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REVIEW ARTICLE

Fetal development of the vesico-ureteric junction, and immunohistochemistry of the ends of refluxing ureters

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Dedicated to Prof. Georg Bartsch, mentor and promoter in Pediatric Urology

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Immunohistochemistry;
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Abstract There is still misunderstanding about the normal fetal development of the vesico-ureteric junction (VUJ), the region that is most important for preventing VUR. There is little information on the causes of primary VUR and on the mechanisms of maturation of refluxing ureteric endings. Some studies show that the ratio of the intravesical ureteric length to diameter is obviously lower than had been assumed. It is doubtful that the length and course of the intravesical ureter is the sole factor in preventing reflux, as previously reported.

The intravesical part of refluxing ureters shows dysplasia, atrophy and architectural derangement of smooth muscle fibres. A pathologically increased matrix remodelling combined with deprivation of the intramural nerve supply has been confirmed. Consequently, symmetrical contraction of the distal ureteric smooth muscle coat, creating the active valve mechanism to prevent reflux, is impossible.

We reviewed publications using Medline, with the keywords 'human fetal development', 'embryology', 'ureterovesical junction', relevant 'growth data', 'vesico-ureteric reflux', 'children', 'immunohistochemistry', 'extracellular matrix', and 'nerve supply', respectively. Priority was given to articles that correlated

Abbreviations: VUJ, vesico-ureteric junction; MMP, matrix metalloproteinase; ECM, extracellular matrix.

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specific embryological findings and basic research on possible mechanisms to the genesis and maturation of the VUJ.

Background

The ureter fuses with the urogenital sinus by day 37 of fetal development [1]. Originally the ureteric bud evaginates from the metanephric (Wolffian) duct. There are few published data about the subsequent caudal growth, although this region is crucial for the development of an antireflux mechanism. The VUJ represents an important area between the low pressure of the upper urinary tract and the variable pressure of the lower urinary tract. The mechanism of ureteric motility, and detailed physiology of the normal valve mechanism of the VUJ, has been examined in some detail. Clinical and anatomical publications do not provide a consensus on the topography and development of the intravesical ureter in fetuses.

Mackie and Stephens [2] postulated that in congenital anomalies of the kidney and urinary tract the ureteric bud arises in an abnormal position on the metanephric duct. After day 37 caudal growth of the ureter remains indefinite, mainly being the distension and intravesical submucosal enlargement which is supposed to be responsible for the antireflux mechanism. Migration of the ureteric orifice in the bladder is in a cranial and lateral direction, with the final position of the orifice at risk of reflux.

Two antireflux mechanisms, one passive and one active, have been discussed. Important factors in passive valve function are the diagonal course and length of the submucosal portion of the ureter; this intravesical course of the ureter is considered to be important in preventing reflux [3]. The active valve function of the human ureter is thought to consist of a 'VU sphincter' which contracts in response to vesical contraction and relaxes after contraction of the external urethral sphincter [4]. As a histologically definable sphincter could not be identified, a 'physiological sphincter', like that described in the rectosigmoid junction, is postulated [5].

While King and Stephens [6] considered the VU valve to be activated by the intrinsic muscle of the ureter, Tanagho et al. [7] attributed this function to contraction of the muscle of the superficial trigone of the bladder. This theory of trigonal attachment of the ureteric function was weakened by Hannan and Stephens [8], who excised the canine trigone except for the mucosal layer, and found reflux in only one specimen of the series.

Regardless of which of these models of valve function is correct, the effectiveness of both depends on functioning ureteric muscle. Thus, it seems clear that the precondition of a patent VUJ is the timely and complete development of the smooth muscle coat of the intravesical ureter. Little attention has been paid to a possible congenital muscular inadequacy of the very distal refluxing ureter, with meticulous attention to general morphology, smooth muscle architecture, chronic inflammatory markers and the distribution of collagen composition, respectively. Also, only limited data on changes of the extracellular matrix (ECM), the remodelling process and altered innervation of the intravesical part of the ureter are available. The ECM is a biologically active and dynamic composition of structural, adhesive and counter-adhesive fibrous proteins embedded in a hydrated ground substance of glycosaminoglycans and proteoglycans. Smooth muscle cells participate in ECM transformation by the local production of various proteinases and their inhibitors. Possibly these ureteric smooth muscle cells participate in repair and re-synthesis of structural matrix proteins, influencing the proteolytic activity of other cell types. They might therefore have an effect on the progression of potential maturation processes in refluxing ureters [9].

Based on morphological data confirming muscle deficiency in the VUJ [10] changes of innervation in association with matrix remodelling have been reported. Impairment of smooth muscle function and changes of the ECM microenvironment can compromise the nerve supply in this area.

The major purpose of these studies was to describe the chronology of development of these tissues, especially to investigate the growth curves of the VUJ and of the mesenchymal and muscular distal and intravesical wall, the meticulous description of the morphology of refluxing ureteric endings, and finally the analysis of ECM remodelling processes, combined with the nerve supply in these specimens, to gain an insight into possible maturation processes.

Fetal developmental studies

Fetal studies based on plastinated sections of whole pelves allow the study of the anatomy of

all pelvic structures in a condition closely resembling the in vivo status. Architectural and cellular integrity are preserved, cytomorphological alterations with artificial tissue shrinkage are avoided by using plastination and a special fixation protocol [11]. Because plastinated sections are transparent, it is possible to determine the exact width and borders of the mesenchymal and muscle structure of the ureter wall, allowing precise sequential measurements.

Baskin et al. [12] reported that mesenchymal–epithelial interactions are necessary for the development of bladder smooth muscle. These changes in the configuration of the ureter indicate a progressive transformation of the mesenchyme around the epithelial tube to smooth muscle. Other observations [11] showed a continuous linear increase of the mesenchymal ureter wall to smooth muscle in human fetal ureters. Statistical analysis showed an almost linear growth profile. The distal and intravesical ureteric thickness increased at 8.98 μm and 11.25 μm per week, respectively, and the intravesical tunnel grows at 97.4 μm per week [11] (Fig. 1).

The timing of smooth muscle differentiation in the distal ureter is unknown. During a first or mesenchymal period (8–12 weeks), the wall of the pre- and intra-vesical metanephric duct consists of undifferentiated mesenchyme. Subsequently a second period (13–20 weeks) can be differentiated, in which condensed mesenchyme predominates. Paquin [13] provided normal values for the length of the submucosal segment of the ureter (orifice to hiatus in the bladder) and in infants aged 1–3 years he found a 7-mm long intravesical ureter. Hutch estimated intravesical ureteric lengths of 5 mm in neonates [14]. His method of using blunt-nosed cylindrical probes or ureteric catheters to measure the VUJ was not as good as previous methods. The mean (range) length of the intravesical ureter over gestational weeks 20–30, as described by Cussen [15], was 3 (2–5) mm, with a 1–3 mm submucosal and a 1–2 mm intramuscular segment. At weeks 30–40 of gestation the mean intravesical ureteric length is 4 mm. A shorter intravesical human fetal ureter with a mean (SD) length of 271.7 (191.01) μm at 9–12 weeks and 3017.2 (388.9) μm at birth was ascertained by another group [11] (Fig. 1). The tunnel length relative to its diameter is thought to be important in preventing reflux by closing the VUJ valvular mechanism in children. Children aged 1–3 years had an intravesical ureteric length of 7000 μm with an intravesical ureteric diameter of 1400 μm. The mean ratio of tunnel length to ureteric diameter at the VUJ was 5:1 [13]. In children with reflux a pathological length to diameter ratio of 1.4:1 was reported [16]. The mean values of the intravesical length in another series of nonrefluxing ureters were somewhat lower, at 3017.2 (388.9) μm, implying an intravesical ureteric length to intravesical ureteric diameter of 2.23:1 [11] (Fig. 2). In contrast to the newborn, in 11- and 20-week-old fetuses the ratio of the intravesical ureteric length to ureteric diameter decreased to 0.69:1 and 1.23:1, respectively. This might be one reason for the higher prevalence of VUR in newborns and fetuses. Even so, the standard ratio of 5:1 for reimplantation surgery seems sufficient, albeit that this relationship does not mimic nature in the theory of the passive antireflux mechanism. Possibly a lower ratio might be sufficient to obviate reflux [17]. Other factors of the antireflux mechanism, such as the active valve mechanism, the intravesical pressure or the connection with the trigone and posterior urethra, must be considered in re-evaluating the function of the VUJ. This might explain why neonatal reflux is a different

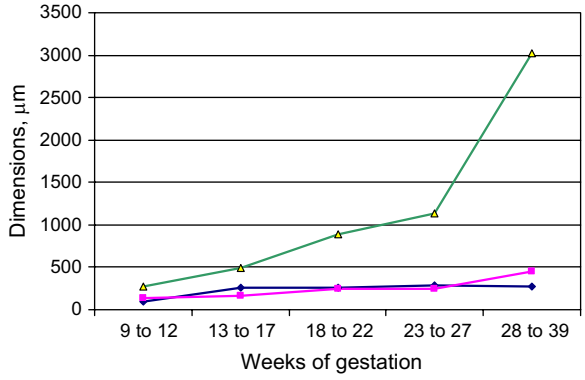

Figure 1 The distal (rhombus), intravesical ureteric wall thickness (square) and length of the intravesical ureter (triangle) in different age groups.

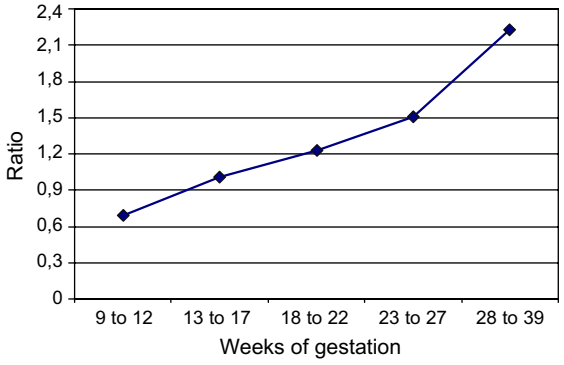

Figure 2 The ratio of tunnel length to ureteric diameter with gestational age.

entity with a much higher tendency to disappear spontaneously than the reflux found in older children.

Morphological changes in refluxing ureteric endings

Spontaneous resolution of reflux in children is explained by the growth of the bladder, during which the submucosal tunnel elongates [18]. On the other hand, Cussen [19] showed that the intravesical ureter grows simultaneously in relation to the surrounding tissue components. Correlating length, muscle mass and muscle population of the intravesical ureter with height, weight, body surface area and age, he found definite growth in all of these elements with time. The morphological and functional integrity of the uretero-trigonal unit providing the active muscular control of the ostial valve mechanism seems as important as the so-called 'passive' antireflux mechanism, most notably as we showed that the ratio of the intravesical ureteric length to the ureteric diameter is obviously lower than assumed so far [11]. The distal ends of refluxing ureters clearly have less expression of smooth muscle α-actin, myosin and desmin [10]. This very distal part had a high degree of muscle atrophy and degeneration, as well as a disordered, disrupted and scattered arrangement of fibres associated with changes in ECM collagen composition (Figs. 3—6). The proportion of muscle to collagen decreased from the normal 1:0.3 to 1:3. Also, in lower reflux grades, there were disintegrated or no smooth muscle filaments either at the periphery or at the interior part of the muscular wall. The increase in type I collagen and less type III collagen could indicate greater collagen synthesis by interstitial fibroblasts, typical for later the stages of fibrotic lesions [20].

Interestingly, there was no significant correlation between the degree of ureteric muscle wall damage and the clinical reflux grade, whereas some degree of damage can be found in all cases of reflux [10]. Further research is needed to determine whether the degree of muscle wall impairment can alter clinical outcome with regard to the so-called spontaneous maturation of the VUJ in childhood.

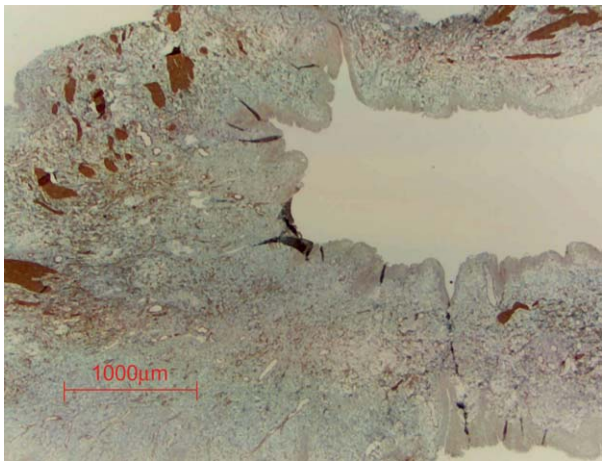

Figure 4 Massive derangement of a refluxive intravesical ureter showing no smooth muscular wall (α-actin smooth muscle staining).

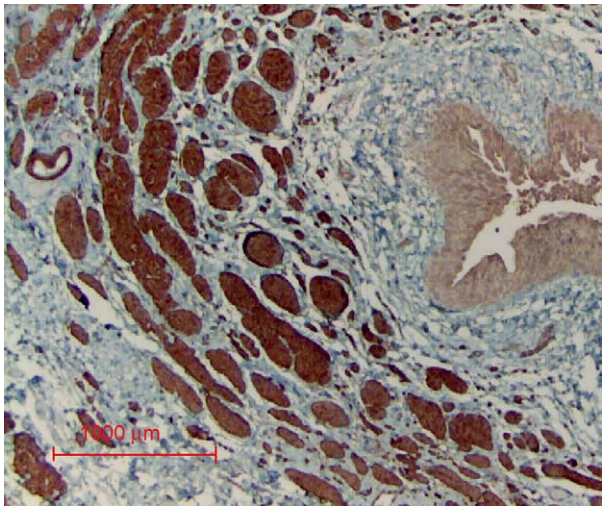

Figure 3 α-Actin staining of a normal ureteric orifice with predominantly inner longitudinal and slight outer circular layers of smooth muscle.

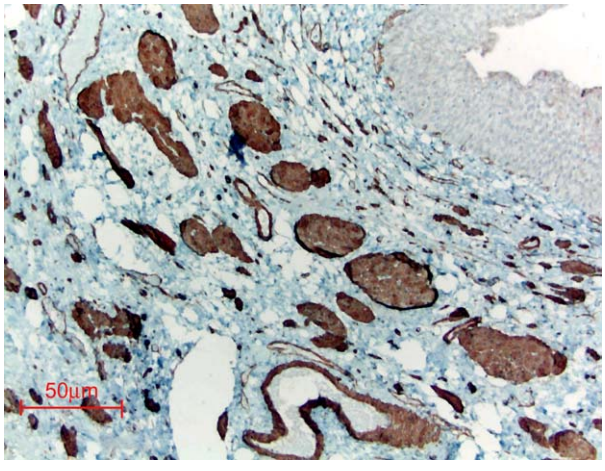

Figure 5 A smooth muscle fascicle showing replacement of deteriorated smooth muscle by connective tissue in perimysial and endomysial regions (myosin smooth muscle staining).

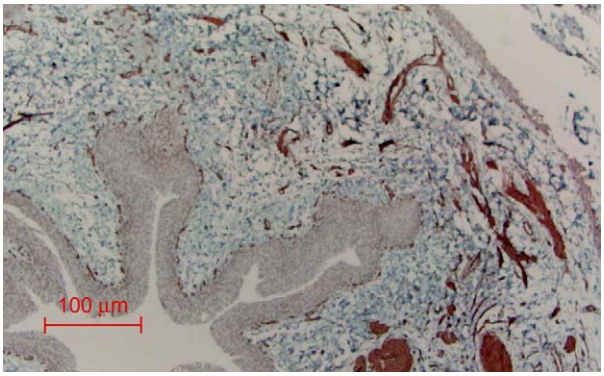

Figure 6 Immunohistochemical staining for desmin, showing massive derangement of the smooth muscle wall, with disintegration and dissolving muscle fascicles.

Studies also showed that matrix metalloproteinase-1 (MMP-1) production was almost four times (161 vs 42 units) and the CD68+ macrophages twice as common than in controls (30 vs 18), in the very distal part of refluxing ureteric specimens [21], both in the ECM and in the fascicles of the longitudinal muscle coat. Cells undergoing programmed cell death are cleared rapidly in vivo by phagocytes without inducing inflammation [22]. Because there are no inflammatory cells, which are present in necrosis, apoptotic cell removal is an admissible hypothesis. The increased activity of CD68+ cells accounts for the efficient deletion of damaged cells, often considered a counterpoint to apoptosis. As the patients in that study had no signs of UTI at the time of surgery, an inherent anatomical or developmental difference from children without reflux must be considered. It is evident that in cases of muscle injury or muscle dysplasia, CD68+ cells infiltrate the damaged tissue and scavenge cellular remnants, as well as replace muscle bundles with connective tissue [23]. The ability of these macrophages to detect small differences in the specific combination, concentration and distribution of matrix components suggests that disturbance of the matrix homeostasis may lead to remodelling of the ureteric ending. In this ECM environment there is adhesion of macrophages, and modification or denaturation of the major ECM component (type I and III collagen) [24]. Once in the underlying tissues, monocytes differentiate into macrophages, which are essential for proper immune function, and ECM transmutation and production of collagen [25]. Above all, in collagen-rich regions, there is an increased secretion of MMP-1, cleaving collagen types I and III. This simultaneous synthesis and secretion of matrix proteins and a degrading enzyme by the same cell indicates a switch from a collagen-producing to a collagen-degrading phenotype [26]. MMPs have several physiologically important functions in wound-healing processes and angiogenesis. The increased synthesis of MMP-1 is stimulated by various cytokines, e.g. TGF-β, released from macrophages [27]. Increased ECM turnover has an influence on the neuronal network within the ureter wall. Some MMPs are neurotoxic, by degrading ECM proteins like collagen type I. They are normally able to protect cultured neurones from cytotoxic cell death. Cytotoxicity by MMP-1 on the ureteric neurones at the VUJ is probable; discrimination from a congenital absence of neuronal nerves is not feasible at present [28]. There was a significant decrease in S-100-positive neuronal cells (from 98 to 30 units) [21] as a result of potential neuronal harm, suggesting that defective innervation may have an additional impact on the pathogenesis of the impaired active antireflux mechanism. Based on this significant reduction in S-100-positive myelinated nerves, an intact innervation may be one of the prerequisites for normal morphogenesis of the intravesical smooth muscle wrap [21]. There was no correlation between the degree of MMP-1 expression, accumulation of CD68+ macrophages or degradation of the neuronal supply and the clinical reflux grade. However, at present it is unclear whether a congenital absence of an intact innervated muscle wall, or an additional deterioration in the framework of tissue remodelling as a result of restoring damaged tissue, combined with increased MMP activity, is the main cause of the significant reduction within the neuronal network at the VUJ. In the maturation process of refluxing ureters it is postulated that somatic growth of the child causes a morphological development of the pathological VUJ, resulting in a restoration of the antireflux valve mechanism [29]. As the ratio of the intravesical ureteric length to ureteric diameter is obviously lower than assumed previously [11], the morphological and functional integrity of the intravesical longitudinal muscle coat, which provides the active muscular control of the ostial valve mechanism, seems to be a crucial factor to prevent reflux [10]. The mechanisms responsible for this rearrangement of the VUJ have never been elucidated. It can be assumed that the increased concentrations of MMP-1-positive cells and CD68+ macrophages in the ECM of refluxing ureteric endings represent a causal correlation of these MMPs to tissue degradation and defective healing. Replacing the smooth muscle fibres by collagen types I and III on the basis of an increased ECM turnover can lead to scarring and fibrosis [30]. The structural changes observed in

refluxing ureteric endings indicate a tissue response that culminates in cell and matrix turnover, as well as in matrix remodelling and maturation, which finally may cause tissue shrinkage analogous to wound contraction. Possibly this ureteric wall shrinkage results in an adequate ureteric diameter to ureteric length ratio, enabling the passive antireflux mechanism to work.

Conclusion

There is little information on the possible causes of primary VUR and on the mechanisms of the so-called 'maturation process' of refluxing ureteric endings. Using continuous gestational age series, there is a clear significant positive linear relationship between gestational age, distal and intravesical ureteric wall thickness of the mesenchyme, and smooth muscle growth and the length of the intravesical ureter in 9–39-week-old fetuses and newborns. The intravesical tunnel does not develop before the late fetal period and the ratio of the intravesical ureteric length to ureteric diameter in newborns is obviously lower than assumed so far. It seems that in nature the passive antireflux mechanism may not be as important as previously thought.

Primary VUR is the consequence of a congenital abnormality of the VUJ. A deficiency of the longitudinal muscle coat leading to dysfunction of the ostial valve mechanism may play some role in the active antireflux mechanism. The muscular wall of the very distal ureter in children with reflux is diminished. Disorganized muscle fibre architecture leads to a dysfunction of the active valve mechanism. The atrophic and dysplastic smooth muscle cells packed in a thick basal lamina are increasingly separated by expanded ECM. These morphological changes in the ureteric wall frequently result in deformation of the smooth muscle wall structure. Refluxing ureters are characterized by reduced and degraded muscle fascicles of the distal ureteric wall, leading to an insufficient active valve mechanism, and hence sufficient contraction of the muscular conduit to close the ostium and prevent VUR is unlikely.

Furthermore, MMP-1 expression and CD68+ macrophages are significantly greater in refluxing ureters than in healthy controls, whereas there are fewer S-100-positive nerve fibres, showing some deprivation of the intramural nerve supply. The enhanced activity of CD68+ cells, together with an increased synthesis of MMP-1, suggests a pathologically increased remodelling of the ECM. The increased collagenolytic activity results in the appearance of collagen cleavages, which may be responsible for triggering mesenchymal proliferation. Possibly, the spontaneous maturation of the primary refluxing ureteric orifice appears to be associated with the modification of the ECM. Further research must focus on smooth muscle cell–matrix interactions, matrix degradation and turnover mediated by cell proteases such as the MMP family, to clarify the mechanism of reflux maturation.

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