



ORIGINAL ARTICLE

# Diagnostic value of combined static-excretory MR Urography in children with hydronephrosis



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ABSTRACT

The aim of this study was to determine the feasibility, accuracy and diagnostic potential of combined static-excretory MR Urography in children with sonographically detected hydronephrosis. We prospectively evaluated 28 children (11 girls and 17 boys), mean age 8.3 years (range 2 months–16 years). Static-excretory MR Urography was performed in all cases. The results of MR Urography were compared with the results of other imaging modalities, cystoscopy and surgery. In 28 children, 61 renal units were evaluated by MR Urography (the renal unit is the kidney and its draining ureter). The final diagnoses included: normal renal units ( $n = 23$ ); ureteropelvic junction obstruction ( $n = 14$ ); megaureter ( $n = 8$ ); midureteric stricture ( $n = 1$ ); complicated duplicated systems ( $n = 5$ ), post ESWL non-obstructive dilation ( $n = 2$ ), extrarenal pelvis ( $n = 4$ ), dysplastic kidney ( $n = 4$ ). Complex pathology and more than one disease entity in were found in 7 children. The MRI diagnosis correlated with the final diagnosis in 57 units, with diagnostic accuracy 93.4%. In conclusions static and excretory MRU give both morphological and functional information in a single examination without exposure to ionizing radiation and iodinated contrast agent. It is a valuable imaging technique for children with upper urinary tract dilatation; especially in cases of complex congenital pathologies and severely hydronephrotic kidney.

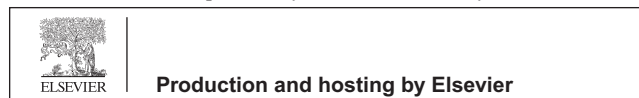
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**Introduction**

Imaging of the urinary tract (UT) is an essential part of pediatric radiology. Early diagnosis and adequate treatment and management of congenital urinary tract malformations have improved prognosis and long-term outcome in some of these entities [1].

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At present, this work-up is performed by ultrasound (US), intravenous urography (IVU), voiding cysto-urethrography (VCUG) and scintigraphy [2]. However these well established methods suffer from some restrictions: the US does not allow assessment of the renal function, and it does not visualize normal caliber ureter, in addition it is an operator dependent [3]. IVU should not be performed in neonates because of the risk of contrast nephropathy; VCUG can only help in patients with vesico-ureteral reflux (VUR); scintigraphy allows for functional evaluation, but offers poor anatomical resolution [4]. In addition most of these modalities also impose radiation burden on the children and, diagnosis is usually made in synopsis of the findings of all these investigations [4].

Because of the limitations of each method, the use of MR imaging has gained progressive acceptance for the evaluation of the UT in children [5]. The potential advantages of MR Urography (MRU) for evaluating renal tract abnormalities in children are that it has multiplanar capabilities, offers excellent anatomic resolution and soft tissue contrast, and does not use ionizing radiation [6].

The most common MRU techniques used to display the urinary tract are: (a) static-fluid MRU and (b) excretory contrast enhanced MRU [7]. The two are complementary sequences, both needed for UT imaging can be achieved with MR Urography. The combined static-excretory MRU provides a complete morphologic and functional assessment of the urinary tract in a one stop-shop manner without the use of ionizing radiation [8].

In present study, we prospectively assessed the feasibility, accuracy and diagnostic potential of static-excretory MRU in children with hydronephrosis.

## Patients and methods

### Patients

During 2 years duration, we prospectively evaluated 28 children (11 girls and 17 boys), they range in age from 2 months to 16 years (mean age 8.3 years). *Informed consents were taken from the children's parents. The study was approved by the ethical committee of Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University.*

These children with dilated UT on US imaging underwent MRU additionally to their standard imaging protocol. Three patients were diagnosed by antenatal US, the remaining patients underwent US because of UT infection ( $n = 10$ ), abdominal mass ( $n = 2$ ), flank pain ( $n = 9$ ), voiding dysfunction ( $n = 4$ ).

### Methods

All children were evaluated using US, MRU and Diuretic Renal Scintigraphy (DRS). VCUG was performed in 13 patients, whereas IVU was performed in 7 patients. All studies were performed within 4–6 weeks of the MRI.

MRU examinations were performed on a two different 1.5-T MR scanner (Gyrosan Entera, Philips medical systems and (Signa Horizon, General Electric Medical Systems). Patients were placed in supine position. Head and body coils were used depending on the patients' size.

Patient preparation included oral hydration was required prior to the examination. Ten out of the 28 children were sedated for MRI. The oldest child required sedation was 6 years. Sedation was performed with oral chloralhydrate (50–80 mg/kg) in children up to 24 months; whereas older children underwent IV sedation. Sedated children were monitored throughout the procedure (heart rate; breathing and oxygen saturation, pulse-oximetry).

### MRU techniques

#### Static MRU

Initial axial T2-FSE sequence was performed through the bladder base to identify abnormalities of the bladder base

including ectopic ureteric insertion. This was followed by heavy T2 weighted 3D FSE sequence performed in a slightly angulated coronal orientation over the kidneys, ureters, and bladder. Imaging parameters were: TR/TE 8000/260 ms, flip angle = 90°, turbo factor = 128, NSA = 2 respiratory triggering was used for motion artifact suppression and the effective imaging time was from 2 to 3 min.

#### Excretory MRU

1 mg/kg of Furosemide was given 1 min before contrast. A bolus of 0.1 mmol/kg of gadopentetate dimeglumine-DTPA (Magnevist; Schering, Berlin, Germany), was administered followed by 10 ml saline injection.

Excretory MRU was performed using coronal T1-weighted fat suppressed gradient-echo (GRE) sequence (TR/TE = 7/2.8 ms, flip angle = 30°, NSA = 2). After the administration of contrast medium (CM), early images were performed for assessment of the renal parenchyma. MRU sequences were repeated 5, 10 and 15 min to image the UT during the excretory phase. Delayed images were performed when necessary. Additional Gd-enhanced MRA images were obtained in 3 children with atypical presentation of UPJO, who presented with recurrent flank pain at older age to exclude the presence of crossing renal vessel.

Images were obtained during continuous breathing in infant and young children, whereas in older cooperative children images was obtained during breath hold. The total examination duration averaged approximately from 30 min in non-obstructed units prolonged to 45 min in obstructed units.

#### Image analysis

Evaluation of MRU results was performed. The images were assessed using both anatomic and functional criteria. Anatomic assessment included renal size, the level and degree of dilatation of the collecting system and ureter according to the Society for Fetal Urology (SFU) grading system [9]. The degree of dilatation was compared between MRU, US and IVU. For functional assessment of the renal units subjective impression based on IVU criteria was employed such as the quality and timing of enhancement of the renal parenchyma, time and duration of the renal collecting system enhancement and assessment of pelvico-ureteral drainage.

The final diagnosis used as reference was based on clinical findings, clinical course, and constellation of findings of different imaging modalities, cystoscopy and surgery.

## Results

The diagnoses were grouped according to renal units (the renal unit is the kidney and its draining ureter). In 28 children, 61 units were evaluated by MRU (1 patient had bilateral and 3 patients had unilateral duplicated system). The final diagnoses of the units are shown in Table 1. Complex pathology and more than one disease entity were found in 7 children.

Thirty-four out of 61 units were dilated. For anatomical assessment the degree of hydronephrosis and level of obstruction were assessed. There was complete agreement in the grading of hydronephrosis between MRU, US and IVU. Static MRU accurately identified the level of hydronephrosis in all cases. It can visualize the ureter down to its lower end. In 3

**Table 1** Final diagnosis in 28 patients (61 units) evaluated by MRU.

Diagnosis	No. of units
UPJO	14
Midureteric stricture	1
Megaureter	8
Complicated duplicated system	5
Post ESWL non-obstructive dilatation	2
Extrarenal pelvis	4
Dysplastic kidney	4
Normal	23
Total	61

cases US could not determine the level of obstruction, due to difficult visualization of the ureter. Three patients believed to have UPJO on renal scan, however on MRU 2 cases were diagnosed as UVJ obstruction and one case was diagnosed as midureteric stricture. In addition renal scan could not identify the level of obstruction in 2 cases with ectopic pelvic kidneys. The diagnostic accuracy of MRU, US and renal scan in identification of the level of obstruction was 100%, 91.2% and 85.3% respectively.

For functional assessment the following parameters were assessed: pattern of enhancement of the renal parenchyma, and urinary excretion.

The pattern of enhancement of the renal parenchyma was assessed as either good symmetrical or poor. Good parenchymal enhancement was noted in 54 units (88.52%), and poor enhancement was found in 6 units (9.84%). No identifiable renal parenchyma was detected in 1 unit (1.64%).

Urinary excretion was assessed in the delayed images. Delayed excretion of the contrast and opacification of the collecting systems were noted in 21 units (34.43%). Normal contrast excretion was noted on 37 units (60.65%). In addition 3 non-functioning units (4.92%) with non-opacification of the collecting systems were identified.

The diagnostic accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of combined static-excretory MRU in the differentiation between obstructed and non-obstructed dilated units was 88.2%, 100%, 63.6%, 85.2% and 100% repetitively.

The patients were assessed and placed in 5 groups according to the cause of dilation of the collecting system. These groups were as follows; UPJO, midureteric stricture, megaureter, complicated duplicated system, and post ESWL non-obstructive dilatation.

### UPJO

UPJO was the final diagnosis in 13 children (14 units) as one patient had bilateral disease. MRU corrected the diagnosis of 2 patients (4 units) with extrarenal pelvis, who misdiagnosed at US and IVU as UPJO. The dilated renal pelvis and level of obstruction were equally seen by US, and MRU in all cases and IVU in 3 cases. An additional sign of UPJO was detected in 2 cases, in whom swirling of CM in the dilated renal pelvis was identified (Fig. 1).

MRA was done in 3 patients; to exclude the presence of crossing renal vessel. No vessels was identified, however situs inverses totals was detected in one case, with MRA accurately

identified the origin of the renal artery from the right sided aorta. Pyeloplasty was performed in 11 patients.

### Midureteric stricture

One patient was diagnosed as midureteric stricture. US, IVU and renal scan could not determine the level of obstruction. Both static and excretory images accurately identified the level of obstruction and proximal dilation (Fig. 2).

### Megaureter

Eight units in 5 patients were diagnosed as megaureter. The final diagnosis was primary megaureter (1 unit in 1 patient), obstructing megaureter due to VUJ obstruction (1 unit in 1 patient) (Fig. 3), obstructing megaureter due to neurogenic bladder (2 units in 1 patient) and refluxing megaureter (4 units in 2 patients). VCUG was done in all cases and cystoscopy was done in 3 cases.

In 2 patients of refluxing megaureter, excretory MRU and DRS diagnosed correctly diagnosed one case as non-obstructive dilatation (based on good contrast excretion on MRU and tracer wash out on renal scan). Whereas both techniques misdiagnosed the other case as obstruction based on delayed contrast excretion and tracer wash out.

In 2 cases there were marked dilation of the collecting system, tortuous ureter, marked delay in the opacification of the calyces and ureters, with superior visualization of the dilated collecting system in static than excretory MRU.

MRU was considered adding in cases of obstructive megaureter (accurately identified narrowed segment at the distal end of the ureter, and insufficient in 2 cases of refluxing megaureter as VCUG only diagnosed reflux. Ureteric re-implantation was done in 3 cases and dextranomer/hyaluronic acid copolymer injection was done in 1 case.

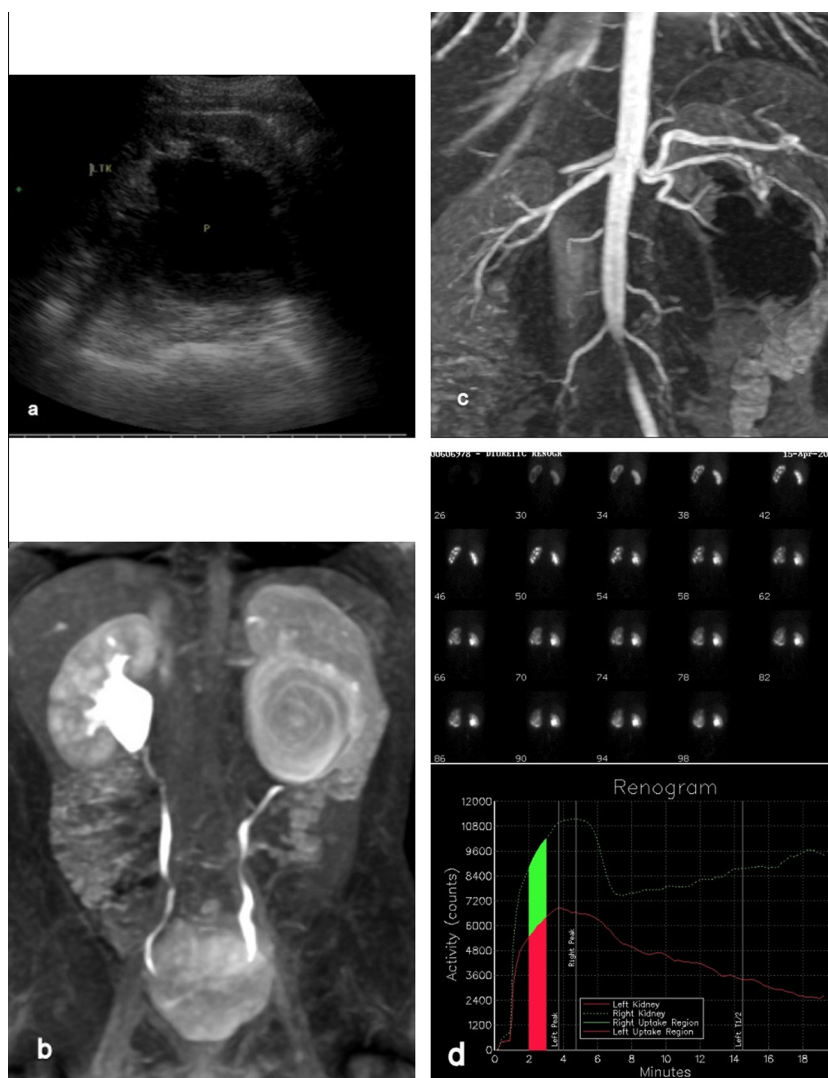
### Duplicated system

Thirteen duplicated collecting systems in 4 patients were included in the study (One patient had bilateral disease). In all cases the presence of complicated duplex system, type of complication and distal ureteric insertion were assessed by different imaging modalities. IVU was performed in 2 cases, VCUG was performed in all cases.

Upper moiety complications were detected in 3 units: obstructing megaureter with intravasical ectopic insertion of the ureter at the bladder neck ( $n = 1$ ) (Fig. 4), and refluxing megaureter ( $n = 2$ ). Two units showed lower moiety complications: refluxing megaureter ( $n = 1$ ) and UPJO ( $n = 1$ ). Segmental MCDK detected in one upper moiety system.

US failed to identify ectopic ureteric insertion. IVU and renal scan misdiagnosed 2 cases of duplicated kidney as single kidney, due to markedly dilated non-functioning moiety, showing non-function on renal scan and IVU studies. Out of 3 units with refluxing megaureter, DRS and MRU misdiagnosed 2 refluxing upper and lower moiety ureters as obstructive megaureter. Only one unit of refluxing upper moiety ureter, was accurately diagnosed as non-obstructive dilatation.

Upper partial nephrectomy was done in 2 cases; dextranomer/hyaluronic acid copolymer injection was done in another 2 cases.



**Fig. 1** Left UPJ obstruction. (a) US image showed moderate dilatation of the left renal pelvis, and accurately detected the level of obstruction at the UPJ. (b) Delayed excretory MRU image showed ballooning of the left renal pelvis and swirling of the contrast medium. (c) MRA image showed absence of crossing renal vessels. (d) DRS images and renogram curve showed good tracer uptake by the enlarged left kidney, with impaired tracer wash out. The renal scan accurately determined the level of obstruction. The patient underwent left pyeloplasty.

#### *Post ESWL non-obstructive dilatation*

MRU was done in two cases after ESWL, in whom US showed mild hydronephrosis. MRU diagnosed non-obstructive dilatation of the renal pelvis and ureter, with no signal void stones. The findings of MRU were confirmatory to other modalities.

The information obtained from the MRU was compared with the results of other imaging modalities. The MRU was marked as adding when its findings gave additional information. In this study MRU provided additional information in 13 units in whom other imaging modalities had failed to make the definite diagnosis. The confirmatory results of MRU also helped to establish the final diagnosis in 40 units. The findings of MRU were insufficient in 8 units.

The MRU diagnosis correlated with the final diagnosis in 57 units, with diagnostic accuracy 93.4%.

The diagnostic accuracy of US and DRS in determining the final diagnosis correctly was 68.8% and 78.7%.

#### **Discussion**

MRU has been widely accepted as a substitute to IVU for investigating children with a dilated urinary tract after preliminary assessment by US and VCUG. Hydronephrosis is by far the main indication for MRU because upper tract dilatation is a frequent condition in infants and children [10].

In most cases hydronephrosis is the consequence of obstruction of the urine flow at any point from the kidney to the bladder. The most severe consequence of obstruction is the renal function deterioration. It is important to differentiate between obstructive and non-obstructive dilatation to choose the proper therapy in order to prevent the loss of renal function [11].

Static and excretory MRU are the most commonly used MR urographic techniques. In heavy T2-weighted static MRU, the static-fluid (urine) is viewed as intrinsic CM. This sequence is very fast and is completely independent of renal



**Fig. 2** Left midureteric stricture, and right MCDK. (a and b) US image showed dilated left ureter, with the level of obstruction could not be determined by US. Small sized right kidney with multiple small non-communicating cysts (MCDK). (c) IVU image showed moderate dilation of the left renal pelvis and ureter. Non-visualized right kidney (non-functioning). Note the examination is degraded due to distended gas filled bowel loops, with inadequate assessment of the level of obstruction. (d and e) Coronal T2WIs images showed small sized right MCDK (involved). Compensatory hypertrophy of the left kidney with moderate dilatation of the proximal ureter and transition in caliber at the mid-ureter. (f) Delayed excretory phase image provided accurate anatomical localization of the level of the stricture, with good visualization of the post stenotic normal caliber ureter.

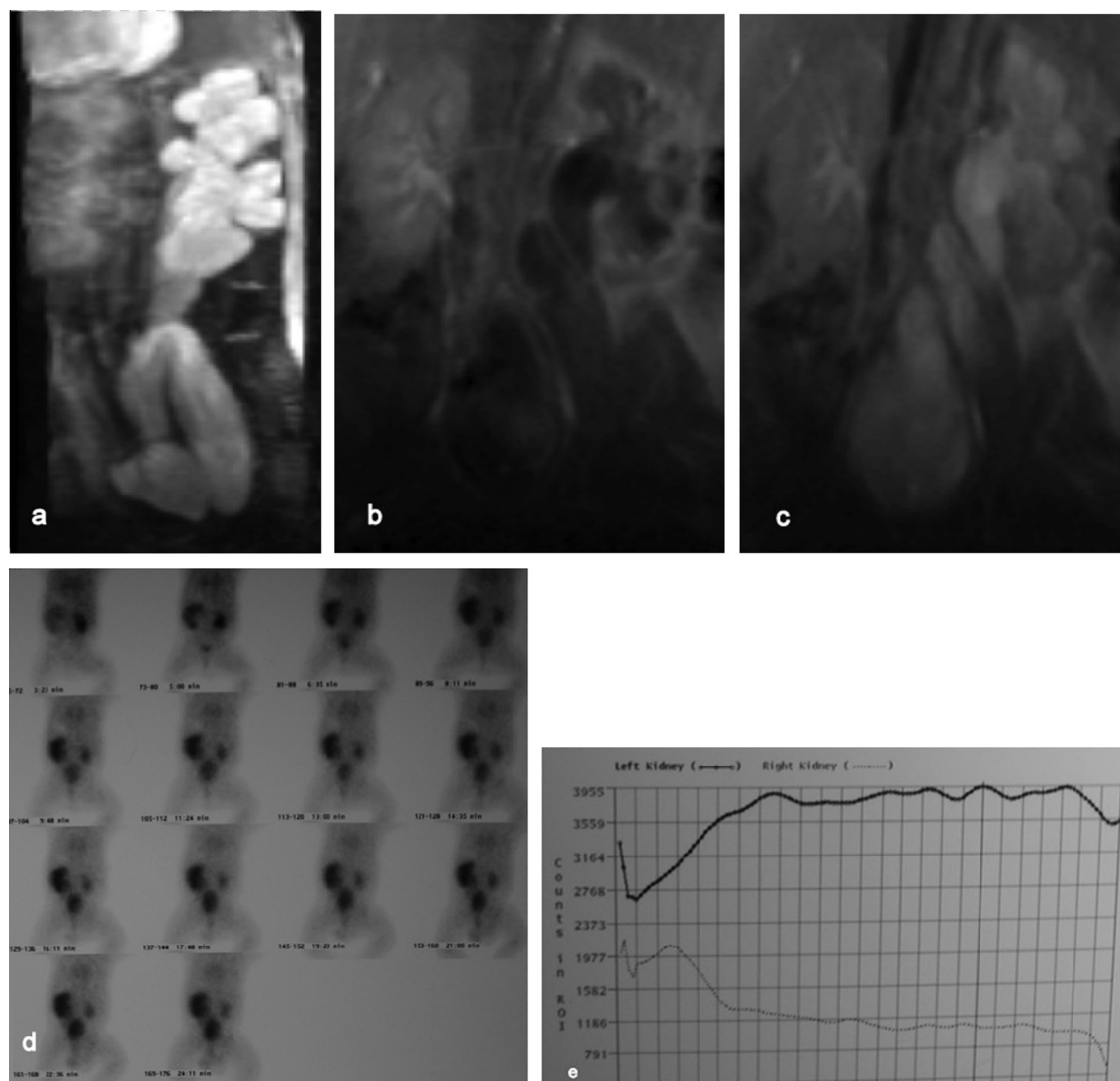
function. It provides excellent urographic images that resemble IVU images. It is particularly useful for in cases of hydronephrosis as it is quickly identifying the level of urinary tract obstruction [6]. However static MRU is often insufficient for identifying the non-dilated ureters and it is unable to provide information about the renal function [12].

Excretory MRU was developed and improved to overcome the limitations of static MRU and to provide functional data [13]. Excretory MRU utilize 2D or 3D T1-weighted sequences to image the UT during the excretory phase after administration of gadolinium-based CM [14]. It is roughly analogous to CT urography and IVU. The collecting systems are imaged

during the excretory phase, thus generating images similar to excretory urograms. However excretory MRU have greater contrast and temporal resolution [7].

The combination of static-fluid and excretory MRU can be useful in the evaluation of obstructive uropathy because T2-weighted images can show the extent of dilatation of the obstructed system and excretory MRU can provide information on the functional effects on excretion [15]. However non-dynamic post contrast imaging can provide limited subjective functional observation [16].

In this study 28 children have been examined by combined static-excretory MRU. We confirmed the feasibility of MRU



**Fig. 3** Left megaureter and UVJ obstruction. (a) Static MRU image showed moderate left hydronephrosis with dilated tortuous ureter down to its lower end. (b) Contrast enhanced excretory MRU images showed faintly enhancing narrow renal parenchyma on the left side and delayed excretion of contrast. (c and d) DRS images and renogram curve showed good tracer uptake by the enlarged left kidney, with impaired tracer wash out and visualization of its ureter (denoting obstruction). The renal scan could not determine the level of obstruction. The patient underwent resection of the distal ureteral segment and re-implantation into the urinary bladder.

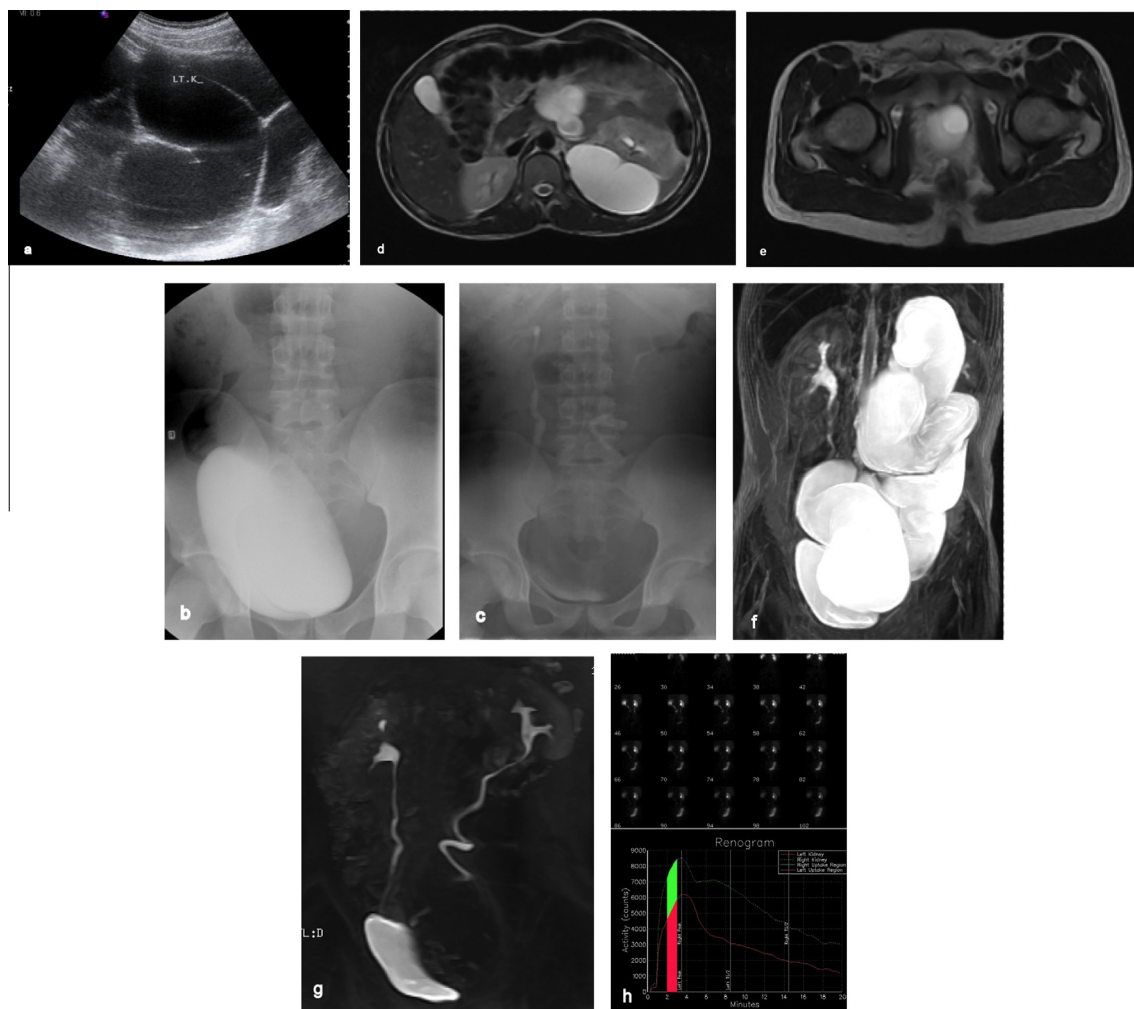
in children with sonographically detected dilated urinary tract. There was excellent correlation with final diagnosis as determined by constellation of findings of other imaging modalities, surgery or clinical follow up, with diagnostic accuracy (93.4%). Our findings were in accordance with the previous study by Payabvash et al. who reported (86%) diagnostic accuracy of MRU [17].

Previous reports had proved the superior anatomic imaging of the UT with MRU compared with different imaging modalities [8,17–20]. In our study, MRU provided superior morphological imaging of the urinary tract in all cases compared to US or scintigraphy. MRU showed greater spatial and contrast resolution, with the MIP images accurately demonstrated the anatomy of the collecting systems and ureters. In addition static MRU was particularly valuable for assessment of severe hydronephrosis and poorly functioning systems.

In this study we had observed obstructed system morphologically by the presence of persistent narrowing with proximal dilatation. For functional assessment we obtained subjective impression of the urinary excretion based on IVU criteria. We differentiated between obstructed and non-obstructed system based on delayed excretion of contrast into the collecting system and ureter.

The diagnostic accuracy, sensitivity, specificity, positive predicative value (PPV), negative predictive value (NPV) of excretory MRU in the differentiation between obstructed and non-obstructed dilated units was 88.2%, 100%, 63.6%, 85.2% and 100% respectively. Our findings were in accordance with previous reports, that confirmed the feasibility of MRU in children with urinary tract dilatation [17–19].

Changes in ureteric caliber are useful indicators of the level of obstruction that most commonly occur at the ureteropelvic



**Fig. 4** Duplicated left collecting system with non-functioning obstructed ectopic upper moiety ureter. (a) US image showed dilated tortuous cystic structure filled the entire abdomen. (b) VCUG image showed no reflux with displaced urinary bladder. (c) IVU image showed displacement of the left pelvicalyceal system and ureter. (d) Axial T2WI through the upper pole of the left kidney showed non-identifiable renal parenchyma of the upper moiety. (e) Axial T2WI through the pelvis showed ectopic insertion of the upper moiety ureter at the bladder neck. (f) Static MRU showed markedly dilated left upper moiety ureter down to its lower end. (g) Coronal delayed excretory image showed excellent imaging of the normal caliber ureters but invisible non-functioning massively dilated obstructed upper moiety ureter. (h) DRS images and renogram curve showed relatively reduced function of the left kidney yet with patent drainage. A large cold area was noted below the kidney displacing the left ureter medially. The renal scan misdiagnosed the case as single kidney. The patient underwent left upper polar heminephrectomy.

junction followed by vesicoureteric junction and rarely the midureter [21].

In UPJO, MRU clearly delineated pelvicalyceal dilatation and narrowing of the UPJ without extrinsic compression in all patients. MRU was mainly confirmatory to US and renal scan. Yet it provided crucial data for diagnosis in 3 patients by excluding the presence of crossing renal vessels in older children diagnosed with UPJO.

MRU corrected the diagnosis of 4 units with extrarenal pelvis, misinterpreted by US and IVU as UPJO. This was an agreement with the previous findings of Wildberett et al. [22].

In the present study, MRU was of great importance in evaluation of megaureter. Static MRU identified dilated ureters along their entire length till the vesicoureteral junction in all cases. However MRU was considered insufficient in 4 units of refluxing megaureter, this was supported with previous find-

ings of Payabvash et al. [17] who had found that although MRU revealed the presence of megaureter in children, only VCUG could confirm the diagnosis of reflux.

In a previous study, Riccabona et al. [4] had demonstrated that increased ureteral diameter, and increased ureteral enhancement in later stage of the examination (with filling of the bladder) are indirect signs of reflux. However, reflux cannot be shown directly because MRU cannot be performed during urination.

Ultrasonography now plays a valuable role in the diagnosis of VUR, however the most definite diagnosis of VUR is made with VCUG or radionuclide cystography (RNC), sonography may be used as the first step in evaluation for VUR, especially in high-grade VUR [23]. Recently Duran and his colleagues proposed the use of Voiding Ultrasonography (VUS) as US examination using US contrast agent (microbubbles) as part

of initial diagnosis of reflux [24]. The common selection criteria for VUS as the primary examination for VUR currently include (a) follow-up studies, (b) first examination for VUR in girls, and (c) screening high-risk patients [25]. In this study we used VUCG as a reference for diagnosis of VUR.

Another group of pediatric pathologies that may pose difficulties in terms of conventional imaging examinations is duplicated collecting system. It presents with subsequent complications, obstruction, ectopic ureter, and ectopic ureterocele or VUR that may be seen in one of the systems [26]. The obstructed upper moiety is likely to excrete IV contrast material slower than the lower moiety, if at all [15]. In the 4 children with duplicated collecting systems, MRU delineated the renal parenchyma associated with each moiety and it was able to visualize the course and insertion of the ureters in all cases. MRU was superior to US and renal scan in the detection of ectopic ureter insertion and occult upper pole moieties.

Four upper moiety ureters with inferior and medial position of the ureteric orifice were accurately demonstrated by MRU. One upper moiety dysplastic megaureter with ectopic ureteric insertion at the bladder neck was accurately identified by static MRU. MRU shows an increasing role in the evaluation of ureteral ectopia [20]. Based on the previous experience by Avni et al., once abnormal duplex kidney with ectopic ureteric insertion is suspected, the anomaly should be confirmed by MRU [26]. This was supported by the previous findings of Perez-Brayffild et al. who had proved that in case of non-function upper poles of duplex systems, which do not show any contrast uptake, scintigraphy is of no further value [27].

In this study MRU permitted more detailed evaluation of the contralateral kidney and detected abnormalities that may predispose patients to future problems. MCDK and renal dysplasia were detected in 4 cases. MRU was able to detect the characteristic imaging features of renal dysplasia including small kidney (hypoplasia), cystic changes apart from the classic appearance of MCDK, disorganized architecture and dysmorphic calyces [21,28].

MCDKs are generally diagnosed by US, the main consideration in the differential diagnosis of a MCDK is a UPJO [29]. In a study done by McMann et al. the 4 patients found to have MCDK on MRU were originally suspected to have hydronephrosis on US [30]. In our study 1 unit was misdiagnosed by US as UPJO, however MRU was able to differentiate between MCDK and UPJO.

The study limitations included the need for sedation and anesthesia in children younger than 6 years, yet there is still need for anesthesia in DRS examination in some patients. In addition the need for bladder catheterization in some cases which not widely accepted by the parents of the children.

The cost and access problem are other disadvantages of MRI. However the total cost of conventional techniques frequently exceeds the cost of the MRU because usually more than one imaging modality is required for the diagnosis. Besides MRU considered the only the diagnostic method in some pathologies as complex congenital anomalies.

One of the drawbacks of this study that we used subjective functional evaluation of the dilated urinary tract. Objective functional data and quantitative evaluation of the renal function are recently employed. Functional data, similar to the information generated with DRS, may be obtained by contrast enhanced dynamic MRU. However specific post-processing must be performed, which is time consuming and needs dedi-

cated software that is not available in conventional workstations.

Despite MRU has always been known as an extremely safe procedure, there are now growing concerns regarding the application of gadolinium-based CM in patients with renal failure [7]. Recent reports linking gadolinium administration to a disorder known as nephrogenic systemic fibrosis (NSF) have resulted in new recommendations to avoid (whenever possible) the use of gadolinium based CM in patients with moderate to severe renal insufficiency [31]. We did not observe adverse effect of cases of NSF following Gd administration in our study group.

## Conclusions

In conclusion, static and contrast enhanced excretory MRU give both morphological and functional information in a single examination without exposure to ionizing radiation and iodinated CM. It is valuable examination for children with upper UT dilatation; especially in cases of complex congenital pathologies and severely hydronephrotic kidney. Both techniques are complementary and can be alternatively employed according to the degree of urinary tract dilatation and renal function. Based on our findings we suggest that in patients with mild dilatation of the urinary tract, both techniques are employed, whereas in cases of marked dilatation and impaired excretory function, static MRU is only used.

## Conflict of interest

*The authors have declared no conflict of interest.*

## Compliance with Ethics Requirements

*This article does not contain any studies with human or animal subjects.*

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