Original Article

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Histogenesis of Muscularis Mucosae of Human Urinary Bladder

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Abstract

Background: Urinary bladder serves an important function of temporary storage of urine without changing its constituents. Many studies are available for the gastrointestinal tract [1,2,3] illustrating the muscularis mucosae presence and its role in local contractility. But only few studies are available to describe the development and role of muscularis mucosae layer of urinary bladder. Current study is on the urinary bladder involving the microscopic examination of wall of 50 aborted human fetuses urinary bladders of different gestational age from 9th week onwards. *Aims:* 1. To note presence, structural differentiation and maturity of muscularis mucosae layer which it attains at different stages of development to show the adult picture. 2. To compare and contrast differences between different age groups and with previous studies and available literature. *Study Design:* Observational (Qualitative) study. *Material and Method*: 50 aborted human fetuses (29 females and 21 males) of different gestational age from 9th week onwards were collected, urinary bladder were taken out and fixed in a fixative. Blocks of tissues were made from bladder wall proper, trigone & bladder neck and processed to get sections which were stained with 1) Haematoxylin and Eosin [4], 2) Masson's trichrome stain [4]. *Statistical Analysis*: No measurements have been taken as it is an Observational study, so statistical analysis is not applicable. *Results:* The stained sections were examined under light microscope at 10 X and 40 X magnifications. Muscularis mucosae was not differentiable between 12-16 weeks. At 17th week, it was first seen as a thin, discontinuous layer of spindle shaped cells. 24th week onwards muscularis mucosae was thicker, wavy and stained dark eosinophilic. Further it showed adult picture and stained bright red with special muscle stain Masson's Trichrome. *Conclusions:* Muscularis mucosae was discernible from 17th week and increased in thickness and differentiated in subsequent weeks. It was found in 75% of studied aborted fetuses.

Keywords: Histogenesis; Muscularis Mucosae; Fetus; Urinary Bladder.

Intoduction

All vertebrates do not have urinary bladder, as birds and most reptiles (snakes and crocodiles)[1]. Urinary bladder is present in all mammals and in ostrich. The present paper describes some light and electron microscopic observations on the smooth muscle cells comprising the muscularis mucosae.

The Urinary tract is composed of four layers. Each layer has different tissues and functions. From the inside out they are called: lining epithelium (Urothelium), lamina propria, muscularis propria and serosa.

The muscularis mucosae is a thin layer of muscle in the gastrointestinal tract and also in urinary tract from the renal pelvis to the bladder; but as it is discontinuous, it is not be regarded as a true muscularis mucosae [5]. In the gastrointestinal, the muscularis mucosae has been shown to exhibit spontaneous phasic contractility and also to respond to nerve-induced stimulation. In the colon, spontaneous phasic activity of the muscularis

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mucosae has been suggested to play a role in modulating mucosal secretion, reducing mucosal surface area in response to noxious stimuli, and facilitating the release of acids into the stomach [1].

Motor activity of the muscularis mucosae shows a great regional and species difference. In urinary bladder,the muscularis mucosae has been identified between the basement layer of the urothelium and the detrusor muscle [6,7]. Although the existence of the muscularis mucosae is well established in the medical literature, particularly with respect to its role in the development of urinary bladder cancer, its functional role in normal urinary bladder contractility and physiology is still not clear [8,9].

There is little information on the innervation of the muscularis mucosae [10]. Some studies say, the muscularis mucosae is nonneurogenic and contracts in rhythmic bursts, suggesting local regulation.This suggest that the muscularis mucosae is functionally distinct from the detrusor [11]. The physiological role of Spontaneous phasic contractions in urinary bladder had been the focus of numerous studies; however, the precise role of spontaneous phasic contractions remains unclear and said to be contributing minimally to micturition [11].

Material and Method

Collection of Materials

After approval from the institutional ethical committee, during period of 2 years, 50 aborted human fetuses (29 females and 21 males) of different gestational age from 9th week onwards were collected from the department of Obstetrics and Gynaecology, Aurangabad (Figure 1). Written consent from parents of aborted fetuses was taken.

Inclusion Criteria

Spontaneously aborted fetuses from 9th week onwards, stillborn fetuses, and terminated fetuses under the Medical termination of Pregnancy Act of India 1971.

Exclusion Criteria

Fetuses less than 9 weeks, twins, presence of any congenital anomalies, post mortem decomposition were excluded from the study.

Fetuses were obtained within 1-2 hrs of abortion to avoid post-mortem decomposition changes and preserved immediately in 10% formalin. Gestational age was calculated from Body weight and Crown-rump length (CRL). They were dissected within 2 hrs of collection by taking a midline vertical incision extending from umbilicus to pubic symphysis (Figure 2). Bladder was then carefully removed along with its neck. Subsequently bladders were passed through following procedures [4]:

1. *Fixation of Bladder*: in Bouin's fluid for 4-5 days. Longitudinal and transverse sections of specimen were taken from bladder wall proper, trigone region and bladder neck region, each section being 3-4 mm thick.
2. *Dehydration*: The tissue was processed in ascending grades of 50%, 70% and 90% alcohol.
3. *Clearing*: done to remove alcohol from tissue. Tissue was placed in xylene for about 30 minutes. It also increases the refractive index of tissues.
4. *Paraffin bath*: It involves soaking of tissue in molten soft paraffin wax (melting point 45-50^{0}C). Tissue was subjected to two changes of paraffin wax each for three hours.
5. *Casting (block making)*: The blocks were prepared by pouring molten paraffin wax (melting point 55-60^{0}C) into a mould. Using two 'L' moulds, suitable size bocks were prepared and wax impregnated tissue was placed eccentrically and oriented so that it could be sectioned in the right angle plane.
6. *Microtomy (Section Cutting)*: The block was cut with the section thickness of 5-7 microns in the form of ribbon with the help of rotary microtome.
7. *Fixing Sections on the Slide*: The ribbon of sections was placed on the surface of warm water in the flotation bath. This removes all wrinkles from the tissue and wax (flattening). The glass slide was smeared with egg albumin and sections were mounted on it and slides were placed on the hot plate at 45^{0}C - 50^{0}C for 2 hours or more as per the requirement for drying.

The sections were stained with the following stains:

- *Haematoxylin and Eosin Staining* [4]

Technique

1. Removal of paraffin wax from the sections was done by dipping the slide into two changes of xylene for one to two minutes each.
2. Removal of xylene done by dipping the slide into two changes of absolute alcohol for one to two minutes each and then treated with descending grades of alcohol- 90%, 80%, 70% for one minute each.

3. The slide was kept under running tap water for 2-3 minutes.
4. The slide was stained with Haematoxylin for about five to seven minutes followed by washing under running tap water for 2-3 minutes. This leads to bluing of the section.
5. Excess stain is removed (Differentiation) by dipping the slide in acid alcohol for few seconds. This changes blue color to red because of the acid.
6. The blue color was regained by washing in running tap water for 5 minutes and it was checked under the microscope, for nuclear staining.
7. The section was counterstained with 5% aqueous solution of eosin for about 5 minutes and dehydrated by dipping in ascending grades of alcohol as 70%, 90%, and absolute alcohol (100%) for one minute each.
8. Clearing was done in two changes of xylene for one minute each.
9. The slide was mounted in DPX (Distrene Plastsizer and Xylene) and coverslip was applied and the slide was kept at room temperature for some hours for firm adhesion of the coverslip to the section.

Result

Nuclei-blue, cytoplasm- pink, muscle cells- pink, collagen fibres- light pink.

• *Masson's Trichome Staining* [4]

Technique

1. Wax was removed and section was brought to water.
2. Nuclei were stained with Weigert's Iron Haematoxylin and then slide was washed well in water.
3. It was stained with diluted Ponceau Acid Fuschin for five minutes.
4. The slide was rinsed in distilled water.
5. Section was differentiated in 1% Phosphomolybdic acid until collagen was decolorized and again rinsed in distilled water.
6. Section was counterstained with light green or aniline blue for two minutes.
7. Light green was differentiated in water.
8. Slide was dehydrated and cleared.
9. Lastly the slide was mounted.

Result

Nuclei- blue to black.

Muscle, red blood cells, fibrin and some cytoplasmic granules– red.

Collagen, some reticulin, basement membrane, amyloid and mucin– green or blue according to counterstain used.

Observations and Results

The slides were stained with Haematoxylin and Eosin and Masson's Trichome stain and observed under light microscope at low (10X) and high (40X) magnifications for thin layer of smooth muscle fibers in lamina propria – Muscularis mucosae.

Starting with fetus of 12th week, muscularis mucosae was not demarcable between 12-16 weeks. At 17th week, Muscularis mucosae was first seen as mesenchymal condensation under low power. Under high power it was seen as a thin interrupted layer of spindle shaped cells with elongated nuclei (Figure 3). Lamina propria was distinct with abundant connective tissue fibres, stellate cells and consisted of blood vessels as endothelium lined spaces. Muscularis mucosae failed to take specific muscle stain Masson's Trichrome. Muscularis mucosae was well demarcated, more condensed, eosinophilic and appeared as a wavy layer and it was found in 10 out of total 16 cases of 19-23 weeks duration (Figure 4,6,7). At this stage, Muscularis mucosae stained bright red with Masson's Trichrome (Figure 5). 24th week onwards Muscularis mucosae was thicker, wavy and stained dark eosinophilic (Figure 8,9). Between 31-38th week it was more distinct and wavy (Figure 10). Among all 50 fetuses studied, muscularis mucosae was found in 75% of bladder wall proper. It was missing at bladder trigone and bladder neck regions as lamina propria layer is negligible (Figure 11,12).

Fig. 1: Fetuses of differentage group

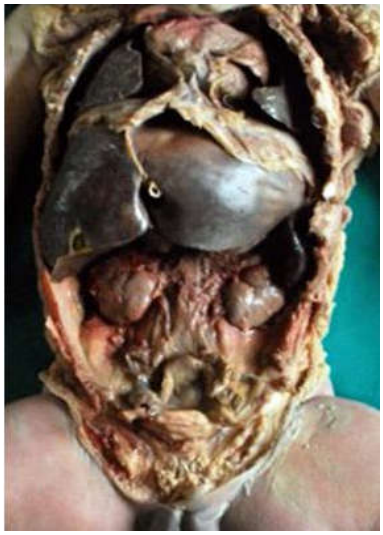

Fig. 2: Urinary bladders of fetuses of different age groups

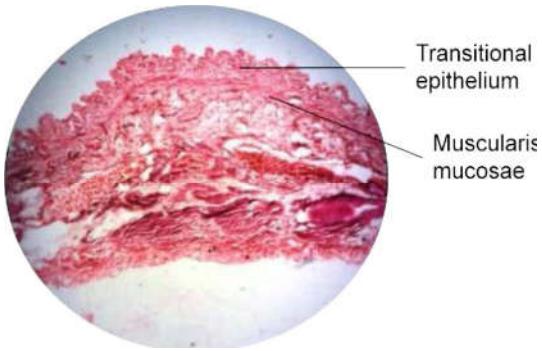

Fig. 3: 17 Weeks: 10X H&E

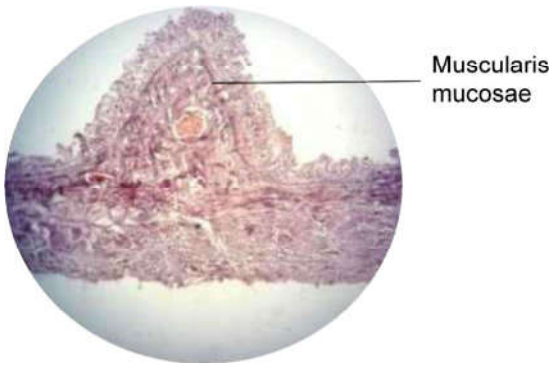

Fig. 4: 19 Weeks: 10XH&E

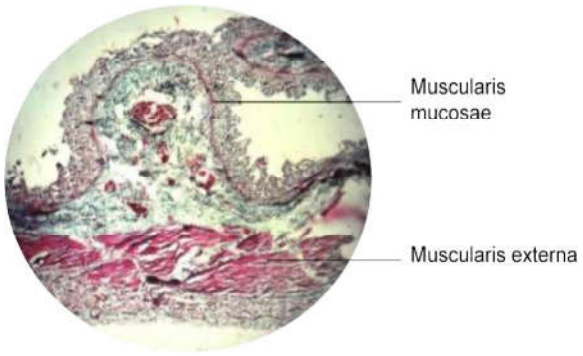

Fig. 5: 19 Weeks: 10X Masson's trichrome

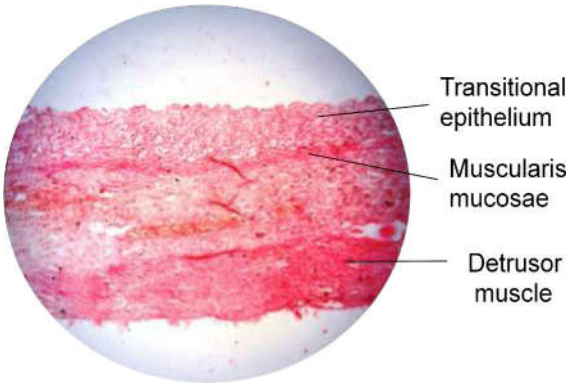

Fig. 6: 20 Weeks: 10 X H&E

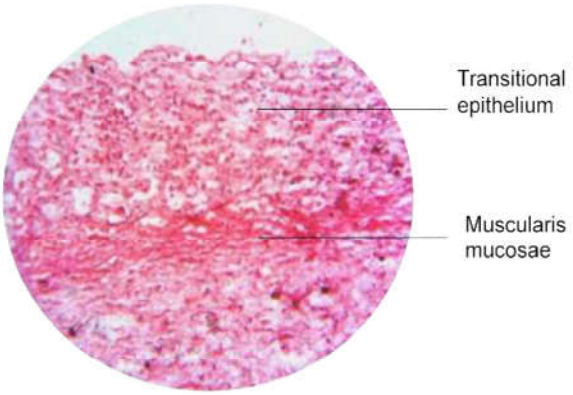

Fig. 7: 20 Weeks: 40X H&E

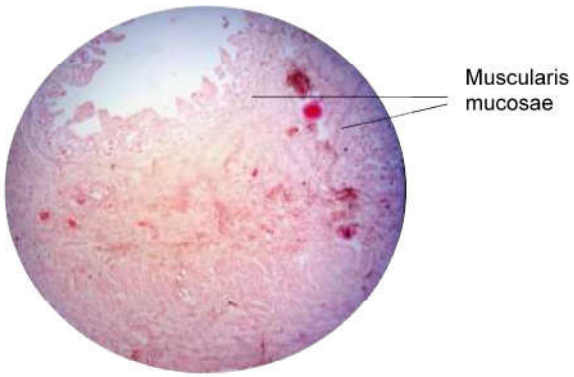

Fig. 8: 24 Weeks: 10 X H&E

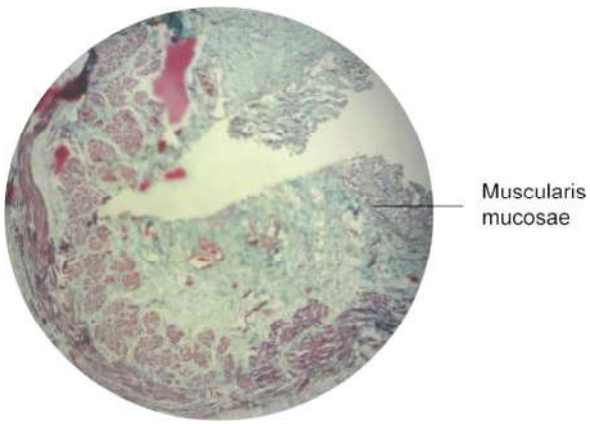

Fig. 9: 24 Weeks: 10 X Masson's trichrome

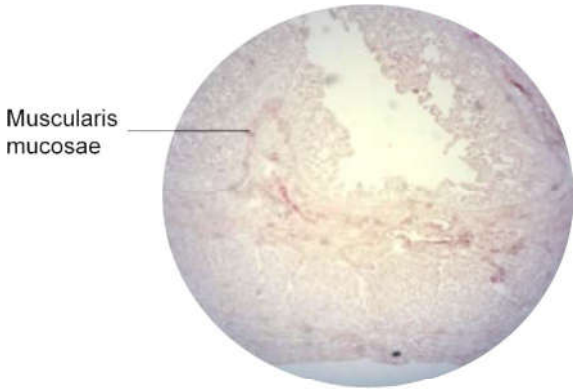

Fig. 10: 34 Weeks: 10 X H&E

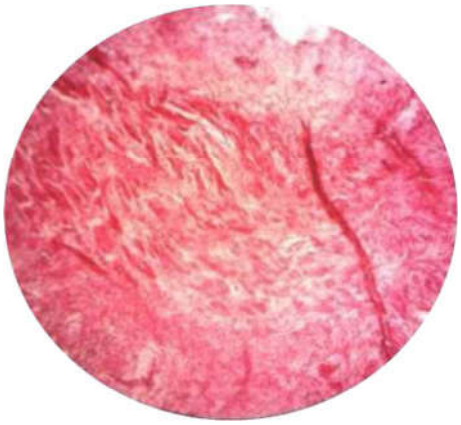

Fig. 11: 20 Weeks: 10X H&E Trigone

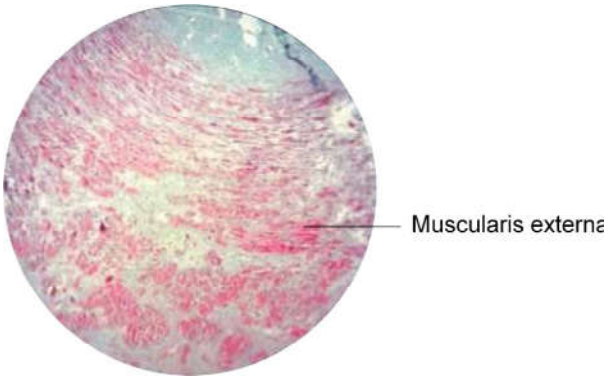

Fig. 12: 32 Weeks: 10X Masson's trichrome–Bladder neck

Disscussion

According to Dixon JS and Gosling JA (1983), mucosa of 78% human bladder possessed distinct muscularis mucosae in all regions of bladder wall midway between urothelium and detrusor. It was present as irregularly arranged bundles of muscle fibres in discontinuous manner [13].

The present study findings coincides with above findings where muscularis mucosae was not demarcable between 12-16 weeks. Muscularis mucosae made its appearance by 17th week as an interrupted layer of spindle shaped cells with elongated nuclei and was present in about 75% fetal bladders.

According to Thomas J. Heppner et al (2011) study done on guinea pig urinary bladder, a type of smooth muscle the muscularis mucosae had been identified between the basement layer of the urothelium and the detrusor layer. They found that mucosal strips of muscularis mucosae exhibited spontaneous phasic contractions (SPC). These SPCs likely result from bursts of Ca^{2+} (flashes) that elevate Ca^{2+} in the mucosal layer. The force of SPCs generated by the mucosal layer was equivalent to that of detrusor SPCs; however, the peak force of detrusor contractions evoked by 60 mM K^{+} was < 40-fold greater than that of the muscularis mucosae. However, the precise role of SPCs remains unclear [11].

Like urinary bladder, in gastrointestinal tract the muscularis mucosa appeared first at about 18 weeks of gestational age [13]. While in other study Muscularis mucosae is discernible from 13th week [14].

Conclusion

Muscularis mucosae was demarcable from 17th week, become more thick and wavy in subsequent weeks. It was found in about 75% fetal bladder walls.

These findings suggest that the muscularis mucosae is functionally distinct from the detrusor and may contribute to bladder physiology.

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Conflict of interest: Nil

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