



Changes in Collagen Type 3, Elastin, Fibrosis and Cajal Cell in Congenital Ureteropelvic Junction Obstruction

Üreteropelvik Bileşke Obstrüksüyonunda; Kollajen Tip 3, Elastin, Fibrozis ve Cajal Hücrelerindeki Değişiklikler

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What's known on the subject? and What does the study add?

Histopathology of ureteropelvic junction obstruction is not fully understood whether these histopathological changes are present from birth and result in UPJO or whether the histopathological changes in this region develop later, associated with a mechanical obstruction. In this study, it was attempted to explain this situation by histopathological comparison of UPJ segments excised from paediatric and adult patients.

ABSTRACT

Objective

To compare changes in collagen type 3, elastin, fibrosis and cajal cells through histopathological examination of the ureteropelvic junction (UPJ) segments of pediatric and adult patients who had undergone Anderson-Hynes pyeloplasty for ureteropelvic junction obstruction (UPJO).

Materials and Methods

Histopathological evaluation was made of the UPJ segments of 52 patients who underwent Anderson-Hynes pyeloplasty for UPJO between January 2005 and January 2008. Patients were separated into 2 groups as pediatric, aged ≤ 15 years (group 1, n=10) and adult, aged >15 years (group 2, n=42). UPJ segments of both groups were compared histopathologically (collagen type 3, elastin, fibrosis and cajal cells). Radiographic evaluations were made with diuretic renogram and/or intravenous pyelography.

Results

Mean age of group 1 patients was 8.12 ± 2.6 years (1-12 years) and of group 2 patients, 38.16 ± 5.91 years (16-62 years). Mean follow-up period was 18 months (9-24 months). No statistically significant difference was determined between the groups in respect of collagen type 3, elastin, fibrosis or cajal cells ($p > 0.05$).

Conclusion

As the pathology in the UPJ obstruction is a congenital defect, the histopathology does not change with age.

Key Words

Ureteropelvic junction obstruction, histopathology, pediatric, adult

ÖZET

Amaç

Üreteropelvik bileşke darlığı (ÜPBĐ) nedeniyle Anderson-Hynes pyeloplasti yapılan pediatrik ve erişkin hastaların üreteropelvik bileşke (ÜPB) segmentlerini histopatolojik olarak incelemek ve bu hastalarda; kollajen tip 3, elastin, fibrozis ve cajal hücrelerindeki değişimleri karşılaştırmak.

Gereç ve Yöntem

Üreteropelvik bileşke darlığı nedeniyle Ocak 2005-Ocak 2008 tarihleri arasında Anderson-Hynes pyeloplasti yapılan 52 hastanın ÜPB segmentleri histopatolojik olarak değerlendirildi. On beş yaş ve altı olan hastalar pediatrik grup (grup 1; n=10), 15 yaş üstü hastalar erişkin grup (grup 2; n=42) olmak üzere iki gruba ayrıldı. Her iki grubun ÜPB segmentleri histopatolojik (kollajen tip 3, elastin, fibrozis ve cajal hücreleri) olarak karşılaştırıldı. Radyografik değerlendirmeler diüretik renogram ve/veya intravenöz pyelografi ile yapıldı.

Bulgular

Hastaların ortalama yaşı; grup 1 için, $8,12 \pm 2,6$ (1-12 yıl), grup 2 için, $38,16 \pm 5,91$ (16-62 yıl) idi. Ortalama takip süresi 18 ay (9-24 ay) idi. Gruplar karşılaştırıldığında; kollajen tip 3, elastin, fibrozis ve cajal hücrelerinde istatistiksel fark yoktu ($p > 0,05$).

Sonuç

ÜPBĐ'de, üreteropelvik bileşkedeki patoloji doğumsal bir defekt olup, yaşla birlikte histopatoloji değişmez.

Anahtar Kelimeler

Üreteropelvik bileşke darlığı, cajal hücreleri, elastin, kollajen tip 3, fibrozis, pediatrik, erişkin

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Introduction

In children, ureteropelvic junction obstruction (UPJO) is the most frequently seen cause of urinary tract obstruction, seen at the rate of 1/1000- 1/2000 births (1). The cause of the pathology in UPJO remains unclear despite embryological (2), anatomical (3), functional (4) and histological (5) research. In the histopathological evaluation of UPJ, a reduction in smooth muscle cells, an accumulation of abnormal collagen and a reduction in cajal cells and neuronal elements have been determined (6,7). It is not fully understood whether these histopathological changes are present from birth and result in UPJO or whether the histopathological changes in this region develop later, associated with a mechanical obstruction. In this study, it was attempted to explain this situation by histopathological comparison of UPJ segments excised from paediatric and adult patients.

Materials and Methods

The study comprised 52 patients who underwent pyeloplasty for UPJO in our clinic between 2005 and 2008. Patients were excluded if the obstruction was associated with extrinsic causes such as aberrant vascular pressure or peri-ureteral fibrosis. The patients were separated into 2 groups as paediatric, aged ≤ 15 years (group 1, n=10) and adult, aged >15 years (group 2, n=42). The UPJ segments of both groups were compared histopathologically (collagen type 3, elastin, fibrosis and cajal cells). Preoperative evaluations were applied to all cases with diuretic renogram and/or intravenous pyelography. Anderson-Hynes pyeloplasty was applied to all patients.

Pathological Evaluation

All excised UPJ segments were examined at Pathology Lab. Sections 4 μm thick were obtained from formalin-fixed paraffin blocks, stained with hematoxylin & eosin, and examined under a light microscope. Reticulin and Masson's trichrome staining was performed to determine collagen type 3 status in the submucosa and the presence of fibrosis.

Immunohistochemical Method

Cross sections 4 μm thick obtained from the paraffin blocks of selected patients were transferred to positively charged slides for immunohistochemical examination using a CD117/c-kit (catalogue no. CME 296 AK Biocare Medical USA) and Elastin (catalog no. GTX29519 Genetex, Inc.USA antibodies). Sections were deparaffinized. Dehydration was performed in 96-degree ethyl alcohol and antigen recovery was carried out in a microwave oven in pH 6.0 citrate buffer solution. The sections were cooled for 20 min at room temperature and kept in phosphate buffered saline (PBS) solution for 10 min. The tissues were circled using a hydrophobic pen, and then maintained in protein block solution (Ultra V Block) for 5 min. The sections were then washed with PBS and incubated for 40 min using a CD117/c-kit and elastin. Next, they were washed with PBS, maintained at room temperature for 20 min with coenzyme Value Primer Antibody Enhancer, and then washed with PBS solution. Afterwards, they were treated with Value HRP Polymer for 30 min in a dark environment. They were then washed with PBS, maintained in AEC (3-amino-9-ethylcarbazol) single solution, and then washed with distilled water. Contrast staining was performed for 2 min using Mayer's hematoxylin. After drying at room temperature they were covered with aqueous

mounting material (Ultramount, Labvision), and then evaluated under a light microscope.

Evaluation of CD117, Elastin, Masson's Trichrome, and Reticulin Staining

Cajal cells between the muscle layers stained with CD117 were enumerated in the 10 Times Enlarging Area (TEA) and evaluated as follows: n=0-1 (-); n=2-5 (+); n=6-10 (++); n \geq 11 (+++). Macrophages in the mucosa and submucosa were evaluated as positive controls. Evaluation of elastin staining was positive (+) or negative (-). The blood vessel wall was evaluated as a positive control. Masson's trichrome staining evaluation was performed according to fibrosis in the submucosa. Elastin couldn't be separated, as the collagen, elastin, and other connective tissues were stained blue; staining was considered positive (+) if there was blue coloring under the epithelium and negative (-) if there wasn't. Reticulin was evaluated according to the thickness of the fibers in the submucosa, as positive (+) or negative (-); reticulin fibers observed via low magnification were considered positive (+) and those that were not were considered negative (-).

Statistical Analysis

Statistical analysis of the data was performed using SPSS v.15.0 for Windows (SPSS Inc., Chicago, IL, USA). Frequencies and means for all data in both groups were calculated. Between-group comparisons were made using the Chi-square test with Yates correction. The level of statistical significance was set at $p < 0.05$.

Results

The mean age of group 1 patients was 8.12 ± 2.6 years (1-12 years) and of group 2 patients, 38.16 ± 5.91 years (16-62 years). No statistically significant difference was determined between the groups in respect of collagen type 3, elastin, fibrosis or cajal cells ($p > 0.05$) (Table 1, Figure 1). The mean follow-up period was 18 months (9-24 months).

Discussion

The cause of congenital ureteropelvic junction obstruction is not fully known. Several studies have suggested that congenital obstruction in the uteropelvic junction originates from changes in the structure of collagen and smooth muscle cells (7).

Table 1. The results of statistical analysis

		Pediatric Group (Group 1) n (%)	Adult Group (Group 2) n (%)	p Value
Cajal Cell	-	1 (10.0)	5 (11.9)	1.0
	+	5 (50.0)	23 (54.8)	1.0
	++	3 (30.0)	8 (19.0)	0.740
	+++	1 (10.0)	6 (14.3)	1.0
Elastin		1 (10.0)	7 (16.7)	0.970
Fibrosis		5 (50.0)	10 (23.8)	0.210
Collagen Type 3		2 (20.0)	6 (14.3)	1.0
P= Chi-square test				

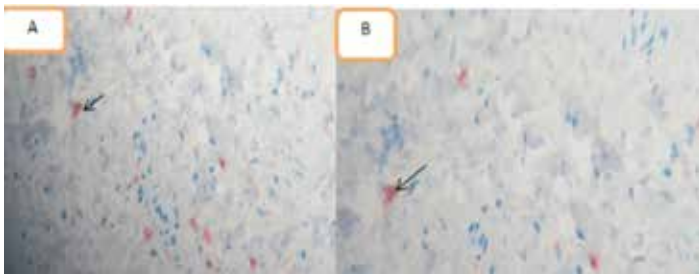


Figure 1. A) Immunoperoxidase x400 Cd117 positive cajal cells. In pediatric group of a male patient age 1, B) In Adult group of a male patient age 38

In a study by Star et al. comparing normal UPJ segments and UPJO segments, it was reported that in UPJO the lamina muscularis was thicker, there was a greater amount of interfascicular and intrafascicular collagen and more deterioration in elastin fibres (8). Yoon et al. compared normal UPJ segments with UPJO segments from both paediatric and adult patients and the amount of collagen was found to be high in both groups and at a significantly high rate in the adult group (9). Masashi et al. also compared normal UPJ and UPJO segments and showed an increased amount of collagen. It was suggested that due to the impaired innervation in the area, smooth muscle cells had become atrophied and been replaced by collagen, which caused both a functional and anatomical obstruction (7).

In the current study, there was no difference between the paediatric group and the adult group in terms of the amount of collagen type 3. This can be considered to be due to collagen accumulation not forming secondary to the obstruction as the pathology in congenital uteropelvic junction obstruction is from birth.

In a study by Özel et al. (10), in which normal UPJ segments were compared with UPJO segments, fibrosis was found to be greater in UPJO patients. In the current study, both groups had congenital UPJO and there was no control group of normal UPJ segments. It is our opinion that greater fibrosis can be seen in patients with UPJO but that this change has been present from birth and has not developed at a later date.

That cajal cells in the UPJ provide peristalsis through electrical waves in the smooth muscle cells was first defined by Solari et al. (6,11,12). There are many studies in literature which have researched cajal cells in the histopathological examination of congenital UPJO segments. In a study by Solari et al. comparing normal UPJ segments with UPJO segments, the number of cajal cells was found to be significantly low in the UPJO segments and therefore in that patient group peristalsis was inadequate in that region as there was an insufficient number of cajal cells, which then resulted in the development of obstruction (6).

In contrast, Koleda et al. determined a higher number of cajal cells in UPJO segments than in normal UPJ segments. This was explained by an increase in the number of cajal cells in the obstruction area to compensate for the obstruction. In the same study, the number of cajal cells were seen to decrease with increasing age in a paediatric group (13). Kuzgunbay et al. (14) suggested that there was an increase in the number of cajal cells in the early stages of an obstruction, but in the late stage, the cajal cell number decreased. In contrast to the studies of Koleda and Kuzgunbay, the results of the current study showed no decrease with age in the number of cajal cells. No statistically significant difference was determined between the two groups of the current study in respect of the number of cajal cells.

In conclusion, the results of this study have shown that the histopathological impairment in the uteropelvic junction formed from congenital UPJO, has not developed secondary to the obstruction and there is no change in the histopathological structure over time. In the current study, the number of cases was low and there was no control group for evaluation of normal UPJ segments. Therefore, there is a need for further studies on this subject with a greater number of cases and including a control group.

Conflicts of Interest

There are no conflicts of interest.

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