronal dysfunction has been thought to be play a part in congenital PUJ obstruction but the mechanism and primary defect have not been precisely defined.[1, 3] Expression of various neuronal markers have been studied in cases of primary PUJ obstruction with variable and contradictory results adding to the grey zone.

The present study was planned to assess the morphological changes in PUJ obstruction and immunohistochemical evaluation of neuronal markers using c-kit (CD117) S-100 and Synaptophysin to assess neuronal dysfunction in cases of PUJ obstruction.

MATERIAL AND METHODS

Present study was conducted in Department of Pathology in collaboration with Department of Pediatric Surgery, Pt. B D Sharma PGIMS, Rohtak (Haryana). Forty five specimens of resected portion of pelviureteric junction during pyeloplasty from cases of primary pelviureteric junction obstruction constituted the study group. Patients with pelviureteric junction obstruction other than primary obstruction such as obstruction secondary to stones, external pressure, previous surgery etc. were excluded. Eleven specimens of normal PUJ obtained at autopsy of age matched pediatric population constituted the control group. All the control cases had no history of any previous urinary tract disease or any other disease of longer duration.

The specimens were fixed and examined grossly for any macroscopic abnormality. Specimens were processed from pelviureteric junction and upper and lower resected margins. An attempt was also made to process the whole specimen and sections included both the cut ends. Sections were processed by routine histological technique for paraffin embedding.

On H&E stained sections, histopathological changes were assessed in detail and changes in urothelium, lamina propria, muscle coat and adventitia were assessed for any deviation from normal with special emphasis on inflammation, muscular hypertrophy, interstitial fibrosis and nerve fiber changes. Inflammatory cell infiltrate in lamina propria was graded as Grade I (normal, (≤15 inflammatory cells/HPF), II (mild, >15 to <30 inflammatory cells/HPF), III (moderate, ≥30 to <60 inflammatory cells/HPF), IV (severe, ≥60 inflammatory cells/HPF) as described by Chiou et al[7]. Muscular hypertrophy was graded as mild, moderate and severe depending upon the thickness of muscular layer and loss of differentiation into different layers (longitudinal and circular). Representative sections were subjected to immunohistochemical (IHC) staining for c-kit, S-100 protein and Synaptophysin.

PROCEDURE FOR IHC STAINING[8]

Cross sections of 3-4 µm thick were taken on slides which were coated with tissue adhesive (Poly-L–Lysine).The slides were incubate at 65 ºC in incubator, then sections were deparaffinization in xylene, rehydration through graded alcohols andwashed in running tap water.The slides were put in tris EDTA (ethylene diamine tetra acetate) buffer (PH 9) in pressure cooker as an antigen retrieval, cool at room temperature and then rinse the sections in PBS (Phosphate buffered saline).The slides were kept in 4% hydrogen peroxide for 15 minutesfor block the endogenous peroxidase.Then slides werewashed with running tap water and PBS for 5 minutes.Optimally diluted primary antibody was applied on slides for 40 minutes and then washed in PBS for 5 minutes.Then slides were incubated with polymer (biotin-avidin combination) for 30 minutes at room temperature and washed in PBS. After that slides were incubated in DAB (3,3diamino benzidine tri hydrochloride) solution for 10 minutes.Slides were dipped in PBS and transferred in running water.After that counter staining was performed with Harris hematoxylin.Then dehydration in graded alcohols, clearing in xyleneand mounting in DPX (dibutyl phthalate xylene) were done.

CD117 was used to assess the number and distribution of ICC. The staining is membranous and cytoplasmic.[9] The mean number of ICCs/HPF were calculated from 10 random high power fields. S-100 protein was used to delineate schwann cells to assess nerve fibres. The staining is cytoplasmic and nuclear.[9] The mean number of S-100 positive cells/fibers perHPF were calculated from 10 random high power fields. Synaptophysin, another neural marker used to assess presynaptic vesicles was interpreted as positive by granular cytoplasmic staining.[10] The mean number of Synaptophysin positive cells/ HPF were calculated from 10 random high power fields. The result were presented as positive, mean and percentage.

STATISTICAL ANALYSIS

The histological changes and expression of neuronal markers; c-kit, S100 and Synaptophysin observed in PUJ obstruction specimens were compared with the

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DOI: 10.69605/ijlbpr_13.6.19

controls and statistically analysed using Statistical package for social science (SPSS) version 20.0, chi-square test was used. p value <0.05 was accepted as significant.

RESULTS

The age of patient ranged from 7 days to 13 years with an average age at presentation being 47.4 months (3.95 years).The age of control group varied between 4 to 11 years with an average age of 8.4 years. There were forty males and five females in PUJ obstruction cases with male: female ratio being 8:1 showing male preponderance which is highly significant (p value <0.0001).Left side involvement by PUJ obstruction was more (n=24; 53.3%) as compared to right side (n=17; 37.8%). The disease was bilateral in 4 (8.9%) cases. Twenty cases (44.4%) were detected antenatally on routine ultrasound examination of mother which showed fetal hydronephrosis. Amongst the cases which were detected postnatally or later in life pain abdomen was most common symptom (n=23; 51.1%) other less common symptoms included fever (4 cases), abdominal mass (2 cases), oliguria (2 cases), hematuria (1 case), dysuria (1case) and vomiting (1 case). Ultrasonography was done in all cases and all of them showed varying degree of hydronephrosis from grade II to grade IV. The clinical diagnosis was based mainly on features detected on ultrasonography.

On gross examination, the wall of PUJ obstruction specimen in all cases appeared rigid and round as compared to control specimens which were soft. The lumen of stenotic portion was round to oval.

On routine histopathological examinations of sections from PUJ area revealed varying degrees of changes in all the layers of wall of pelviureteric junction.Urothelium was normal in 8 (17.8%) cases while in rest (n= 37; 82.2%) cases there were varying combination of ulceration (42.2%), degeneration (33.3%), atrophy (6%) hyperplasia (17.8%), squamous metaplasia (2.22%) columnar cell change (2.22%). Dysplasia was seen in 2 (4.4%) cases. All the cases in control category showed normal urothelium. Urothelium showed lymphocytic and neutrophilic exocytosis in varying combination in 13 (28.9%) cases while controls showed only lymphocytic exocytosis in 2 (18.2%) cases. Neutrophilic exocytosis thus was seen only in cases of PUJ obstruction.

TABLE-1: GRADING OF INFLAMMATION IN LAMINA PROPRIA

Grade	Cases		Controls	
	N	Percentage (%)	N	Percentage (%)
Grade-I (no or rare: ≤15cells/HPF)	15	33.3%	11	100%
Grade-II (mild: >15 to <30 cells/HPF)	16	35.6%	-	
Grade-III (moderate: ≥30 to <60 cells/HPF)	10	22.2%	-	
Grade-IV(severe: ≥60cells/HPF)	04	8.9%	-	
Chi-square =15.8	p value = 0.001			

Lamina propria showed varying degrees of inflammation in two third of cases. The number of inflammatory cells in lamina propria in PUJ obstruction cases were less than 15 in one third of cases which was taken as not significant, while the inflammation was significant in rest 30 cases with number of cells varying from 15 to more than 60 cells/HPF which was highly significant when compared to controls (p=0.001). Significant transmural inflammation was seen in 2 cases while 2 other cases showed lymphoid follicles formation in the wall (table 1).

All the eleven cases taken as control showed lymphocytes and mast cells in the lamina propria but the number was less than 15 cells/HPF which was taken as normal/ insignificant. The cases of PUJ obstruction in addition to lymphocytes and mast cells showed presence of neutrophils, plasma cells, eosinophils and marcophages in varying combinations. The presence of cells other than lymphocytes and mast cells was significant in the cases of PUJ obstruction. Lamina propria was significantly congested in 24 cases with areas of hemorrhage in 1 case with significant edema in 5 cases. The congestion which appeared due to surgical procedure (without associated inflammation) was not included in the congestion category. Three cases amongst control also showed mild degree of congestion which was probably passive congestion in postmortem state. Lamina propria showed significant fibrosis in 7 cases (15.6%) and was seen only in cases of PUJ obstruction so it was a highly significant finding (p < 0.001).

TABLE-2: GRADING OF MUSCULAR HYPERTROPHY AND INTERSTITIAL FIBROSIS

Grade	Cases: Muscular hypertrophy		Cases: Interstitial fibrosis	
	N	Percentage (%)	N	Percentage (%)
Nil /absent	02	4.4%	10	22.2%
Mild	20	44.5%	27	60%
Moderate	17	37.8%	7	15.6%
Severe	06	13.3%	1	2.2%

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Chi-square = 45.3, p value = 0.0001	Chi-square =18.9, p value = 0.0001

Muscular hypertrophy of varying degree was seen in 43 out of 45 cases of PUJ obstruction. None of the control showed muscular hypertrophy. Thus the finding of muscular hypertrophy in PUJ obstruction was a highly significant finding (p value=0.0001). Varying degree of interstitial fibrosis (in the muscular layer) was seen in 35 cases while 10 cases of PUJ obstruction and all controls showed no significant interstitial fibrosis. The presence of interstitial fibrosis was a highly significant finding in PUJ obstruction (p=0.0001) (table 2)

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Expression of CD117 was evaluated in muscle layer. The care was taken not to include mast cells which appear rounded with more abundant cytoplasm and also show positivity on CD117 IHC staining. Only the cells with elongated or ovoid positivity were considered as positive interstitial cell of Cajal (ICC).Number of ICC in PUJ obstruction cases ranged from 0-5 /HPF with an average of 2.3±1.2/HPF. Ten cases showed very sparse positivity from 0-1/HPF and 2-3/HPF in 28 cases. In the controls majority of cases (10 out of 11) showed 4 or more ICC/HPF with an average of 6.45±1.75/HPF. The difference of mean positivity was highly significant (p value=0.0001) and presence of 3 or less ICC was significantly associated with PUJ obstruction (38 of 45 cases).

TABLE- 3: CD117 POSITIVE ICCs IN MUSCLE LAYER

Range/HPF	Cases		Controls	
	N	Percentage (%)	N	Percentage (%)
0-1	10	22.2%	-	0%
2-3	28	62.2%	01	9.1%
4-5	07	15.6%	02	18.2%
6-7	-		05	45.4%
8-9	-		03	27.3%

Mean number of S-100 positive points (which included nerve twigs and nerve fibers) were counted in various layers of wall of pelviureteric junction and tabulated for muscle layer. The number of positive points varied from 2-25/ HPF in both controls and cases. However, mean S-100 positivity was less (8.9±4.87/HPF) in PUJ obstruction cases as compared to controls (12.54±6.3/HPF).However difference was not statistically significant. On an average nerve fibers were morein adventitia as compared to muscular layer as seen normally/ in controls. Five out of forty five cases (11.11%) showed nerve bundle hypertrophy mainly in adventitia which was not seen in controls. So nerve fibers hypertrophy could be secondary to or related with PUJ obstruction(table:4).

TABLE -4: S-100 POSITIVE NERVE FIBERS IN MUSCLE LAYER (n= 45)

Range/HPF	Cases		Controls	
	N	Percentage (%)	N	Percentage (%)
1-5	11	24.5%	01	9.0
6-10	24	53.3%	04	36.4
11-15	06	13.3%	03	27.3
16-20	03	6.7%	02	18.2
21-25	01	2.2%	01	9.1

TABLE -5: SYNAPTOPHYSIN POSITIVE CELLS IN MUSCLE LAYER (n=45)

Range/HPF	Cases		Controls	
	N	Percentage (%)	N	Percentage (%)
0-3	25	55.55%	03	27.3%
4-6	15	33.33%	04	36.3%
7-10	04	8.9%%	03	27.3%
>10	01	2.22%	01	9.1%

Synaptophysin immunoreactive cells were evaluated in the muscle layer. Number of Synaptophysin positive cells ranged from 0-20/HPF in both cases and controls. Although not significant statistically (p value = 0.172), the mean number of Synaptophysin positive cells was less in PUJ obstruction cases (3.84±3.4/HPF) than controls (6.36±5.36/HPF), (table 5).

Seven cases in addition showed diffuse background staining. The significance of which could not be determined but it was definitely not procedural error as other sections stained in the same batch under similar conditions did not show such type of stain.

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DISCUSSION

In the present study, age of the patients at the time of surgery ranged from 7 day to 13 years with mean and median age at presentation being 47.4 months (3.95 years) and 24 months. In our study one third cases being diagnosed and operated upon before 3 months of age and 42.2% by the age of 12 months. This probably was due to better mother-child health awareness and antenatal check-ups in our set of population. Similar age distribution were observed by Kim et al[11], Eken et al[12] and Gunduze et al[13] in which mean age of PUJ obstruction was 3.6 years, 3 years and 2.8 years respectively. However, different age distribution were observed in studies conducted by Kajbafzadeh et al,[14] Mehrazma et al,[15] Soleri et al[16], Koleda et al,[17] and Kaya et al[18] in which mean age of PUJ obstruction was1.4 years, 1.7 years, 2.3 years and 8.1 years respectively. It could be due to difference in the sample size, range of age, suspicion on part of clinician and availability of modern imaging techniques.

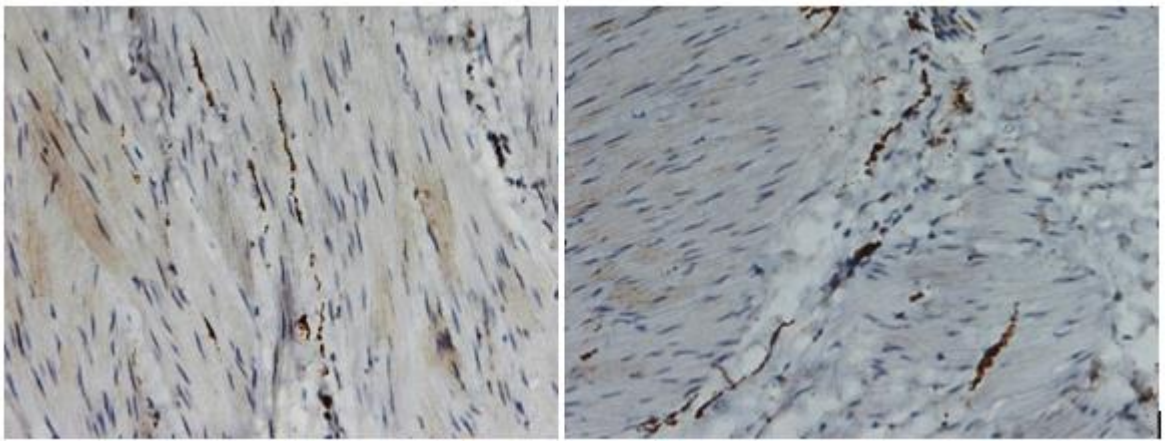

Fig 1: Immunostaining for S-100 showing intramuscle nerve fibers, parallar to muscle fibers (IHC, 200X)
Fig.2: S-100 positive nerve fibers in interfascicular space (IHC, 200X)

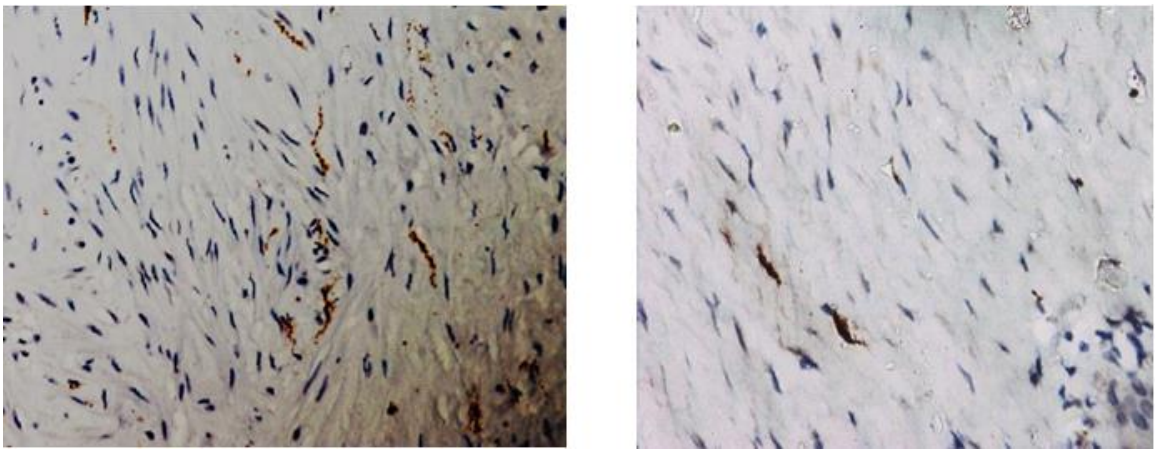

Fig.3 : PUJ obstruction: Immunostaining for Synaptophysin (Granular cytoplasmic) in nerve terminals (IHC, 200X) , Fig.4 : C-kit positive Cajal cells parallal to muscle fibers with spindle like cytoplasmic extension, (IHC, 400X)

PUJ obstruction affects males more commonly than female child. The male:female ratio in current study was 8:1 (40 male and 5 female). Other studies by Senguttuvan et al[19], Chiou et al[7], Ozel et al,[20] Kaya et al[18], Demirbilek et al[21] and Gunduze et al[13] have also reported male preponderance (from 2:1 to 5.5:1) but most study groups have small number of cases except for one by Senguttuvan et al[19]with 45 cases as ours. So probably in other studies the male:female ratio although showing male preponderance, was falsely low due to lesser number of cases.

The present study showed PUJ obstruction involved left side more (n=24; 52.3%) commonly than right side (n=17; 37.8%). The involvement was bilateral in 8.9% (n=4) cases. This was similar to the distribution of cases seen in study conducted by Senguttuvan et al.[19]In present study 40% cases were detected antenatally by routine sonography which was also in concordance with study conducted by Senguttuvan et al[19] Amongst cases which were detected postnatally, pain abdomen was the most common presenting symptom in our study. Similarly Kim et al also observed pain abdomen was the most common

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DOI: 10.69605/ijlbpr_13.6.19

presentation.[11] In most studies the common symptoms included pain abdomen, fever, abdominal mass and hematuria.

Wall of PUJ obstruction specimen in all cases appeared rigid and round as compared to control specimens which were soft. The lumen of stenotic portion was round to oval. Findings were similar to reported by Murakumo et al.[22]

In our study urothelium was normal in 17.8% cases while in rest cases varying combination of degeneration (33.3%), ulceration (42.2%), hyperplasia (17.8%), squamous metaplasia (2.22%) columnar cell change (2.22%) and dysplasia (4.44%) were seen. Chiou et al demonstrated urothelial changes as hyperplasia in 60% cases with few degenerative changes and mononuclear cell infiltration.[7] In a study by Ozel et al, epithelial proliferation was seen.[20]Other epithelial changes observed in our study included lymphocytic and neutrophilic exocytosis in 13 (28.9%) cases. No such detailed study was available for comparison.

Lamina propria showed significant inflammation in 30 (66.7%) cases in present study when 15 lymphocytes/HPF was taken as cut off level. Lymphocytes were present in all cases (100%) with other inflammatory cells like neutrophils, eosinophils, mast cells, plasma cells and macrophages being present in varying combinations. In present study lymphocytes and mast cells were found in controls but the number of cells was <15/HPF (mean=7.2) which was considered insignificant. Other author have also reported presence of inflammation in lamina propria in case of PUJ obstruction,[7, 23, 22, 20, 24] while Chiou et al reported lymphocytes in 100% cases and eosinophils in 60% cases as the type of inflammatory cells[7]; while Hanna et al found mast cells and polymorphs in mucosa and submucosa.[23]

In our study, varying degree of muscular hypertrophy (mild in 44.5% cases, moderate in 37.8% cases and severe in 13.3% cases) was observed in most PUJ obstruction cases (43 of 45); whereas no muscular hypertrophy was seen in control group and it was statistically highly significant. Interstitial fibrosis of varying degree was observed in 35 of 45 (77.8%) cases which again was highly significant.Presence of muscular hypertrophy with interstitial fibrosis was a significant consistent morphological alteration observed in PUJ obstruction. Wang et al,[25] Harish et al,[24] Demirabilek et al[21] and Gunduze et al[13] have also reported similar result i.e. muscle hypertrophy with increased interstitial fibrosis. While Murakumo et al,[22] Kajbafzadeh et al,[14] Ozel et al[20], Mehrajma et al[15] and Kaya et al[18] reported reduced thickness of muscle layer or atrophy or resolution of smooth muscle coat but all of them reported significant increase in interfascicular collagen fibrosis.

c-kit positive ICCs were seen as spindle shaped cells parallel to muscle fibers. The number of ICC in obstruction cases ranged from 0-5/HPF with an average of 2.3/HPF while the corresponding figures for controls were 2-8/HPF and 6.45/HPF respectively which was statistically highly significant. Results were in concordance with those reported by Soleri et al,[16] Yang et al,[26] Eken et al,[12] Mehrajma et al,[15] and Kuvel et al.[27]In a study of Ozel et al, c-kit did not stain ICC. However, mast cells stained prominently at smooth muscle layer in PUJ obstruction.[20] This difference may be reflecting some technical difference or subjectivity in interpretation and other still undefined factors. However, Koleda et al reported an increase in c-kit positive ICC in PUJ obstruction cases and explained it on the basis of animal studies and duration of obstruction.[17] The difference may be due to case selection and other still unknown compounding factors. However, from the present study and majority of other studies evaluating c-kit expression in PUJ obstruction, it can be inferred that reduced number of ICC as determined by c-kit positivity is a consistent finding. Although grey zone with overlap between normal PUJ and PUJ obstruction exist.

In our study, the number of S-100 positive points (which included nerve twigs and nerve fibers) in muscle layer varied from 2-25/HPF in both cases and controls. However, mean S-100 positivity was less (8.9/HPF) in PUJ obstruction cases as compared to controls (12.54/HPF). On an average nerve fibers were more in adventitia as compared to muscular layer as seen normally in controls. Similar trends were reported by Mehrazma et al,[15] Harish et al,[24] Kaya et al[18] and Kuvel et al[27] while Wang et al[25] and Demirablek et al[21] found no difference of S-100 positive points in controls and PUJ obstruction cases. However, they also reported on an average nerve fibers were more common in adventitial layer.In present study five out of forty five cases (11.11%) showed nerve bundle hypertrophy mainly in adventitia which was not seen in controls which could be secondary to or related with PUJ obstruction. No study was available for comparison. However, Gunduz et al showed significantly more intense staining for S-100 in PUJ obstruction specimens compared with normal specimens.[13] The finding suggest depletion of nerve fibers in intrinsic obstruction but whether it is the cause of PUJ obstruction or is secondary to loss during fibrosis in obstruction could not be determined unequivocally.

Synaptophysin immunoreactive cells were evaluated in the muscle layer. Number of Synaptophysin positive cells ranged from 0-20/HPF in both cases and controls. The mean number of Synaptophysin positive cells were found less (3.84/HPF) in PUJ obstruction cases as compared to controls (6.36/HPF). Wang et al and Kuvel et al observed reduced expression of Synaptophysin in PUJ obstruction specimens compared with normal PUJ specimens.[25, 27] On the contrary Dimirbilek et al observed a marked nuclear staining of cells with Synaptophysin in all layers of PUJ obstruction specimens while control did not stain for Synaptophysin.[21] In a study by Gunduz et al

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DOI: 10.69605/ijlbpr_13.6.19

cytoplasmic staining of cells by Synaptophysin were found in PUJO specimens which was totally absent in normal PUJ specimens.[13] This could be due to use of different antibody to Synaptophysin (polyclonal versus monoclonal) and other technical procedural differences. Seven cases in present study also showed diffuse low intensity staining. The significance of which could not be determined but it was definitely not procedural error as other sections stained in the same batch under similar conditions did not show such type of staining.

This study has been directed precisely to the aspect of primary PUJ obstruction in the pediatric age group. Present study and some other recent studies have tried to look into some aspect of morphological changes and neuronal dysfunction in primary PUJ obstruction cases. Although, the results are contradictory in some of the studies but the consensus emerges that defective neuronal function or innervation play an important role in pathogenesis of PUJ obstruction which secondarily lead to morphological alteration in urinary conduit.

CONCLUSION

The present study demonstrated muscular hypertrophy with or without fibrosis in most cases of PUJ obstruction and variety of urothelial changes which seem possibly due to shear stress on the epithelium with secondary changes in the lamina propria. Neuronal markers showed consistent decreased expression of CD117 with a tendency for other neuronal markers (S-100 and Synaptophysin) to be expressed at level lower than normal indicating thereby neuronal dysfunction with reduced neuronal drive as primary defect in PUJ obstruction and the muscular changes being secondary as a compensatory mechanism. What leads to these neuronal changes needs to be investigated further to arrive at the final cause of disease.

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DOI: 10.69605/ijlbpr_13.6.19

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