

Original Article

Histogenesis of Urinary Bladder in Human Fetuses

Yanglem Elizabeth Devi¹, N Saratchandra Singh², Ch Rajendra Singh², Laishram Cindy³

Background : The Urinary Bladder starts functioning during fetal life, It fills every hour with minimal increase in pressure (5-10 cm H₂O) and empties by contraction over a few seconds. The proper functioning of an organ depends on its histological maturation. There are only few literatures of study on microstructure of the development of Urinary Bladder. For this reason, this study was done to look into the histological development of urinary bladder in different human fetuses.

Materials and Methods : Fifty fetuses of different gestational ages ranging from 15 weeks to 40 weeks were studied after staining with Haematoxylin and eosin stain, Van Gieson, Masson Trichrome and Verhoeff stain.

Results : In the specimen of 15 weeks, Urinary Bladder wall consists of 3 layers ie, inner mucosa comprising of lining epithelium and lamina propria, middle muscular layer and an outer adventitia. Mucosa layer appears thickest comprising about half of the thickness of the bladder wall. The lining epithelium assumed adult urothelium by 22 weeks.

Conclusion : Human fetal bladder undergoes a series of vital developmental changes during 15-40 weeks of gestation both morphologically and histologically finally acquiring the typical adult shape with a typical urothelial lining and well developed muscle coat.

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Key words : Urinary Bladder, Urothelium, Lamina Propria, Detrusor Muscle Layer.

The Urinary Bladder is a temporary reservoir of urine brought from the kidneys by the ureter¹. In a normal adult, internal surface of the bladder is lined with urothelium, deep to this is the lamina propria and underneath the lamina propria is the detrusor muscle layer².

The Urinary Bladder starts functioning from fetal life³. The impermeability of transitional epithelium, particularly during early fetal development is possibly a function of umbrella and intermediate transitional cells⁴.

MATERIALS AND METHODS

Fifty fresh human fetuses without any gross anatomical abnormality, ranging from 15 to 40th Gestational Weeks (GWs) were procured from Obstetrics and Gynaecology Department, RIMS Imphal. Permission for the study on fetuses was obtained from the Institutional Ethics Committee. The age of the fetuses were assessed from the obstetrical history, Crown Rump Length (CRL) and gross features before fixation. The fetuses were kept immersed in 10% formalin for 10 to 15 days.

Editor's Comment :

- Functional maturity of an organ largely depends on its histological maturity.
- In case of urinary bladder also, its primary function as urinary storage organ and its impermeability depends on the proper development of urothelium and detrusor muscle layer.

The fetuses were categorized into different age groups according to their gestational age as follows for easier study and observation :

- Group I : Upto 15 weeks
- Group II : 16 – 20 weeks
- Group III : 21 – 25 weeks
- Group IV : 26 – 30 weeks
- Group V : 31 – 35 weeks
- Group VI : 36 – 40 weeks

After proper fixation (for about 2 weeks), the fetuses were dissected, the Urinary Bladder was identified. Appearance of the Urinary Bladder in situ was studied in detail and then isolated. Sections of these specimens were fixed in the neutral buffer formalin for 10-15 days. After proper fixation and trimming, the tissue was processed for parafin sections. Serial sections of 7 micrometer thickness were cut with Leika RM 2125 RT rotary microtome. The prepared sections were stained with Haematoxylin and Eosin stain. Special staining was done with Van Gieson's stain, Masson's Trichrome for differentiation between the collagen fibres, muscle fibres and Verhoeff's stain for elastic fibre. All the stained slides were studied under trinocular compound light research microscope and

Department of Anatomy, Regional Institute of Medical Sciences, Imphal, Manipur 795004

¹MD (Anatomy), Senior Resident and Corresponding Author

²MD (Anatomy), Professor

³MD (Paediatrics), Senior Resident, Department of Paediatrics

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microphotography was done with USB camera. All the digital microphotographs were stored and analysed.

RESULTS AND OBSERVATIONS

Group I : 15 Weeks

At 15 GW, Urinary Bladder wall consists of an outermost adventitia, middle muscular layer and an innermost mucosa layer comprising of lining epithelium and lamina propria. Connective tissue cells are present in the Lamina Propria with some undifferentiated mesenchymal cells. Few blood vessels have developed in the Lamina Propria. Lots of mucosal foldings are seen indicating that the epithelial growth is faster than the rest of the wall. Mucosa layer appears thicker than muscular layer. Muscular layer consists of three indistinctly formed layers (Fig 1).

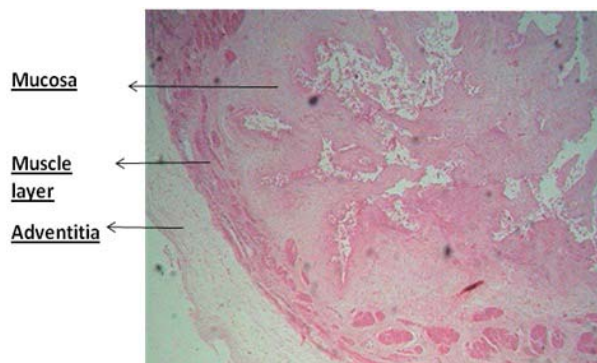


Fig 1 — Photomicrograph of urinary bladder from a 15 weeks fetus (H&E x 10)

Note : Lamina propria thicker than the muscle layer

Group II : 16 - 20 Weeks

Well formed lumen is seen. Lining epithelium looks like stratified cuboidal and basal cells are present. Intermediate polygonal cells are present (Fig 2). Superficial cuboidal cells are darker stained than the underlying cells (Fig 4). Lamina Propria is well developed with presence of mesenchymal cells and fibroblasts. Smooth muscle fibres are well developed with varying thickness at different sites and are oriented in multiple directions as longitudinal, circular and oblique muscle fibres. More blood vessels have developed in the Lamina Propria than in the previous group (Fig 3). Blood vessels are also present in between the muscle fibers. Adventitious coat consisting of collagen fibers is seen.

Group III : 21 - 25 Weeks

By this time, epithelium is four to six cell layered. Surface cells are darker stained going towards adult epithelium, some of them are binucleate. Lamina Propria is well developed, muscle layer have grown

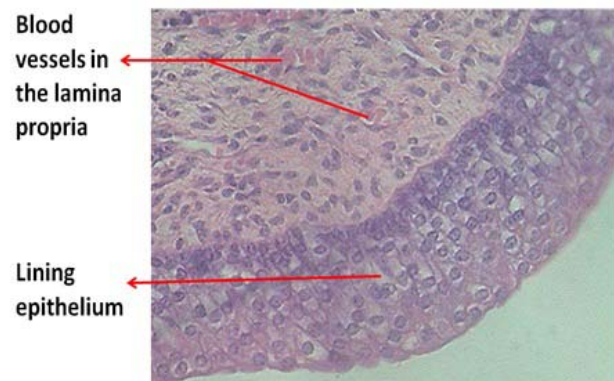


Fig 2 — Lining epithelium and part of lamina propria of 18 weeks urinary bladder (H&E x 40)

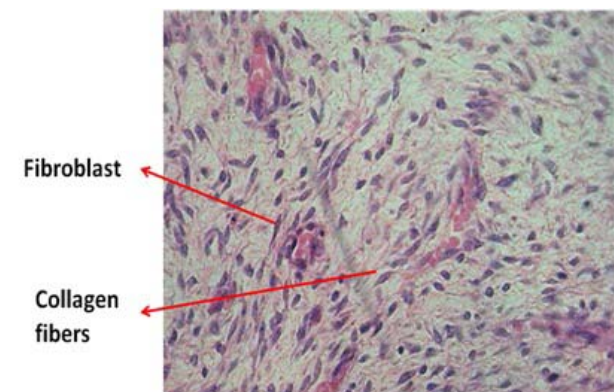


Fig 3 — Lamina propria in a 18 weeks urinary bladder showing laying down of collagen fibers by the fibroblasts

Note : Cellular elements predominate fibers element

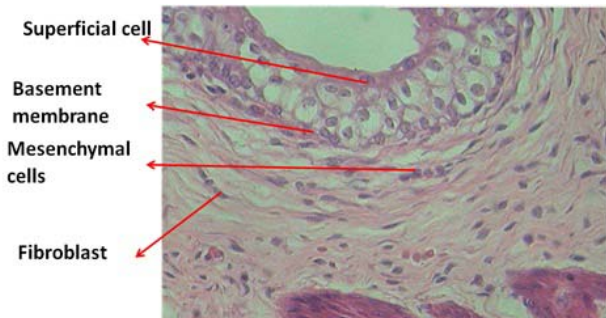


Fig 4 — Cytoarchitecture of a 20 weeks urinary bladder (H&E x 40)

Note : (a) Superficial cells stained darker, (b) Basement membrane is ill defined (c) Fibers element starts predominating cellular element in the lamina propria

more so it appears to dominate in thickness. Fibroblast cells dominate in the Lamina Propria whereas mesenchymal cells are less. Mesenchymal cells are seen to be clustered more near the basement membrane (Fig 5). Elastic fibres couldn't be seen in the Lamina Propria though it is visible in the wall of arteriole in sections stained with Verhoef's stain (Fig 6).

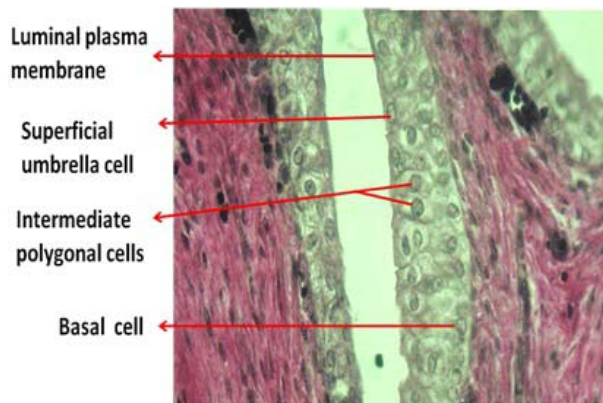


Fig 5 — Photomicrograph of a 22 weeks old urinary bladder showing the lining epithelium assuming the adult urothelial form (Verhoeff's stain x 40)

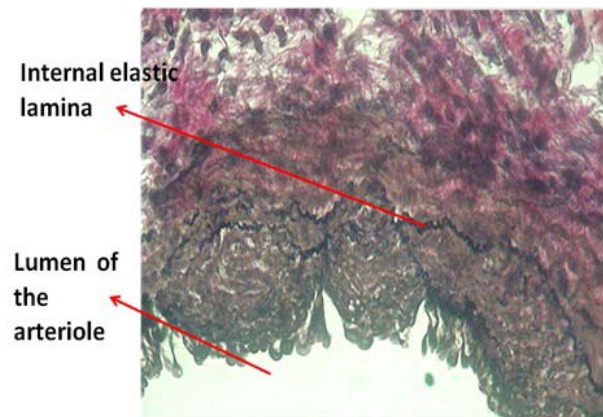


Fig 6 — Photomicrograph showing the elastic fibers in the internal elastic lamina of an arteriole present in the adventitia of a 22 weeks urinary bladder (Verhoeff's stain x 40)

Group IV : 26 - 30 Weeks

There is spurt of growth so that the cells are compressed and the rounded intermediate cells assume polygonal in shape. Signs of apoptosis are also seen. In the Lamina Propria, both fibroblasts and fibrocytes are present, as the fibroblasts mature into fibrocytes nuclear shape gradually changes from the earlier tadpole form to spindle shape. Collagen fibres being laid down by the fibroblasts are seen. Mesenchymal cells are also present which are differentiated from the fibroblasts by their oval nucleus and are not associated with collagen fibres. Mesenchymal cells are seen more near the basement membrane and possess oval nucleus. Muscle wall has also increased in thickness. Elastic fibers start appearing in the Lamina Propria (Fig 7). Abundance of collagen fibres are also visible in the Lamina Propria (Fig 8).

Group V : 31 - 35 Weeks

Bladder by this time has assumed the adult cytoarchitecture. Lamina Propria is well developed with

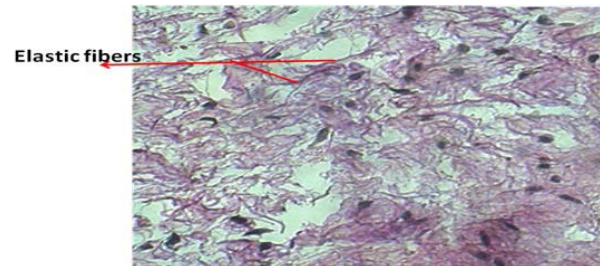


Fig 7 — Photomicrograph of 28 weeks urinary bladder showing the first appearance of elastic fibers in the lamina propria (Verhoeff's stain x 40)

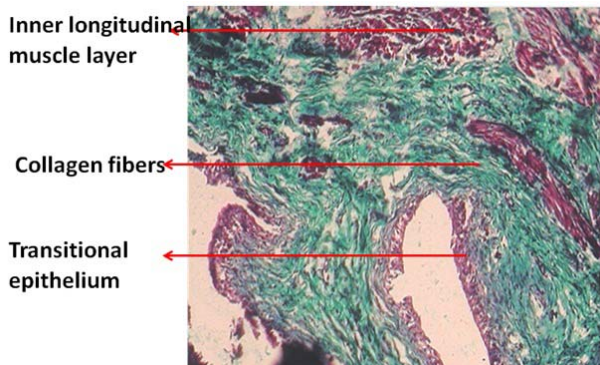


Fig 8 — Cytoarchitecture of a 28 weeks urinary bladder showing abundance of collagen fibers in the lamina propria (Masson's Trichrome stain x 10)

more fibrous element than the cellular element. Subepithelial capillaries are also visible. Mesenchymal cells are still present in the Lamina Propria. Muscle wall is well developed but some connective tissue elements are present in between the muscle layers.

Group VI : 36 - 40 Weeks

In this series, well developed Lamina Propria is seen which is more compact in the subepithelial zone and is loose close to the muscle layer, fibrous element predominates the cellular element. Detrusor muscle layer is well formed and is thicker than the Lamina Propria.

DISCUSSION

The epithelial lining of the urinary bladder is derived from the endoderm of the vesical portion of the urogenital sinus, while the Lamina Propria of the bladder mucosa, the muscle coat and the adventitia all develop from the adjacent splanchnic mesenchyme. Initially the entire vesico-urethral anlage is lined by a single layer of low cuboidal to tall columnar epithelium with prominent vesicular characteristics.

Above the openings of the ureters this single layer becomes bi or trilayered by the end of the 6th week according to Felix (1912) or by the 7th week (Gray and Skandalakis, 1972). Below the ureteric orifices it becomes bi- or trilayered and later, by the 9th

week, 4 to 5 cells thick (Felix 1912). According to Felix the urothelial characteristics appear in the 11th week embryo while according to Gray and Skandalakis (1972) they appear in the 12th week. While according to Newman J, *et al* the typical ultrastructural urothelial characteristics appeared by 21 weeks⁵.

According to Hoyes AD, in the 4th month, the bladder epithelium was bilaminar and in the 5th month, a third layer of cells was sometimes present. Differentiation of the luminal plasma membrane was evident by the end of the 3rd month⁶. During second trimester, the epithelial lining of the bladder becomes transitional in type⁷.

In our present study, we observed the adult urothelial characteristics by 22 weeks as defined by the presence of specific features – large binucleated umbrella shaped surface cells with prominent darker staining luminal plasma membrane, two to three layers of intermediate polygonal shaped cells and a basal layer of cuboidal cells lying over an ill defined highly undulating basement membrane.

The musculature of the bladder wall begins to develop at the 13th week and is very prominent by the 16th week⁸. Muscle thickness progressively increased and the relative collagen content in the muscle decreased during gestation. The ratio of thick-to-thin collagen fibers also decreased, whereas elastic fibers increased⁹.

In our present study, in the earliest available fetus of 15 weeks, muscular layer consists of three indistinctly formed layers which are infiltrated with abundant connective tissue elements in between the muscle fibers. The muscle layer can be more or less differentiated into inner longitudinal, middle circular and outer longitudinal layers by 18 weeks. This detrusor muscle layer gradually thickened as age advances and is associated with the progressive decrease in the connective tissue elements infiltrating the muscle fibers.

The highest level of expression of RNA products of elastic fiber genes occurred in the urothelial – Lamina Propria fraction during the late second – early third trimester⁹. In the present study, Verhoeff's elastic tissue stain was used to identify the elastic fibers, internal elastic lamina in the wall of arteriole could be identified by 22 weeks, but elastic fibers are not yet visible in the Lamina Propria. By 28 weeks, few elastic fibers are identifiable in the Lamina Propria and the elastic fibers distribution gradually increases as age advances and at term plenty of elastic fibers could be seen in the Lamina Propria.

CONCLUSION

On histological examination, in the early specimen of 15 weeks, urinary bladder wall consists of 3 layers ie, inner mucosa comprising of lining epithelium and lamina propria, middle muscular layer and an outer adventitia of which mucosa layer appears thickest comprising about half of the thickness of the bladder wall. By this time, in the Lamina Propria there is abundance of mesenchymal cells and fibroblast cells which have started laying down few collagen fibers. By 18 weeks, lining epithelium appears stratified cuboidal with the superficial cells more darker stained than the underlying cells. The lining epithelium assumed adult urothelium by 22 weeks.

There is lack of literature regarding development of Lamina Propria, available literatures just mention that there is increase in fibers element with increase in the gestational age but specific age of this change has not been mentioned. In our study, in the Lamina Propria, there is gradual change in the proportion between the cellular element and fibers element. In the earlier week of fetus less than 20 weeks, cellular element predominates the fibers elements whereas in the later fetal life, fibers element predominates the cellular element. This is associated with the increase in both elastic and collagen fibers of which the collagen fibers appear earlier. Elastic fibers could be seen in the wall of arteriole by 22 weeks while it appears in the Lamina Propria by 28 weeks.

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