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Morphological study of congenital megaureter

M. VLAD1, N. IONESCU2, A. T. ISPAS3, E. UNGUREANU3, CLAUDIA STOICA1

1)Department of Anatomy, Compartment of Clinical Anatomy and Surgical Techniques
2)Department of Histology
3)Department of Anatomy
“Carol Davila” University of Medicine and Pharmacy, Bucharest

Abstract
Congenital anomalies of the kidney and urinary tract are frequent. They can be detected once every 500 ultrasonography fetal examinations. Causes that determine a dilated ureter compared to the rest of the urinary tract are still partly unknown. Concerning the exploration and the clinical diagnosis of these anomalies important progresses have been made, the morphological research is still able to bring forth data that, together with genetic researches, may help reveal the pathogeny of the disease and may ease the planning of the screening genetic tests for early diagnosis.

Material and methods
During the surgical operations realized in order to recalibrate the ureter, ureter fragments have been harvested, colored with 1% tetrocin, in sections thinner than 1 micron and examinated through immersion, ob. 100×. Other fragments have been prepared and examinated through electronic microscope. We have followed the structural modifications of the muscular tissue, nervous tissue, connective tissue and the rapports between these components.

Results
Congenital megaureter presents qualitative and quantitative anomalies of the connective tissue and muscular tissue, and also structural modifications of the nervous tissue. Connective tissue. We have noticed the abundance of the connective tissue in congenital megaureter. Connective tissue, with an important representation of typical elements, such as fibroblasts, mastocytes and plasmocytes is partly hyalinized, dissociating muscular fiber bundles and nervous fiber bundles. Connective tissue/muscular tissue rapport is evidently increased.

Muscular tissue
Muscular fibers are hypoplastic, smooth endoplasmic reticulum is present in the tubular form, rare mitochondria are vacuolized. Dense corps are increased in number, plasmatic membranes are folded. Sarcomplasm contains vacuolized organites. The nuclei are rigged with visible nucleoli.

Nervous structures
We have noticed varied alterations of the axons and myelin. In some axons there are multiple cavities that may produce the opacification of the entire axonal structure. The proliferation of the myelin sheath under the nodular form or vortex form produces the destruction of the axonal structure. Mitochondria are condensed, partially vacuolized. The modifications in the axonal structure and those of the myelin sheath determine modifications of the nervous excitability and conductivity. All the lesions we have pointed out in congenital megaureter participate in compromising of the peristaltic. Nervous and muscular structures lesions indicate a process of incomplete development of the ureter. They are structures that do not achieve functional maturation. We may consider congenital megaureter as a digenesis with hypoplasia.

Keywords: congenital megaureter, electronic microscopy, disgenesis, hypoplasia.

Introduction
The term „megaureter” is used for an abnormally dilated ureter compared to the rest of the urinary tract.

Congenital megaureter means the congenital dilatation of the ureter usually associated with the dilatation of the renal pelvis. The pelvic part of the ureter is very dilated or the whole ureter is dilated. The ureter also presents a tendency to extend and to curl, this anomaly being frequently associated with malformations of the ureter-bladder junction.

The congenital anomalies of the kidney and urinary tract are common for humankind – about 1 per 500 fetal ultrasonography examinations. It is very clear that those complex development models are under genetic control. The association between urinary tract malformations and kidney dysplasia known as CAKUT (Congenital Anomalies of Kidney and Urinary Tract) may be determined by a single error of embryoniary development of kidney and urinary tract [1].

The increased frequency and severity of malformations for males might be explained by linkage disequilibrium or X-linkage transmission [2]. For example, AGTR2 gene (X chromosome) mutations have been noticed on laboratory animals, but have not been certified for humans [3]. ACE gene polymorphism is associated with increased rate of dysplasic congenital kidneys and is an important risk factor for progressive renal disorders [4].

Recent hypothesis concerning CAKUT ontogeny start from notions concerning biologic border spaces [5, 6]. It is known that the ontogeny of many organs incriminates cellular apoptosis or programmed cell death (PCD). This phenomenon is important because it allows the elimination of old distorted cells and also the elimination of unwanted tissues or those necessary only in initial stages of the ontogenesis.

Embryonary urinary tract is surrounded by undifferentiated mesenchymal cells. These are
ues at the ureteric bud and the Wolffian duct are surrounded by frequent undifferentiated mesenchymal cells presenting the AT2 receptors. The induction of the ureteric bud development is produced by biological signals from the metanephros and is conditioned by a strong interaction between the inductor – metanephrogenic blastema – and the receptors of the Wolffian duct. It is possible that a failure of the undifferentiated mesenchymal cells apoptosis may slow the interaction between the metanephrogenic blastema and ureteral bud [9].

This might produce the ectopic budding followed by an abnormal localization of the ureteral foramen and the apparition of vesicoureteric reflux. The ureteric bud will be in contact with parts of the metanephrogenic blastema which contain rare undifferentiated mesenchymal cells, resulting in the apparition of a dysplasic or hypoplastic kidney. The transmission of the signal from ureteric bud to the metanephros must penetrate the undifferentiated mesenchymal cells layer. An abnormal persistent population of undifferentiated mesenchymal cells might interfere in the transmission of the signal, producing ulcerar anomalies in both organs development. Angiotensin II also has a role, inducing certain cell populations apoptosis through type 2 receptor. The role of the angiotensin in kidney morphogeny is sustained by a broad specter of data, though most of them are of indirect nature.

There are still few certif ied data concerning the molecular patogeny of these diseases. The causes that determine the apparition of a megaureter are subject to future researches. Considered in the past as an aplasy of the urine collecting system, more recent researches have proved that a megaureter may be produced by vesicoureteric reflux, by obstructive disease, by an increased urinary flow from from kidneys which lost the ability to concentrate urine or by lack of development of the ureteral muscularity. Bacteria from body infections may furthermore dilate the megaureter and may produce the dilatation of a normal ureter through toxic paralysis of the muscular cells. An important proportion of megaureters are diagnosed following an urinary infection and frequently more than one of the factors mentioned above is present in any individual case. Because of this the exact contribution of vesicoureteric reflux, infection and ureteral malformation is hard to quantificate in certain cases of megaureter. There are many classifications which varies from simple to complex.

International classification, which seems to be the most adequate is present in and is based upon the evaluation of the urinary tract through intravenous pyelography and cystourethraphy. The dilatation is produced by obstruction or vesicoureteric reflux or without any of them (without reflux, without obstruction). Each group is divided furthermore in primary and secondary. In primary cases the defect is produced by the megaureter directly, while in secondary cases the dilatation is determined by another factor (such as distal ureteral obstruction). In some cases the obstruction of the terminal part of the ureter may coexist with reflux – this is a group which is not mentioned in the classification (modified after Hanna MK, 1988 [12]) (Figure 1).

It is important to consider carefully the factors outlined above, which may induce an ureteral dilatation, and to evaluate carefully the urinary tract. If concerning clinical investigations and precise diagnosis of this diseases important progresses have been made, morphological research is still able to bring important data which, together with genetic researches may contribute to the disclosure of the pathogeny of this disease and ease the genetic screening tests for fast diagnosis. Finding all the cell level anomalies might be the start point in explaining the disfunctionalities which appear in congenital megaureter. This may also establish a check point in the unification of different diagnosis concepts, with the purpose of achieving a real benefit in recover of the functional potential.

5 Material and methods

I. Congenital megaureter fragments harvested intraoperatory from children less than 1 year old.
- fixation in glutaraldehide and osmium tetroxide;
- inclusion in epoxi resins;
- coloration with 1% tionale for semifine sections at ultramicrotome;
- sections under 1 micron examined in immersion ob. ×100.

II. Fragments prepared for electronic microscope examination through the usual technique, overcoloured with uranyl acetate and lead citrate. The pieces were examined with a Philips 403 transmission microscope. Images were magnified between 1850 and 3200×, and photographs were magnified 3×.
Results

Optic microscopy and electronic microscopy studies pursued the structural elements – connective tissue, muscle and nervous tissue, their modifications and structural raports between them.

Connective tissue modifications in congenital megaureter

It is from the beginning easy to notice the abundance of connective tissue in congenital megaureter (Figures 2–5).

The typical cells – mastocytes, fibroblasts, fibrocytes, lymphocytes – are present in great amount between muscular hypoplastic fibers (Figures 6 and 16).

There is an increased amount of connective tissue cells and vascular elements that dissociate the smooth muscular fibers and a clear tendency of the connective tissue to partial hyalinisation (Figure 7).

Smooth muscular fibers in a longitudinal section are separated by partially hyalinised connective tissue (Figures 6–8).

Electronic microscopy reveals the fibrilar collagen with characteristic striations. We noticed the alternation between light areas and dark areas.

Mitochondria are vaculized, jonctional complex type interfibrillar jonctions are reduced around myofilaments, with a lot of dark extended bodies (Figure 11).

Exaggerated increase of collagen fibers is the most important element of the fibro-hyaline atmosphere, which is abundant around the muscular fibers. The excessive collagen fibers around muscular cells are probably responsible of functional discontinity, allowing to take in consideration the failure of the transmission of the impulse from a cell to another, because of inadequate jonctions. Also, the excessive collagen may be the cause [13] that impedes the slackness of the patological part of the congenital megaureter.

Muscle tissue modifications in congenital megaureter

The smooth muscle bundles are grouped, each containing a small number of fibers (Figure 9). They have oval slightly extended nuclei, placed in the center of the cell. The smooth fibers are dissociated by abundant connective tissue (Figure 10). In this connective atmosphere smooth muscular fibers have a marked hypoplastic aspect (Figures 2–4, 9 and 10).

The decreased proportion between muscle and connective tissue has a detrimental effect on the ureteral motility [14, 15]. Electronic microscopy brings essential elements in order to understand functional modifications in congenital megaureter. In muscular fibers in contraction, interfibrillar limits are folded. The dens corps are increased in number, nucleus has a cogged aspect (Figures 12 and 13). Nucleii appear crenelled with hetero- and enterocromaphine. Miofilaments present dens corps, slightly elongated. Mitochondria are grouped, with increased vacualisation. Mitochondrial cista are hard to visualize (Figures 11, 14 and 15). Excess collagen is grouped in fibrillar bundles.

The degree of cellular and muscular disorganisation brings up the idea that the energy from mitochondrial ATP is inadequate for an efficient muscular contraction.

Nervous structures modifications in congenital megaureter

The most spectacular morphological alterations in congenital megaureter are those concerning the nervous structures. Myelinated and unmynelinated nervous fibers are surrounded by perinerve (Figure 17).

Nervous fibers fragments present important modifications of the myelin sheath (Figure 18) that can go to marked alterations (Figures 19–21).

Thickening of the sheath may produce opacification of the entire axonal structure (Figure 20), alternating with multiple axonal vacuolisation (Figure 22), with an increased number of opacities (Figure 23), eventually producing the destruction of the axonal structure or
hazing of the axonal structure through myelin sheath proliferation (Figures 21–23). Electronomicroscopic examination shows myelinated and unmyelinated nervous fiber bundles dissociated by connective tissue.

Myelinated fibers present structural modifications of the myelin sheath that consist in partial thickening of the sheath which determine the dilaceration of the myelin elements, along with axonal vacuolisation (Figures 24–27).

Some unmyelinated fibers have partially preserved structures even for the axons with vacuolisation (Figures 25 and 26). Thickening of the myelin sheath may be under a vortex form, or under a nodular form (Figures 27 and 28).

Myelinated fibers have an irregular disposition encircling axons with condensed mitochondria and dilated neuroutubules. Axons may present vacuolisations (Figure 28).

Myelinated fibers are surrounded by typical fibrillar connective tissue and may present vacuoles at the perifery of the sheath. Mitochondria are partially vacuolized, neurofilaments slightly disorganized and Schwann cells cytoplasm present an increased number of polyribosomes (Figure 29).

Myelinated fibers have an increased diameter and present a marked structural disorganisation under the forms of vortexes or vacuoles (Figure 30).

Marked disorganisation of the structural elements of the sheath circumscribes clear spaces of various dimensions. In some portions the sheath is fragmented, myelin may assemble in vortexes, with unorganized thickenings which alternates with multiple vacuolisations in the sheath (Figure 31).

Segmental thickening of the myelin sheath produce dilaceration of the structure. Neurofilaments have an unorganized disposition, neuroutubules are inflated (Figure 32).

5 Discussions

The essential function of the ureter is to transport the urine from kidneys to the bladder. Ureter peristaltic is the consequence of a coherent nervous and muscular activity. Distension and ureteral dynamics represent a mechanism that participates to realize a decreased intraluminal pressure. The increase of the ureteral diameter determines a decrease of the intraluminal pressure.

In the case of a displasic ureter the elements necessary for the propagation of the nervous influx and those that support an efficient muscular contraction are heavily modified. In congenital megaureter case there is a short adynamic segment outside the bladder, close to the uretero-vesical junction that realizes a functional obstacle at this level, producing the ureteral dilatation proximal of this segment.

The primary etiology is unknown. If we consider the cause is a decrease in number or even absence of the parasympathetic nervous system in the adventitia of the bladder, we can establish a parallel with cardia achalasia where we notice the almost complete loss of the ganglionar cells form the mienteric plexus of esophagus where both the constricted segment and the dilated segment are affected by a neuromuscular disfunction.

The constricted segment of the ureter is localized by Allen at the intersection between the ureter and the umbilical vessels. He considers that in uterus there is a vascular effect which interferes with the development of the muscular tissue which determines the lack of differentiation of mesenchimal cells of the ureteral bud in muscular ibers with an adequate structure for an efficient contraction.

A hypoplasic muscular tissue compromises the peristaltic. Smooth muscular fibers grouped in bundles dilacerated by connective tissue present vascular aspects in cytoplasm, nuclei are hypochromatic, coggad, with visible nucleoli. In equal measure the hypoplasic muscular fibers have transversal and longitudinal direction.

The sections that intersect the smooth muscular tissue surprise a high degree of disorganisation, with the decrease of the fibers/connective tissue raport. The congenital ureteral anomalies can be quantitative, qualitative and combined.

The ureters with poor development of the muscular tissue, with small deforms muscular fibers and reduced intercellular jonctions, with low muscular tissue/connective tissue raport have a decreased or even absent ureteral motility. The excessive connective tissue between and around the muscular fibers presents hyaline areas that dissociate the smooth muscular fibers. In the connective tissue atmosphere there are cellular elements such as fibroblasts, mastocytes and plasmocytes.

In our opinion the relation between the smooth muscle tissue and connective tissue represents the prove of the structural alterations that determines the hypodinamic or even the lack of ureteral dynamics and also the cause of ureter dilatation that produces the impossibility of the ureteral contraction in order to maintain the tonus.

If the ureter does not present structural elements necessary for an efficient muscular contraction, also the nervous influx transmission does not have the necessary nervous structures to transmit a contaction influx, as the nervous fibers bundles are also dissociated by connective tissue.

The myelinated fibers present structural modifications of the sheath, partial thickening of the sheath with the dilaceration of the myelinic elements, up to axonal vacuolizations. The thickened myelin sheaths have variable aspects – vortexes, nodular forms, segmentary thickening or even fragmentation of the sheath. In the axon the mitochondria are condensed or partially vacuolized, neuroutubules are dilated and neurofilaments are disorganized. Schwann cell cytoplasm contain an increased number of polyribosomes.

The main structural modification we have noticed in congenital megaureter participate in a decisive mode in compromising the peristaltic, but we still have to find if the muscular tissue, the connective tissue and nervous structures alterations appear in the same time or they are succesive.
Figure 2 – Smooth muscle bundles dissociated by abundant connective tissue

Figure 3 – Marked dissociation of smooth muscle bundles by connective tissue

Figure 4 – Abundant vascular and connective tissue dissociating hypoplastic muscle fibers

Figure 5 – Hypoplastic muscular fibers dissociated by connective tissue

Figure 6 – Hyaline areas in connective tissue. Connective tissue typical elements: fibroblasts, fibrocytes, mastocytes and capillaries

Figure 7 – Muscular fibers and partially hyalinated connective tissue with typical connective cells: mastocytes, fibroblasts, fibrocytes

Figure 8 – Longitudinally and transversally sectioned smooth muscle bundles fragmented by partially hyalinated connective tissue

Figure 9 – Smooth muscle hypoplastic fibers grouped in small bundles, dissociated by abundant connective tissue
Figure 10 – Smooth muscular fibers with hypoplasic aspect in abundant connective atmosphere

Figure 11 – Smooth muscular cell in transversal section, with smooth endoplasmic reticule in tubular form, miofilaments and dense corps, rare vacuolized mitochondria, surrounded by connective tissue with macrophage and fibroblast. Abundant connective tissue that dilacerates cellular structures.

Figure 12 – Assemble of smooth muscular fibers with miofilaments and dense corps, folded plasmatic membranes, coggd nucleus and visible nucleolus. Sarcoplasm with vacuolized organites.

Figure 13 – Fragments of smooth muscular cells with coggd nucleus, sarcoplasmic organites (vaculized mitochondria, dilated reticule, dense corps increased in number).

Figure 14 – Assemble of smooth muscular fibers with vacuolized mitochondria and dense corps, surrounded by connective atmosphere.

Figure 15 – Abundant fibrillar collagen surrounding a smooth muscle fiber with vacuolized mitochondria and dense bodies in miofilaments.
Figure 16 – Abundant connective tissue with typical cellularity. Mastocytes, fibroblasts, fibrocytes, lymphocytes

Figure 17 – Bundle of myelinated fibers, surrounded by perineur (assemble image)

Figure 18 – Bundle of nervous fibers with marked alterations of the myelin sheaths

Figure 19 – Nervous fiber fragment with important modifications of the myelin sheath

Figure 20 – Important modifications of the myelin sheath producing opacification of the entire axonal structure

Figure 21 – Marked alteration of the myelin sheath with important axonal degeneration

Figure 22 – Marked alteration of the myelin sheath with important axonal degeneration. Multiple axonal

Figure 23 – Complete opacification of nervous fiber including myelin sheath and axon
Figure 24 – Myelinated and unmyelinated nervous fibers bundles dissociated by connective tissue. Myelinated fibers have structural alterations of the myelin sheath – partial thickening of the sheath with dissociation of the myelin. *Axonal vacuolisation*

Figure 25 – Unmyelinated fibers with partially preserved structures. Axonal vacuolization and Schwann cell vacuolization in myelinated fiber

Figure 26 – Thickening and irregularities of the myelin sheath. Axonal vacuolization in myelinated and unmyelinated fibers

Figure 27 – Thickening and irregularities of the myelin sheath. Opacification of the axoplasm

Figure 28 – Myelinated fiber with irregular disposition encircling an axon with multiple axoplasm vacuolisations. Axonal vacuolisation in unmyelinated fibers

Figure 29 – Nervous fiber with modifications at the Schwann cell level – cytoplasm with an increased number of polyribosomes and vacuoles – and axonal level – partially vacuolized mitochondria, neurotubules and endoplasmic reticule are slightly inflated; disorganized neurofilaments
Figure 30 – Nervous fiber with normal myelin sheath (left). Myelinated fiber with increased diameter, with marked lamellar structure disorganization – vortexes, vacuoles (right)

Figure 31 – Thickening of the myelin sheath with important disorganization of myelin sheath elements, circumscribing clear spaces and vortexes

Figure 32 – Thickening of the myelin sheath, nodular aspect. At axonal level dilatations of the neurotubules and smooth endoplasmic reticulum between neurofilaments with irregular disposition

Conclusions

Congenital megaureter represents from an anatomopatological and clinical point of view a disgenesis with hypoplasia of the smooth muscular tissue dissociated by abundant connective tissue and degeneration of the nervous fibres. Congenital muscular anomalies may be qualitative or quantitative. Primary hypoplasia of the muscle fibers, their displacement and dissociation by connective tissue with hyaline zones determines an ineffective contraction of the ureter.

Nervous fibers presents important myelin sheath modifications. Myelin sheath may present densifications producing the opacification of the entire axonal structure or multiple vacuolizations and dissociations. Vacuolizations alternating with densifications in the myelin sheath structure and the alteration of the axonal structures are suggesting lack of the nervous influx conductibility at this levels.

In congenital megaureter connective tissue is excessive, partly hyalinated, between the muscular fibers bundles and surrounding them.

Connective tissue/muscular tissue ratio is raised in connective tissue favor. Structural modifications of the smooth muscle fibers, structural disorganisation of the nervous elements and the abundant connective tissue of the congenital megaureter participate in compromising the normal peristaltic of the organ.

All these structural modifications may be the result of genetic modifications, but we only know little about the molecular pathogeny of these anomalies. Until now there have been identified genetic mutations that determine the apparition of Cakut (Congenital Anomalies of Kidney and Urinary Tract).

Muscular anomalies cannot be corrected. Propulsion deficiencies may be lessened by surgical reducing of the ureteral lumen diameter. Ureters with severely affected muscular fibers are from a morphological point of view organs in terminal phase and are not improved by surgical reconstruction.

Future electronic microscopy and pharmacological studies of congenital megaureter will be able to improve the understanding of the anomalies and disfunctionalities at cellular level in order to improve the benefit of functional screening and potential recovery.

Nervous, connective and smooth muscle tissue lesions suggest in case of congenital megaureter a process of incomplete development of the organ, with the apparition of pathological structures that impede the functional maturation. It is a process of disgenesis associated with hypoplasia.

References


Corresponding author
Marius Vlad, Associate Professor, MD, PhD, Department of Anatomy, Compartment of Clinical Anatomy and Surgical Techniques, „Carol Davila”University of Medicine and Pharmacy, 8 Eroilor Sanitari Avenue, 050 474 Bucharest, Romania; Phone +40745–932 333, E-mail: vlad_marius@hotmail.com

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