

ORIGINAL ARTICLE

Development of the Fetal Ureter: A Fetal Metric and Histological Study

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ABSTRACT

Introduction: This study provides a description of development of various components of the human ureter at different gestational ages. **Methods:** Measurements of the length of the ureter from pelvi-ureteric junction to vesico-ureteric junction, length of its intravesicular portion and angle made by the ureter at the entry into the bladder with respect to the internal ureteric orifice were taken. Transverse sections of the ureter were taken at the upper end, close to the pelvi-ureteric junction, and at the midpoint between the pelvis of the kidney and the vesico-ureteric junction. These were subjected for processing and stained with Haematoxylin Eosin and Masson's Trichrome. They were then studied under the light microscope. **Results:** The length of the ureter from the pelvi-ureteric junction to the vesico-ureteric junction showed a highly positive correlation with the gestational age (GA). The angle of entry of the ureter to the bladder decreased as the GA increased. The microstructure of the ureter in both the sections showed development of transitional epithelium with an increase in layers by 33 weeks. At 18 weeks, the smooth muscle layer was in a single layer with an abundance of interspersed collagen fibers. By 33 weeks, the thickness of the wall increased significantly with a decrease in collagen. There was no trace of longitudinal muscle fibers even upto 33 weeks. **Conclusion:** Epithelial, muscular and connective tissue components displayed significant changes during intra uterine development.

Keywords: Fetal ureter, Masson's trichrome, Smooth muscle, Transitional epithelium.

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INTRODUCTION

Histologically, the ureter has three layers: (a) the mucosal epithelium which is the transitional, (b) the smooth muscle, and (c) the adventitia (1). The development of these three layers begins at around the fourth week of gestation (2). The mesonephric duct just cranial to the cloaca gives rise to a diverticulum or ureteric bud that will develop into the definitive ureter. The bud increases in size and branches into undifferentiated mesenchyme that will form the permanent kidney. The ureteric bud will also give rise to the future major and minor calyces and the collecting tubules of the kidney (2).

Congenital anomalies related to the urinary tract are the most common birth defects in individuals, accounting for almost 1% of the total developmental malformations

(3). As the growth and position of the ureteric bud has an inductive influence on the development of kidney morphogenesis, the accurate and correct placing of the ureteric bud is significant(4).

The muscle layer of the wall of the ureter consists of inner longitudinal and outer circular with an additional layer of outer longitudinal in the lower end.(5) There is an intermixing of fibers between the layers as the ureter enters through the wall of the bladder (5).

Antenatal management of pregnancy with routine fetal ultrasound has made detection of congenital anomalies easy and calculation of gestational age (GA) accurate. Often, however, the discoveries deliberated as malformations are mostly normal variations without having actual clinical significance(6). Hence, it is imperative that the radiologist as well as the obstetrician understand the various nuances of development and have reference data to avoid unnecessary false diagnoses.

Objective: This study provides a description of development of various components of the human ureter and provides reference data for correlating it to the GA of the fetus.

MATERIALS AND METHOD

Twenty five fresh and unfixed fetuses which were spontaneously aborted or stillborn ranging from 18 to 33 weeks were procured from Department of Obstetrics. The fetuses had no visible external deformities or history

of chromosomal or genetic disorders, and showed no signs of maceration. Appropriate clearance from the ethical committee of the institution was obtained (ECR/146/Inst/KA/2013 IEC115/2017). Their GA was known prior to dissection from the obstetric history.

The abdomen was dissected and the kidneys and ureters were identified. The whole of the ureters along with the kidneys and the urinary bladder were procured from the fetuses. The lengths of the ureters were measured using the vernier calipers (Fig. 1.1). Two transverse sections of the ureter were taken: one at the upper end, close to

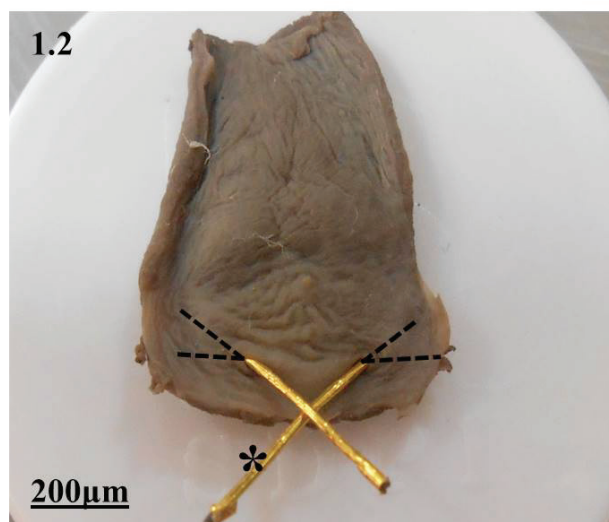
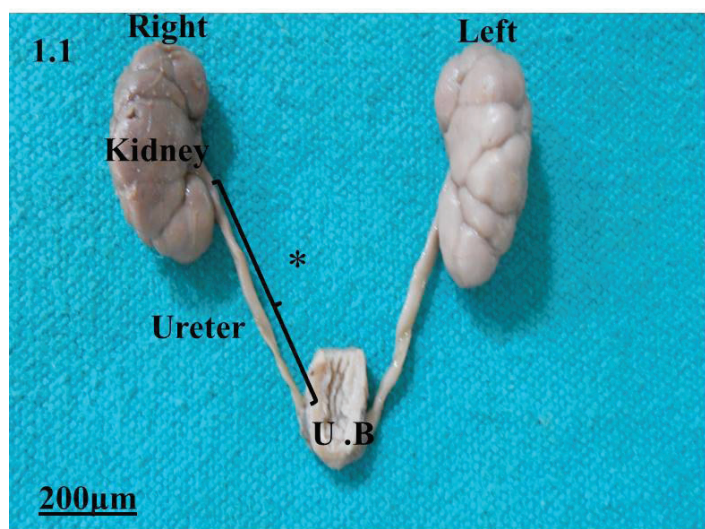


Figure 1.1 *Length of the ureter from the Pelvi-ureteric junction to the Vesico-ureteric junction.

Figure 1.2 Showing the posterior wall of urinary bladder with the measure of the angle of entry of the ureter with respect to the internal ureteric orifice. *Probe passed through the internal ureteric orifice.

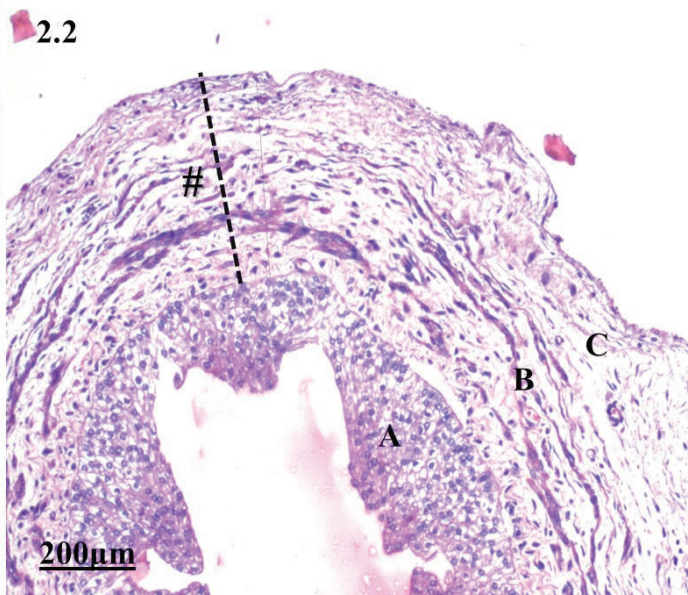
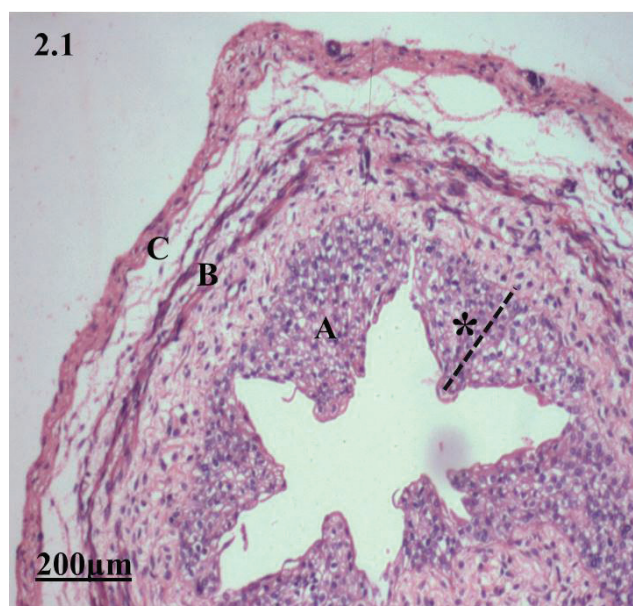


Figure 2.1 Transverse section of the ureter at the upper end, at 18 weeks of gestational age,

Figure 2.2 Transverse section of the ureter at the middle portion at 18 weeks of gestational age, Haematoxylin & Eosin stain, 10X. A- epithelium, B- smooth muscle layer, C- Adventitia, * with dotted line - Thickness of epithelium;# with dotted line - Thickness of ureteric wall (excluding epithelium)

the pelvi-ureteric junction and the other at the midpoint between the pelvis of the kidney and the vesico-ureteric junction. As the variability between the upper and mid portion in the arrangement of muscle is maximum these two sites were selected. Also literature shows abundant study done in the lower end of the ureter. So this was not considered for the present study. A vertical incision was given through the anterior wall of the urinary bladder to visualize the internal ureteric orifices and the inter-ureteric ridge. A probe was passed along the intravesical segment of the ureter through the internal ureteric orifice. Photographs were taken and the angulation of the intravesical segment with the inter-ureteric ridge was measured using Image J software (Fig. 1.2).

The collected sections of each ureter were processed using paraffin embedding, and sections of 5 μm thickness were acquired at 100 μm recesses. The sections were subjected to haematoxylin-eosin staining to evaluate the tissue. Next, a special staining technique, namely Masson's trichrome stain, was done so as to define the connective tissue and smooth muscle component in the ureteric wall. Image Pro software was used to identify the epithelial, muscular and fibrous elements of the ureter and also to measure the thickness of the ureteric wall. The arrangement of muscle fibers was noted in all the sections. The mean and standard deviation (Mean \pm SD) of the parameters was calculated and correlated with the gestational age using Pearson's correlation test. The side differences were calculated using paired t test. The statistical analysis was done using SPSS software version 16.0

RESULTS

The study included 25 fetuses of known gestational age (GA). The GA ranged from 18 - 33 weeks. The length of the ureter from the pelvi-ureteric junction to the vesico-ureteric junction ranged on the right side from 22 ± 2 mm to 59 ± 1 mm and on the left side from 22 ± 1 mm to 61 ± 1.5 mm. The length of the intravesical portion of the right as well as the left ureter ranged from 1 ± 0.5 mm to 6 ± 0.5 mm. The length of the ureter was correlated with the GA using Pearson's correlation test. There was a highly positive correlation, with $r = 0.9$.

The angle of entry of the ureter to the bladder on the right side ranged from $56.310 \pm 0.5^\circ$ to $28.496 \pm 1^\circ$ for GA of 18 to 33 weeks respectively, while on the left side it ranged from $52.431 \pm 2^\circ$ to $24.687 \pm 0.5^\circ$ respectively. There appears to be no difference in ureteric length (Table 1) between the right and the left side, as the p value of the paired t test of ureteric length is 0.18 ($p > 0.05$). However, there is a significant difference between the angulation of the right and left ureter at the vesicoureteric junction, as the p value of the statistical test is 0.007 ($p < 0.05$) (Table 1).

The microstructure of the ureter at the pelvi-ureteric junction at 18 weeks showed transitional epithelium that is 8-9 layers thick. (Fig 2.1). The thickness of the muscle wall with adventitia is $779.35 \pm 0.5\mu\text{m}$ at 18 weeks. (Fig 2.2). As the GA increases, the transitional epithelium increases in size, reaching up to 14-15 layers at 33 weeks. (Fig 3.1). The circular muscle layer similarly

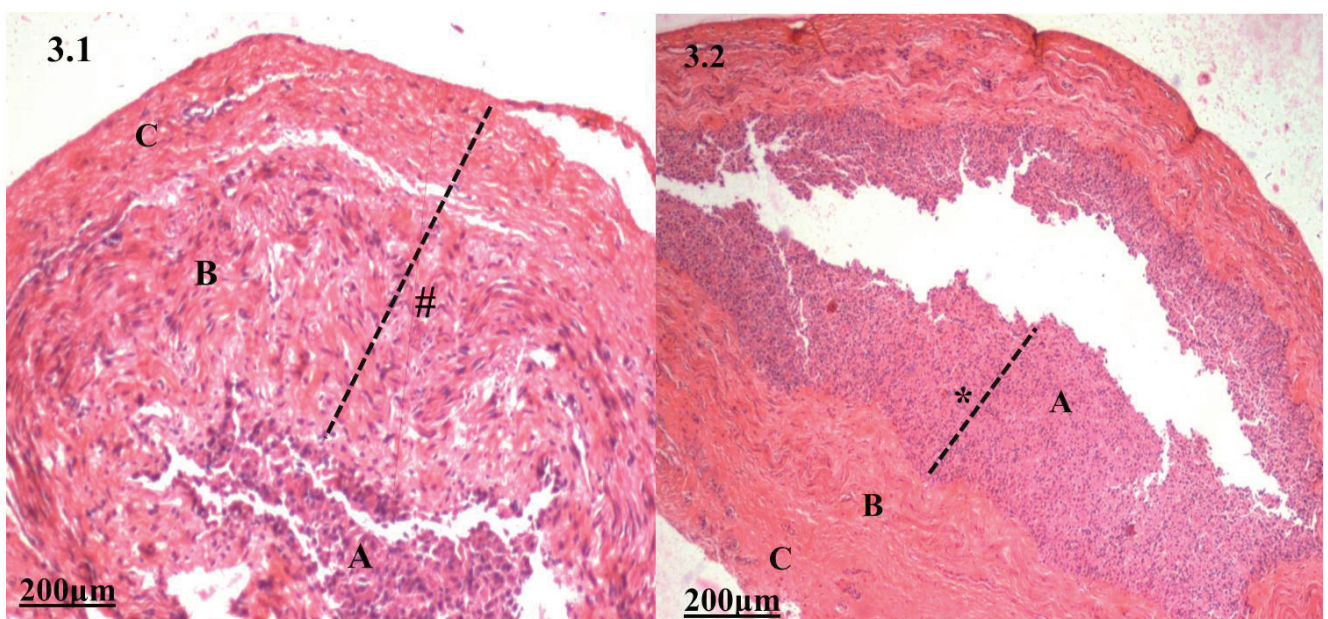


Figure 3.1 Transverse section of the upper end of ureter at 33 weeks of gestational age,

Figure 3.2 Transverse section of the ureter at the middle portion of ureter during 33 weeks of gestational age, Haematoxylin & Eosin stain, 10X. A- epithelium, B- smooth muscle layer, C- Adventitia, * with dotted line - Thickness of epithelium; # with dotted line - Thickness of ureteric wall (excluding epithelium)

increases in thickness however but the collagen fiber reduces. The wall thickness without the epithelium at 33 weeks is $1055.60 \pm 1.5\mu\text{m}$. (Fig 3.2). Large amounts of collagen fibers are present, interspersed between a single layer of thin circular smooth muscle fiber layer, which is highlighted in Masson’s trichrome staining. The adventitia is present, containing loose connective tissue (Fig 4.1). The microstructure at the mid ureter showed the epithelial and the muscle components very similar to that of the pelvi-ureteric junction. There was presence of only circular muscle layer, with no trace of longitudinal muscle layer in all the sections of pelvi-ureteric as well as mid ureter junction up to 33 weeks of GA, as shown by trichrome staining (Fig 4.2).

DISCUSSION

A study carried out by Islam (7) and Magoma et al (8) on adult cadavers in relation to the length and obliquity of the intravesical part, shows the mean intravesical length

of pelvic ureter in males was 18.72 mm compared to 14.67 mm in females. The angle at which ureters lay to the bladder was 27.32° in males and 28.68° in females. It also showed sex differences in morphometry, with the intravesical segment being longer in males, with a more oblique course. The current study in fetuses showed differences between the angulation of the right and the left side, with the left side being more acute and oblique than the right side. (Table 1)

Oswald et al (9) also found that the distal ureteral wall thickness increased throughout the fetal period from $93.91 \pm 64.29 \mu\text{m}$, at 9-12 weeks to $367.4 \pm 149.6 \mu\text{m}$ at 28-38 weeks.

Intravesical ureteral wall thickness increased from $131.77 \pm 113.52 \mu\text{m}$, at 9-12 weeks to $451.5 \pm 77.1 \mu\text{m}$ at week 39. In the present study on fetuses, the intravesical length was found to vary from 1 mm to 6 mm. The angle of the ureter at its entry with respect to the internal ureteric orifice was found to range from

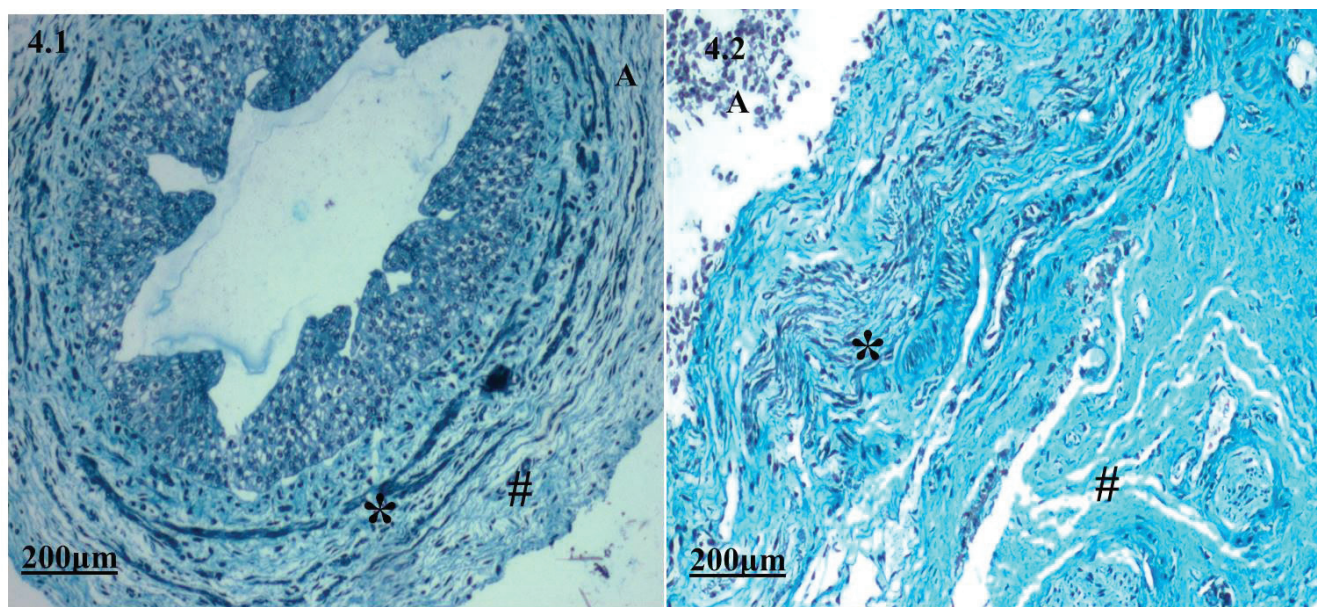


Figure 4.1 Transverse section of the upper end of the ureter at 18 weeks of gestational age, **Figure 4.2.** Transverse section of the upper end of the ureter at 33 weeks of gestational age, Masson’s Trichrome stain, 10X , *muscle tissue, # connective tissue

Table 1. Showing morphometric parameters with respect to the gestational age.

Gestational Age (GA)	Length of the ureter from PUJ to VUJ (mm)		Length of intravesical ureter (mm)		*Angle at the entry of ureter to bladder (°)	
	Right	Left	Right	Left	Right	Left
18-23 weeks	22 ± 2	22 ± 1	1 ± 0.5	1 ± 1	$56.310 \pm 0.5^\circ$	$52.431 \pm 2^\circ$
24-29 weeks	37 ± 0.5	40 ± 1.5	2 ± 1.5	2 ± 2	$35.538 \pm 1^\circ$	$8.18 \pm 1.5^\circ$
30-35 weeks	59 ± 1	66 ± 1.5	6 ± 0.5	6 ± 0.5	$28.496 \pm 1^\circ$	$24.687 \pm 0.5^\circ$

PUJ: Pelvic-ureteric junction; VUJ: Vesico-ureteric junction; ° : Degree; * : p value: < 0.05

56.310 ± 0.5° to 28.496 ± 1° on the right side and 52.431 ± 2° to 24.687 ± 0.5° on the left side during 18-33 weeks of GA. There was a significant increase in the length of the intravesical ureter and a decrease in the angle correlating to the GA (r=0.9). No significant difference has been observed in the morphometry of ureters of the left and the right side.

Costa et al (10) analyzed the structural difference between normal and anencephalic fetal ureters. Ureteral luminal area, thickness, and diameter were measured and smooth muscle cells were quantified. Collagen and elastic fibers were also studied. There was no significant difference in smooth muscle cell concentration, ureteral epithelium, collagen and elastic fiber distribution. The ureteral luminal area, diameter and thickness were significantly smaller in anencephalic fetuses, indicating the possible effect of lesions of the cerebrum with resultant loss of brain control on ureteric nerves supplying the structures of the ureter.

In the present study, the upper end of the ureter showed transitional epithelium 8-9 layers thick at 18 weeks. Large amounts of collagen fibers were found interspersed between a single layer of thin circular smooth muscle fiber layer, as seen in Masson's trichrome staining. The adventitia was present, containing loose connective tissue. The thickness of the muscle wall with adventitia was 779.35 ± 0.5 µm at 18 weeks. As the GA increases, the transitional epithelium increases in size to 14-15 layers at 33 weeks. The circular muscle layer also increases in thickness and the collagen fibers decreases. At 33 weeks, the wall thickness (without the epithelium) is 1055.60 ± 1.5µm.

In a study conducted on 12 fetuses, the authors found the appearance of inner longitudinal muscle coats by 16 weeks. By 36 weeks, the muscle layer was thick at the vesico-ureteric junction with the presence of an outer longitudinal coat (11). In the current study, there was the existence of only a circular muscle layer, without any noteworthy longitudinal muscle layer in the ureteric wall, in both pelvi-ureteric and mid-ureteric sections up to 33 weeks of GA.

In a study conducted by researchers, the ureter showed varied amounts of alpha-SM actin positive smooth muscle cells. It was found to be maximum at the distal ureter, midway at the mid ureter, and minimum at the proximal ureter close to the kidney. Also, the formation of smooth muscle in the ureter was subepithelial compared to the bladder where it was subserosal. Expression of alpha-SM actin was found to be increased in the bladder and the ureter throughout the postnatal life as all the spindle cells present around the epithelium stained positively leading to intensified staining. Differentiation of smooth muscle in both the ureter and the bladder corresponds to that in other visceral and vascular organs during embryonic life

and ensues arising from the bladder to the collecting system within the kidney (12).

Baskin et al (13) demonstrated the relationship between smooth muscle development in the bladder and the epithelial mesenchymal interaction. He found a steady increase in the mesenchymal urethral wall corresponding to the smooth muscle in human fetal ureters. In our study, it can be observed that the smooth muscle thickness is increasing and the collagen fiber amount is correspondingly reducing for GA of 18-33 weeks. The adventitia in the initial weeks is larger in size made of loose connective tissue along with collagen fibres. The smooth muscle layer is only one single layer then (Fig 2). In later weeks of GA the adventitia decreases in size and a proper muscle layer is formed with number of smooth muscle layer increasing (Fig 3). The probable reason for this may be owing to the formation of urine which increases with the development of kidneys. The smooth muscle layer help in the function of ureter which is propulsion of urine, hereafter there is enlargement of muscle layer replacing the collagen fibres (9).

CONCLUSION

Gross developmental morphology of human ureters in fetuses of second and third trimesters was studied in relation to the gestational age. Epithelial, muscular and connective tissue components displayed significant changes during development. Presence of only a circular muscle layer with no traces of longitudinal muscle was a highlighting feature. This data would significantly contribute to the existing literature on the intra uterine development of human ureter.

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