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HIGHLIGHTS IN PEDIATRIC UROLOGY: 2021

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MINI REVIEW
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Current Concepts in Pediatric Robotic Assisted Pyeloplasty

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Robotic surgery in pediatric urology has been gaining popularity since its introduction almost two decades ago. Robotic assisted pyeloplasty is the most common robotic procedure performed in pediatric urology. Advances in robotic technology, instrumentation, patient care and surgical expertise have allowed the correction of ureteropelvic junction (UPJ) obstruction in most patients using this minimally invasive technique. The excellent experience with robotic assisted pyeloplasty has challenged other approaches as a new standard for the treatment of UPJ obstruction. In this review, we will describe the technique as it relates to the different robotic platforms, review the surgical experience and compare its results to other surgical approaches. Also, we will discuss patient and parent satisfaction, cost and financial considerations, along with evaluating the future of robotic surgery in the treatment of UPJ obstruction.

Keywords: pediatric urology, robotics, pyeloplasty, UPJ obstruction, children

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INTRODUCTION

Despite its recent beginnings, robotic assisted surgery has been progressing in the treatment of many conditions in pediatric urology. Since the introduction of laparoscopic pyeloplasty in 1993 in adults and 2 years later in the pediatric population, minimally invasive laparoscopic approach for the treatment of ureteropelvic junction obstruction (UPJO) became an evident viable option. In 1994, the first robotic system used in the urological practice known as AESOP was introduced. Later, the evolution of these devices would bring the Zeus system and finally the Da Vinci system while continuously increasing their precision and effectivity (1). This new surgical approach was embraced by doctors throughout the US and promoted a statistical increase in use throughout the country (2).

When compared to classical laparoscopic surgery, robotic assistance offers several benefits. Tremor cancellation, three-dimensional vision and 7° of freedom allow the surgeon to optimally perform in confined working spaces such as those found during pediatric surgery while executing precise and delicate movements with ease (3). In 2002, the first pediatric robotic procedure performed was the robotic laparoscopic pyeloplasty (4). The high incidence of UPJO combined with the surgeons' previous experience with the laparoscopic approach naturally made it a pioneer procedure for robotics in pediatric urology.

Until now, the gold standard method for treating UPJO is open dismembered pyeloplasty with a success rate between 90 and 100%. Laparoscopic pyeloplasty had gain popularity but it struggled to be adopted by many pediatric urologists because of its technical difficulty and tedious learning curve. However, robotic assisted laparoscopic pyeloplasty (RALP) had all the advantages of the laparoscopic approach with an ease of use and a much shorter learning curve. This allows some surgeons to transition from open pyeloplasty to a minimally invasive robotic approach without any previous laparoscopic experience. RALP has been the most commonly reported robotic

procedure in children to date (5). In this review, we will describe the technique as it relates to the different robotic platforms, review the surgical experience over the last 5 years and compare its results to other surgical approaches. Also, we will discuss patient and parent satisfaction, cost and financial considerations, along with evaluating the future of robotic surgery in the treatment of UPJ obstruction.

BACKGROUND

UPJO is a common cause of pediatric hydronephrosis occurring in 1 per 1,000–2,000 newborns (6). Widespread use of antenatal ultrasonography (US) and the increase availability of postnatal imaging have resulted in earlier and more frequent diagnosis of hydronephrosis. UPJO is found more commonly in boys than in girls with up to 67% of cases involving the left kidney, and up to 10% seen bilaterally (7). Renal dysplasia, multicystic dysplastic kidney, duplicated renal collecting system where the lower pole UPJ is usually the obstructed segment; horseshoe kidney; and ectopic kidney have been found in association with UPJO. The etiology can be described as lesions that involve the UPJ intrinsically, lesions that are extrinsic or a combination of both.

The initial postnatal evaluation is performed with a renal/bladder US in order to determine the presence of pelvocalyceal dilation with or without renal cortical thinning. The most widely used grading systems of the severity of hydronephrosis on US are the Society of Fetal Urology (SFU) system and the Anterior/Posterior (AP) diameter of the renal pelvis. In 2014, a multidisciplinary consensus group developed the urinary tract dilation (UTD) classification system pertinent to antenatal and postnatal evaluation. The new classification incorporated the following six US parameters: AP renal pelvis diameter (APD), calyceal dilation, renal parenchymal thickness, renal parenchymal appearance, bladder abnormalities, and ureteral abnormalities (8).

Diuretic renography is the most widely used non-invasive technique to determine the severity and functional significance of UPJ obstruction (9). Technetium-99 m mercaptoacetyltriglycerine (^{99m}Tc-MAG3) is the ideal tracer for the pediatric population. One of the most useful measurements in diuretic renography is the estimate of differential renal function. This is considered significant when it is <40%. This percentage usually is well-correlated with the half-life (T1/2) washout curve.

Other than US and MAG3 renal scan there are other imaging and diagnostic tests also less commonly utilized for diagnosis of UPJO. Magnetic resonance imaging has been used by some center as their study of choice to evaluate UPJO (10). Developments in magnetic resonance imaging (MRI) technology have made it possible to image kidneys while assessing anatomy, renal transit times as well as intracellular metabolic parameters independent of blood flow and tubular function.

Indications for surgical interventions are ipsilateral UPJO with <40% of differential renal function (DRF) on diuretic renography, bilateral severe UPJO with renal parenchymal atrophy, obstructive pattern on diuretic renography with abdominal mass, urosepsis, or cyclic flank pain with or without vomiting and recurrent UTI under antibiotic prophylaxis.

Robotic Assisted Laparoscopic Pyeloplasty (RALP)

RALP is now a well-established method of correcting UPJO (5). It has the advantage of being able to help overcome the difficulties encountered with laparoscopic dissecting and suturing. The basic principle is similar to that of laparoscopic pyeloplasty but facilitated by 3-D imaging and the help of an articulated instrument. The operative technique has evolved to the point where RALP can be successfully performed in most pediatric patients. Some limitations could be encountered in infants <5 mo. or patients with a small abdominal cavity due to the limited available working space.

Positioning

The procedure starts with a crucial element for success: proper positioning of the patient. The patient is positioned at the edge of the table with the arms resting on a folded arm board. This will allow the robot arms to use their full range without table interference. Our preferred patient position is the 45° or modified flank position. This position with the use of the table rotation allow access and trocar placement in a near supine position avoiding potential complications. Also, this position allows for the intraabdominal contents to move away from the surgical site. We use two large jelly rolls to support the patient's back with the upper arm placed across the body in a praying position. The lower leg is bended at a 90° angle and the upper leg is straight. It is of outmost importance that the patient is well-padded specially between the legs, arms, and face. The patient is secured to the table with wide tape across the arms and shoulders, chest, hip, knee, and ankles. Care is taken not to place tape directly on patient's skin. With the help of the anesthesiologist, proper positioning, and adequate access to the patient's airway and IV lines are confirmed before the start of the procedure.

Initial Access, Insufflation, and Trocar Placement

With the table tilted away from the surgeon' side, the access is performed on a nearly flat patient. Our preferred approach is percutaneous using the Veress needle. Others have preferred the open Hassan technique for smaller children (11). After CO_2 insufflation to 8–10 mmHg, a 5 mm optical trocar is placed under direct vision in the infraumbilical position allowing easy and safe access. The port position for robotic assisted laparoscopic pyeloplasty will be in straight line for most patients using the DaVinci Xi system (**Figure 1**). The 8 mm robotic trocars are placed under direct vision with the last trocar replacing the infraumbilical trocar. With the DaVinci Xi, the trocar should have at least 3 cm of separation in order to avoid robotic arms collision. Other have described the best trocar position for the Si system which needs to be modified depending on the patient's size. Several options include straight line, triangulation, and HIDES (12). The HIDES trocar positioning allows for better cosmetic results with an infraumbilical port and 2 additional

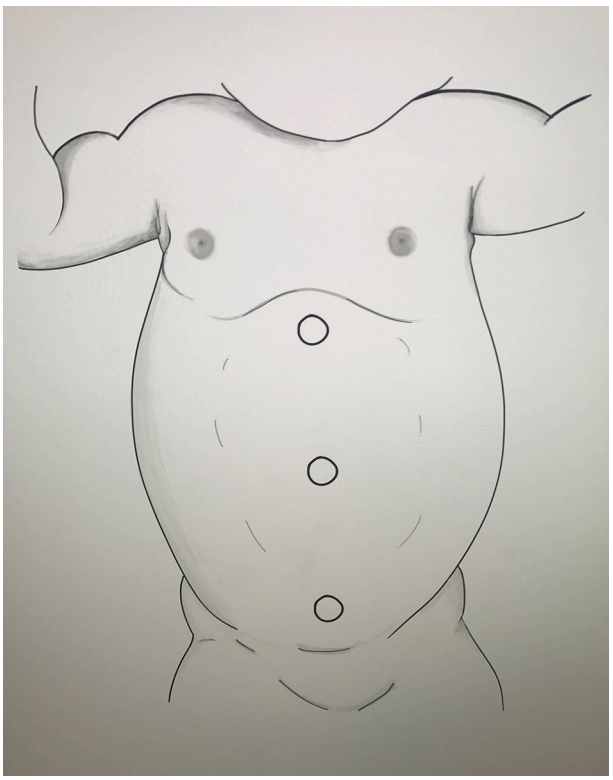

FIGURE 1 | DaVinci Xi port placement for RALP (Drawing by Carla Fernandez).

lower abdominal ports. "Burping" of all ports will give additional intraabdominal space needed in order to successfully perform the procedure on smaller children.

Docking With Si and Xi Systems

RALP requires the Xi robotic system to be docked at a 90° angle to the patient at the level of the infraumbilical port (camera site). We preferred to keep the robot at an established position and rotate the arms of the robot to adjust to the surgical site without moving the operating table. This allows the head of the patient to remain in the standard position and close to the anesthesiologists. Alternatively, the table could be rotated 180° to allow docking without rotation of the robotic arms. With the Si robotic system, the robot will need to be repositioned to come in a straight line with the camera site, trocar and the surgical site. As an example, the HIDES port placement the robot will dock from the inferolateral position (13).

Surgical Technique and Stent

Cystoscopy with a retrograde pyelogram can be performed selectively at the start of the procedure. Indication for cystoscopy with retrograde pyelogram included complex anatomy (ectopic/horseshoe kidney) and need to clarify preoperative testing. Our preferred surgical technique for the corrections of UPJO is the dismembered pyeloplasty. The procedure follows the same surgical principles described in the laparoscopic pyeloplasty (14). Instruments used during the procedure include: 2 dissecting forceps, scissors, 2 needle drivers, and a suction device. The approach to the UPJ area can be transmesenteric for left sided UPJO or with medial mobilization of the colon for Right sided UPJO and with selected complicated left sided UPJO. Caution is needed during the initial dissection of the UPJ area to avoid injury to a lower pole crossing vessels. Tethered stitches using 3-0 prolene on a CT needle can be placed to the renal pelvis and proximal ureter to help with exposure and ease of the operation. We preferred to place a stent in all patients undergoing robotic assisted pyeloplasty. The stent can be placed in an antegrade or retrograde fashion depending on the surgeon's preference.

Postop Care and Follow up

Indwelling urethral catheter is removed on POD # 1. Most patients are discharged within 24 h if they are able to void, tolerate diet and have adequate pain control. The ureteral stent is removed 6 weeks after the operation. Renal and bladder ultrasound is performed at 2 weeks., 3 mo., and 6 mo. post operatively. MAG-3 renal scan is reserved for symptomatic patients or significant residual hydronephrosis after 3 mo. follow up. Asymptomatic patients with residual hydronephrosis and good renal interval growth are followed with a renal and bladder ultrasound until resolution.

EXPERIENCE AND RESULTS

We retrospectively reviewed our experience with RALP using the DaVinci Xi robotic platform. We identified 41 patients with a mean age of 10.9 years (7 mo.−17 years). Ten patients were <1 year of age. Left RALP was performed in 27 patients and a right pyeloplasty in 14 patients. All procedures were performed using a transperitoneal approach. Our mean operative time was 135 min with a mean hospital stay of 1.5 days. The overall success rate for our series was 95%. Two patients had persistent SFU IV hydronephrosis requiring redo laparoscopic pyeloplasty and balloon dilatation, respectively. Four patients had post-operative complications including stent pain in 2 and non-obstructive small renal stone in 2. None of the patients less than a year of age had any complications. Residual hydronephrosis was identified in 29% of the patients.

Multiple authors have reported strong series with RALP (**Table 1**). A series of studies performed over the last decade show that when compared to open and laparoscopic pyeloplasty, the robotic assisted procedure has performed well in the treatment of UPJO (**Table 2**). These studies have shown comparable success rates with no statistically significance between the modalities.

In comparison to open or laparoscopic pyeloplasty, robotic pyeloplasty typically exhibit shorter hospital stay and less use of medication for pain management following the procedure (4). The only consistent negative variable has been the longer operative times exhibited by the robotic approach as compared to other modalities. Operative times seems to improve in center with high volume and surgeon's experience.

TABLE 1 | Series of reported robotic-assisted laparoscopic pyeloplasty cases.

Author	Procedure	# of pts	Mean age (yrs)	Laterality UPJ	Approach	Mean op time (min)	Hospital stay (days)	Complications	Success rate (%)
Kutikov et al. (15)	RALP	9	0.47	n/a	Transperitoneal	122.8	1.4	n/a	78
Avery et al. (16)	RALP	60	0.61	Bilateral (2)	Transperitoneal	232	1	7	91
Asensio et al. (17)	RALP	5	10.59	n/a	Transperitoneal	144	2.6	n/a	100
Olsen et al. (18)	RALP	65	7.9	n/a	Retroperitoneal	146	2	11	100
Minnillo et al. (19)	RALP	155	10.5	n/a	n/a	198.5	1.9	17	96
Singh et al. (20)	RALP	34	12	n/a	n/a	105	n/a	2	97
Atug et al. (21)	RALP	7	13	n/a	Transperitoneal	184	1.2	1	100
Franco et al. (22)	RALP	15	11.9	n/a	Transperitoneal	223	n/a	4	n/a
Perez-Brayfield	RALP	41	10.2	Right (14), Left (27)	Trans	135	1.5	5	95%

TABLE 2 | Series of reported cases comparing open pyeloplasty, laparoscopic pyeloplasty, and robotic-assisted laparoscopic pyeloplasty.

Author	Procedure (OP, LAP, RALP)	# of patients	Mean age (yrs)	Laterality UPJ	Approach	Mean operative time (min)	Hospital stay (days)	Complications	Success rate (%)
Barbosa et al. (23)	RALP	58	7.2	Bilateral (10)	Transperitoneal	n/a	n/a	1	76.9
	OP	154	1.2	n/a	n/a	n/a	n/a	7	67.9
Yee et al. (24)	RALP	8	11.5	n/a	n/a	363	2.4	1	100
	OP	8	9.8	n/a	n/a	248	3.3	0	87.5
Subotic et al. (25)	OP	8	9.8	n/a	n/a	248	3.3	0	87.5
Lee et al. (26)	RALP	33	7.9	n/a	n/a	219	2.3	1	94
	OP	33	7.6	n/a	n/a	181	3.5	0	100
Song et al. (27)	OP	30	8.5	Right (8), Left (22)	Transperitoneal	192.5	6.6	4	96.7
	LP	30	10.5	Right (6), Left (24)	Transperitoneal	197.4	5.8	4	89.7
	RALP	10	11	Right (3), Left (7)	Transperitoneal	254.1	3.2	1	100
Cundy et al. (28)	OP vs. RALP	157,166	7, 8.1	n/a	n/a	RALP (Longer OT)	RALP (shorter HS)	5, 9	88.5, 87.3
	LP vs. RALP	97, 151	6.5, 10	n/a	n/a	no significant diff.	RALP (shorter HS)	10, 10	96.9, 99.3
Salö et al. (29)	OP	92	6.2	Right (38), Left (54)	n/a	167	4.4	25	92
	RALP	31	8.3	Right (10), Left (21)	Retro (15), Trans (16)	249	3.4	9	94

COSTS AND CONSIDERATIONS

Several studies have delved into the evaluation of costs of the treatment options for UPJO. Some studies have even suggested a 2.7 time increase in cost in RALP as compared to other modalities of UPJO (30). In 2017 Jacobs et al. (31) published a cost analysis study in adult patients showing fairly similar costs for open pyeloplasty ($22,421) as compared to minimally invasive pyeloplasty ($22,843). Varda and colleagues evaluated the national trends of UPJO treatment modalities in children including analysis of the available data on cost (32). They reported evidence of an increasing trend toward utilization of minimally invasive pyeloplasty over open pyeloplasty. In the study, minimally invasive modalities had an increased cost with a significant increase in price related to RALP. Operating room costs were by far the greatest contributor to costs, with robotic supplies being the largest contributor to the rising cost. For example, when comparing laparoscopic vs. robotic approaches there was an average increase in costs of over $3,000.

In another study, Varda et al. again demonstrated an increased utilization of the RALP in children (33). They showed that within a 12-year period there was a persistent higher cost when RALP was compared with open pyeloplasty. The increased cost in RALP over open pyeloplasty persisted as the cost of operating room equipment for robotic cases remained high even when considering the cost associated with longer hospital stays related to open surgery. High volumes of RALP may be required for institutions to profit from the procedures as total investment cost is divided between an increased number of procedures performed. An estimated three to five robotic cases per week are necessary to profit from robotic surgery, which is a clear limitation for pediatric centers no matter their size (34). Reaching

the required number of cases needed will be a challenge to children's hospitals with low to mid volume RALP programs.

Based on our analysis and personal experience there appears to be clear evidence that there is in fact a higher cost to RALP as compared to open and laparoscopic approaches. Published data seems to suggest than even with shorter length of stay attributed to RALP as compared to other treatment modalities, the high cost of training, maintenance and materials point to a greater cost as compared to other modalities. In the near future innovation in technology, robotic market competition and market tendencies may see a further normalization of RALP costs that could be comparable to other treatment options.

Parental Capital Gains

Other than the inherent cost analysis necessary for the evaluation and comparison of the treatment modalities of UPJO, there is also a further economic impact related to UPJO treatment as it pertains to parental gains/losses in the pediatric population. A 2011 study by Behan and colleagues evaluated the human capital gains associated to RALP in children (35). An evaluation of 44 patients most of which underwent RALP as compared to open approach was done retrospectively, in which indirect expenses to each procedure was estimated using already published financial models. Although parental work loss is sometimes used as the greatest variable to capital gains/loss other data was analyzed to evaluate the procedures. The results showed that the overall cost savings that are a result of decrease hospital length of stay for RALP may help compensate for the added operative costs previously alluded to. This study suggested that RP is associated with decreased lost parental wages and savings attributed to shorter length of stay, but the results are extremely dependent on the overall costs and amortization related to the robot. Prospective large center studies would be of great value to truly assess the impact of this variable in the treatment modalities of UPJO.

Satisfaction

In the pediatric population satisfaction is not merely based on patient satisfaction and outcomes, but also related to parental satisfactions. Freilich et al. evaluated parental satisfaction based on a modified Glasgow Children's Benefit Inventory (36). Groups of open and RALP were compared based in most part to the responses of the questionnaire. Overall the results of the study showed that even when objective success of surgery were similar between groups (i.e., decreased hydronephrosis on imaging, improved renal scan measures), RALP was favored overall by parents. In regard to specific variables such as postoperative pain, speed to normal activity, speed of return to normal sports, surgery incision scar, impact of surgery on parental life, burden of postop visits/studies, and overall satisfaction, parents seemed to find a greater difference between actual results and expected results within the robotic wing. Based on this study there is increased satisfaction when RALP is undertaken especially in regards to cosmesis and recovery, but expectations as compared to actual results are almost always improved notwithstanding the type of treatment modality employed.

The effect of cosmesis takes greater impact when novel treatment techniques are utilized within the endoscopic treatment realm. For example, hidden incision endoscopic surgery (port sites at level of a Pfannenstiel incision) did show greater satisfaction from patients and parents in regard to cosmetic results in a series of 12 patients published by Gargollo in 2011 (37). In our experience endoscopic approaches are preferred by parents based on the reported considerations as well.

Benefits vs. Risks

Apart from patient benefits like reduced pain, improved cosmetic results, shorter hospitalization, and rapid convalescence there are also added technical benefits to robotic surgery. Extensive published data exists on the benefits of magnified three-dimensional vision, the advantage of having an increased number of working arms, reduced tremors, and overall improved ergonomics. These qualities are an upside on robotic surgery when compared to both open and conventional laparoscopic approaches.

In a review of 5,400 laparoscopic cases performed Peters reported an overall complication rate of 5.4% (38). The greatest predictor of complication rate was surgeon experience.

Braga et al. published a systematic review and metanalysis of RALP vs. conventional laparoscopic approaches (39). RALP showed improved operative time reduction, and a significantly shorter stay at the hospital, but no statistical significance was found with regards to the rates of complications between the treatment modalities. The effects of reduced morbidity in robotic surgery, especially within the pediatric population, is also apparent due to a trend to its utilization in redo cases (40). Up to this point when compared to conventional laparoscopy there is no clear or definitive decrease in morbidity in RALP, especially in experienced hands.

Future of Robotic Pyeloplasty

The future of RP seems to lie both on achieving greater utilization of the currently described technique as well as in the development of new techniques and technology. Single ports, smaller surgical sites, telesurgery, and hidden surgical incisions all seem to be in development and may show promise as they become more available.

Further miniaturization of robotic arms, especially in the form of table mounted systems, will allow for increased dexterity (4). Baek et al. published data regarding the use of 5 mm instruments for RALP in children of different ages (infants and non-infants). Utilization of smaller port sites allowed for safe intervention of RALP in infant children with similar results when compared to older children (41). Improvement in the 5 mm instrument and miniaturization of the robotic arms will facilitate RALP in the smaller infant patients (4).

Another area of particular interest is further development of force-feedback mechanisms to the surgeon that can compensate for the lack of tactile feedback in robotic cases. This in conjunction with newer technologies like virtual reality and augmented reality may not only change robotic surgery as a whole but may also improve education in robotic surgery including RALP.

CONCLUSION

RALP is safe, effective, and well-accepted by surgeons, patients and their parents. There are real concerns regarding the longer operative times and cost associated with this procedure. As surgeons become better trained and have more experience with this technology operative time and associated cost should reduce significantly. Also, as more companies develop additional robotic technology, competition should produce more affordable robotic systems and instrumentations directly reducing the overall cost to the health systems. In the near future, RALP could become our new gold standard in the treatment of UPJO or at least be an equal to the open approach pyeloplasty.

AUTHOR CONTRIBUTIONS

RM-L, MP-M and MPB contributed in all aspects of the manuscript including research, writing, and editing of manuscript.

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ORIGINAL RESEARCH
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Hydronephrosis in Children Caused by Lower Pole Crossing Vessels—How to Choose the Proper Method of Treatment?

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Objectives: Assessment of the efficacy of intraoperative diagnosis between extrinsic and intrinsic UPJO in children. Assessment of the efficacy of laparoscopic vascular-hitch procedure in UPJO caused by lower pole crossing vessels (CV).

Materials and Methods: Between 2008 and 2017, 47 laparoscopic procedures were performed with the CV discovered intraoperatively. CV were translocated cephalad, and the UPJ was carefully inspected. The Chapman's vascular hitch procedure was accomplished in the case of decreasing sizes of the pelvis and clear, visible peristalsis of the UPJ (31 patients). In the other cases, Anderson–Hynes (A-H) pyeloplasty with posterior translocation of the CV was performed (16 patients).

Results: The median age at operation was 6 years (range 1–16) in VH and 6 years (range 2–17) in A-H ($p = 0.4635$). Prenatal dilatation of kidney was diagnosed in 18.7% of VH and 10% of A-H cases ($p = 0.5474$). Success was achieved in 16 (100%) patients in the A-H and in 29 (93.54%) in the VH groups. Two patients (6.5%) in VH required repeated surgery because of a misdiagnosed intrinsic obstruction. Median operation time in VH was 80 min (range 40–105) and was 105 (range 70–225) in A-H ($p < 0.05$).

Conclusions: The intraoperative selection based on intraoperative pelvis and UPJ appearance after vessel transposition is sufficient in majority of cases. Laparoscopic vascular hitch seems to be effective and safe procedure, but can only be performed on carefully selected patients. In case of misdiagnosis, reoperation is possible with the same laparoscopic access.

Keywords: crossing vessel, vascular hitch, pyeloplasty, ureteropelvic junction obstruction, hydronephrosis, children, pediatric

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INTRODUCTION

The classical operative procedure for ureteropelvic junction obstruction (UPJO) in children is dismembered pyeloplasty, which was described by Anderson and Hynes in 1949 (1, 2). In the same year Hellstroem presented a technique, applied in the case of crossing vessels that causes mechanical obstruction of the UPJ (3). The "vascular hitch" procedure means cephalad translocation of the CV

and fixing it to the tissues around the kidney. In the next years, many different modifications of this technique appeared, i.e., the most popular one was introduced by Chapman (4). The author recommended fixing the CV in a tunnel made of the pelvis, away from the UPJ. This technique enabled the surgeon to save the CV covered by pelvic tissues from being punctured with needle and any complications associated with vascular damage. Vascular hitch procedures in comparison to dismembered pyeloplasties are less technically demanding and shorten the operation time (5). The proper choice of operative treatment for UPJO with CV is very important, as simultaneous intrinsic obstruction of the UPJ could be overlooked. In this article, we present our experience in children with transperitoneal laparoscopic approach for the treatment of hydronephrosis caused by CV.

AIM

Assessment of the efficacy of intraoperative diagnosis between extrinsic and intrinsic UPJO with CV in children. Assessment of the efficacy of the laparoscopic vascular-hitch procedure in UPJO caused by lower pole crossing vessels.

MATERIALS AND METHODS

Between 2008 and 2017 in the Department of Pediatric Surgery and Urology in Wroclaw, 47 laparoscopic hydronephrosis operations with intraoperatively diagnosed lower pole crossing vessels at the level of UPJ were performed. Retrospective analysis of the medical history was carried out. As a qualification for surgical treatment in all patients, ultrasound of the kidneys and diuretic renography were scheduled. In questionable cases, CT scans were done. The lower pole CV before surgery were suspected in 18 (38.3%) of the patients on the ultrasound examination and in 30 (63.8%) in computed uro-tomography. In all cases, an enema of the large intestine was performed 24 h before surgery. Laparoscopic, transperitoneal technique with three 5 mm or two 3 mm and one 5 mm ports was used. Access to the left kidney was achieved through a window in the colonic mesentery. On the right side, the colon was mobilized. The UPJ was released by blunt dissection and an electrocautery hook. Detected crossing vessels were translocated cephalad, and the UPJ was carefully inspected. In case of a decreasing pelvis and a clear, visible peristalsis of the UPJ, the Vascular Hitch with Chapman modification was performed. The CV was fixed with 2–3 sutures in a tunnel made from the pelvis, far away from UPJ. This procedure was done in 31 children (VH group). In situations when the pelvis did not decrease or/and there were visible stenosis or no clear peristalsis of the UPJ after the release and translocation of the CV the Anderson–Hynes dismembered pyeloplasty with posterior translocation of the CV was performed. This procedure was used in 16 patients (A-H group). Three patients were operated upon before by using open surgery: one of them twice in another center, and two patients in our department. In these cases, CV was not discovered during the primary surgery. The median follow-up was 4 years (range 0.5–6) in the VH group and 3 years (range 0.5–6) in the A-H group.

To compare numerical samples, both the Student's test and the non-parametric unpaired Wilcoxon test (known also as the Mann-Whitney test) were utilized. The choice of the most suitable test was made based on responses of the Ljung-Box test (independence within each sample), the Shapiro-Wilk test (normality of distributions in each sample), and the F test (equality of variances in two samples). To compare categorical (dichotomic) samples, the test for equal proportions was used. The statistical analysis was performed in R, the language and environment for statistical computing.

RESULTS

Demographic data comparing two groups of patients are presented in **Table 1**. There was no statistically significant difference in any parameter except the side of the kidney. Success was achieved in 16 (100%) patients in the A-H group and 29 (93.54%) in the VH group. Two patients (6.46%) in the VH group required reoperation because of undiagnosed intrinsic obstruction. The retrospective analysis of videos of surgeries in these two patients showed incorrect assessment of renal pelvis emptying after relocation of CV. Both were reoperated using the same transperitoneal laparoscopic access without any technical difficulties. Secondary JJ stent insertion was needed in one patient (3.2%), but after removal of the stent, there were no symptoms of hydronephrosis. There was no need for conversion in any case. The median operative time in the VH group was 80 min (range 40–105 min) and 105 min (70–225 min) in the A-H group ($p < 0.05$). Outcomes in both groups of patients (VH and A-H) are given in **Table 2**. The limitation of the study is that it was performed retrospectively.

DISCUSSION

A very important role in diagnosis of patients with UPJO is played by a carefully gathered clinical history. In most children with CV, there is no history of hydronephrosis in the neonatal period, with a frequency of 75–100% (5–8). In our material dilatation of the kidneys during pregnancy and in neonatal period had 18.7% of children in the VH group and 10% in the A-H group (0.5474). Furthermore, the typical clinical picture for these patients is presenting with colicky flank pain, which is sometimes associated with vomiting. In the literature, the incidence of colic pain in pure extrinsic UPJO is given as 71.8–100% (5, 8, 9). In our patients, in the VH group it happened in 80% but was only in 63.6% of children in the A-H group. The incidence of CV causing obstruction of the UPJ in children increases with age. CVs are very rarely noticed in newborns and infants. According to the literature, the average age of patients with a CV is between 7 and 11 years and is statistically higher than in patients with pure intrinsic obstruction (8–12). In our material, the median age at surgery was 6 years in both groups.

The key to success is adequate patient's selection, but this faces some challenges. It can be difficult task to whether the CV are an incidental finding or play a significant role in the obstruction (13). In our material the decision whether to use

TABLE 1 | Demographic data comparing two groups of patients.

	Method of treatment		*p*-value
	VH (Vascular-Hitch)	A-H (Anderson-Hynes)	
Number of pts	31	16	
Age in years—median (range)	6 (1–16)	6 (2–17)	0.4635
Gender (M—male, F—female)	M–18, F–13	M–11, F–5	0.4751
Side (R—right, L—left)	R–7, L–24	R–7, L–9	0.0264
Prenatal diagnosis [yes/all (%)]	3/16 (18.7%)	1/10 (10%)	0.5474
Symptoms yes—pain [yes/all (%)]	16/20 (80%)	7/11 (63.63%)	0.3191
Ultrasound-dilatation of kidney pelvis in AP (cm) before surgery – median (range)	4,5 (2.5–7)	3 (1.3–6)	0.1944
Diureric renography before surgery-median (range)	41% (23–53)	42% (14–57)	0.9548
Follow up in years—median (range)	4 (0.5–6)	3 (0.5–9)	0.9730

the vascular hitch procedure or dismembered pyeloplasty was taken intraoperatively. The UPJ was carefully inspected after the cephalad translocation of crossing vessel. In the case of decreasing or emptying renal pelvis and a clear, visible peristalsis of the UPJ, the Chapman procedure was performed. Otherwise or in case of apparent UPJ stenosis Anderson-Hynes pyeloplasty with posterior translocation of the crossing vessel was done. Our intraoperative technique to select patients seems to be very successful. Based on it, a 95.74% overall success rate was observed (100% in A-H and a 93.54% in VH). Only in two patients after VH there was a need for reoperation. The retrospective analysis of recorded videos revealed incorrect assessment of renal pelvis emptying after relocation of CV. This could be the reason for failure. In both cases there was no prenatal history of hydronephrosis. There were no other complications. In other studies surgeons using the similar technique of laparoscopic intraoperative selection between intrinsic and extrinsic obstruction reported success rate between 96 and 100% (14–18). However, they had much lower number of cases ranged between 8 and 19. Villemagne et al. on the cohort of 70 children using laparoscopy in 42 cases and robotic-assisted surgery in 28 cases reached a success in 67 (96%) (13). They concluded, that purely extrinsic obstruction can be deduced from a dramatic decrease in the renal pelvis size between the beginning and the end of the PUJ mobilization, and by observing pelvic emptying. With these findings at surgery, in the context of a child with the typical clinical picture of intermittent hydronephrosis, no prenatal history and preserved renal function, the possibility of associated intrinsic stenosis is low.

The advantage of the laparoscopic vascular hitch procedure over dismembered pyeloplasty is the absence of the need to open the collecting system, leaving the PUJ intact, avoiding the technical challenge of pelviureteric anastomosis. It is a quite simple technique compared to pyeloplasty (5). It can also be performed by less experienced surgeons in minimally invasive surgery. The time of the surgery is shorter than in laparoscopic dismembered pyeloplasty. In our material, in the VH group, the mean operative time was 80 min (range 40–105 min) and 105 min (70–225 min) in the A-H group with statistical difference ($p < 0.05$) however the operating surgeon had a great experience in suturing.

TABLE 2 | Outcomes of vascular-hitch-group patients and anderson-hynes-group patients.

	VH (Vascular-Hitch)	A-H (Anderson-Hynes)
Number of pts (%)	31 (100%)	16
Redo surgery	2 (6.5%)	0
Redo JJ	1 (3.2%)	0
Symptoms resolution	29 (93.54%)	8 (100%)
Ultrasound—improvement	29 (93.54%)	16 (100%)
DR—improvement or stable function	11 of 11 (100%)	11 of 11 (100%)
Success rate	29 (93.54%)	16 (100%)

Because of the risk of overlooking a simultaneous intrinsic obstruction some authors recommend additional, intraoperative diuretic-test. In a multicentre study, Esposito et al. used this technique in 51 patients with CV (5). In the laparoscopy before the vessels mobilization, they administered a bolus of normal saline, followed by furosemide. In all patients, they observed symptom resolution and no need for repeat surgery. Miranda et al. performed laparoscopic vascular hitch with modified Whitaker test in 4 of 11 children. A fine needle was inserted percutaneously into the renal pelvis and the ureteral opening pressure was evaluated three times using a water column device. They assumed that if the opening pressure was lower than 14 cm of water, then the junction was considered to be unobstructed. None of their four patients required reoperation (19). Because of the small number of patients, this study is less valuable. Parente et al. calibrated UPJ using a high-pressure balloon inserted by cystoscopy and inflated at the UPJ level in 10 patients with suspected CV (20). They considered intrinsic obstruction to be present where a "waist" was observed at the UPJ on inflation of the balloon and a laparoscopic dismembered pyeloplasty was performed. When no "waist" was observed, laparoscopic vascular hitch was performed. They found no intraoperative and postoperative complications using this technique. In our opinion, there is no need for additional, invasive procedures like the Whitaker test. The puncture of the pelvis causes the risk of complications

like urine leakage and urinoma formation after surgery. The classical intraoperative, diuretic test with furosemide could be taken into consideration. It is simple to perform, does not extend the surgical time and maybe could reduce the risk of misdiagnosis.

The intraoperative selection based on intraoperative pelvis and UPJ assessment after vessel transposition is sufficient in majority of cases. Laparoscopic vascular hitch seems to be effective and safe procedure but can only be performed on carefully selected patients. In case of misdiagnosis, reoperation is possible with the same laparoscopic access.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

ETHICS STATEMENT

The consent was obtained from all participants both informed and written. Ethics board approval number is KB-341/2018 (Ethics Committee by Medical University, University of Wrocław).

AUTHOR CONTRIBUTIONS

MP and DP contributed conception and design of the study. DB, KT, MP, and DJ organized the database. MP performed the statistical analysis. MP wrote the first draft of the manuscript. WA and DP wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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OPINION
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What Term to Choose: Ambiguous Genitalia or Disorders of Sex Development (DSD)?

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Keywords: ambiguous genitalia, disorders of sex development, DSD, feminizing genitoplasty, masculinized genitoplasty, Chicago consensus

The meeting of Chicago, in 2005, which regrouped about 50 experts from 10 countries marked a milestone in the history of genital malformations. However, the two main changes proposed by the Chicago consensus (1), concerned the terminology and the timing of the surgical correction deserves a discussion.

1- The changes in terminology: it is clear that "Terms such as intersex, pseudohermaphroditism, hermaphroditism, sex reversal are perceived as potentially pejorative by patients" (1). However, I think that the term "ambiguous genitalia," widely used in the past is not as pejorative, and this term was in particular more precise in its definition, with clear limits.

We talk about ambiguous genitalia when the appearance of the external genitalia of a newborn, does not resemble that of a girl, nor a boy, but rather a form between the two. However, this definition remains incomplete, and ambiguous genitalia must also include patients with phenotypes that do not correspond to the genetic sex, a discordance between genital appearance and karyotype. Usually, these cases are discovered later in older children and at puberty (2).

Whereas, the definition and limits of the term disorders of sex development (DSD) remains fuzzy, this term refers to a wider range of pathologies completely different by origin, pathogenesis, clinical expression, and their therapeutic method (ex: ambiguous genitalia by congenital adrenal hyperplasia and 46, XY cloacal exstrophy). Thus, some questions remain unanswered, for example; Why include 46, XY cloacal exstrophy and not 46XX bladder exstrophy (with two hemiclitoris'), Why include aphallia and not diphallia…?

I agree with González and Ludwikowski who reports that "Although the term DSD was widely accepted by patients, families and health professionals, it created a new series of problems" (3). In Turkey, a Muslim country, only 2% of parents of DSD patients preferred using the term DSD (4). A nomenclature which remains controversial (5).

I think there are two elements that greatly influence the therapeutic approach: the karyotype and the causes of ambiguous genitalia. Thus, they must be mentioned in the terminology and ambiguous genitalia can be classified into four groups: enzymatic deficiencies, abnormalities in the androgen receptor, abnormalities of gonad development, and maternal androgens excess.

a)- Enzymatic deficiencies
-of glandular origins (testis and adrenal): such as
46XY/17β-hydroxystéroide-déshydrogénase(17 β-HSD3) deficiency
46,XX/ congenital adrenal hyperplasia (CAH)
46XY/StAR deficiency
46XY/3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency
-of peripheral origins: such as 46XY/5-alpha reductase 2 (5-AR2) deficiency

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b)- Abnormalities in the androgen receptor: such as
46XY/Complete Androgen Insensitivity Syndrome (CAIS)
46XY/Partial Androgen Insensitivity Syndrome (PAIS)

c)- Abnormalities of gonad development: such as
46,XX or 46,XX/XY or 46,XY/Ovo-testis (historically called true hermaphroditism)
45X/46 XY/Mixed gonadal dysgenesis
46XY/pure gonadal dysgenesis.
46XY/Leydig cell hypoplasia type1 and 2
45X/Turner syndrome
47XXY/ Klinefelter syndrome

d)- Maternal androgens excess (very rare)
46XX/Virilized by maternal tumor
46XX/Virilized by exogenous androgens

2- Timing of surgical correction: one of the main recommendations of the Chicago consensus was to delay vaginoplasty at adolescence, "when the patient is psychologically motivated and a full partner in the procedure" (1).

There is a surgical reason to delay the vaginoplasty at adolescence, many patients who undergo feminizing genitoplasty in early childhood require surgical correction at puberty, but there is also an innuendo that it is necessary to wait until the patient can participate in the choice of his sex. However, if this is normal or tolerated in the Western world, with a population that represents only about 18% of the world's population, in most Muslim countries, the religious factor plays an important role in the social life of the people, and it is difficult to change the identity of the gender at adolescence, and very difficult, even impossible to do it at the adulthood.

In some western countries, intersex activists have encouraged legislative bodies to ban genital surgery without the individual's informed consent (5). While DSD patients and their parents were not consulted.

Mouriquand et al. reported recently that "Ten years after the Chicago consensus meeting, genital surgery continues to raise questions and criticisms...There is no consensus regarding the indications, the timing, the procedure, and the evaluation of outcome of DSD surgery" (6).

Patients who require vaginoplasty could be divided into two groups (2):

-Patients with vaginal agenesis: such as the complete androgen insensitivity syndrome (CAIS) and some cases of 46, XY DSD, such as Leydig cell hypoplasia type1. These forms, often manifest later, in older children or at puberty, through clinical signs, such as inguinal hernia; virilization or the delayed of primary amenorrhea in a girl; and breast development or apparition of cyclical hematuria in a boy. Thus, the timing of vaginoplasty does not arise and the first recommended treatment of vaginal agenesis at the adolescence is the vaginal dilatation.

-Patients with persistent Müllerian duct: such as 46, XX DSD, Ovotestis DSD, and mixed gonadal dysgenesis are often discovered at birth, they are characterized as having ambiguous genitalia with the presence of a uterus, fallopian tubes, and an upper vagina. The aim of the vaginoplasty is to separate the openings of the vagina and urethra, and to connect the vaginal cul-de-sac to perineal skin.

Early assignment of an appropriate sex is an important step for a good development of gender identity. The current trend is to keep DSD individuals with Y material in the male gender despite the unlikelihood of fertility and an uncertain surgical outcome (6). When the male gender is chosen, things are simpler and the masculinized genitoplasty must be accomplished in early infancy, definitely before the age of 10. During puberty and adulthood, hyper vascularization of the penis which accompanies the hypersecretion of androgens makes the surgical repair more hemorrhagic, and more difficult.

However, in some cases of ambiguous genitalia, choosing the sex gender remains a complex and difficult step, particularly for ovotestis DSD and gonadal dysgenesis with 46,XYor 45,X/46,XY karyotype. Nevertheless, in two forms of ambiguous genitalia, 17 β -hydroxysteroid dehydrogenase (17β-HSD3) deficiency and 5 α-Reductase 2 deficiency, the feminizing genitoplasty must never be performed during childhood. These cases virilize and often change the sexual behavior and gender identity at puberty.

Facing high rates of poor cosmetic results and difficulties in sexual intercourse, reported in women who underwent feminizing genitoplasty for ambiguous genitalia during their childhood, surgeons were the main target of critics at the meeting in Chicago. However, the surgical repair of these urogenital malformations has been considerably improved over the last 20 years. Several cohorts of adult patients who underwent a feminization procedure at various ages have recently been interviewed in different French hospitals, all claimed that early surgery is highly preferable to late surgery (6). Nevertheless, as mentioned at this meeting, it is imperative that these patients be treated in specialized centers where at least 50 surgical repairs of ambiguous genitalia are performed per year.

I think that when the diagnosis of the type of ambiguous genitalia is accurately made, and the feminizing genitoplasty is proposed, which is not always the case, the vaginoplasty should be performed as early as possible, during early infancy, at the same time with clitoroplasty and creating labia minora, preferably between 6 and 24 months (7). It is difficult to accept the sentence reported at the Chicago consensus "Emphasis is on functional outcome rather than a strictly cosmetic appearance" (1). I think that the cosmetic result should be as important as the functional result.

The cosmetic result depends on three important criteria (2):

- The glans should be small, and its apparent part should not exceed 5 mm.
- Presence of the labia minora and its appearance.
- The nature of the tissue used in covering the area located between the two labia minora. This area should be covered by the wall of the common urogenital canal, never by perineal skin.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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ORIGINAL RESEARCH
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The Homeodomain Transcription Factor NKX3.1 Modulates Bladder Outlet Obstruction Induced Fibrosis in Mice

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Fibrosis is an irreversible remodeling process characterized by the deposition of collagen in the extracellular matrix of various organs through a variety of pathologies in children, leading to the stiffening of healthy tissues and organ dysfunction. Despite the prevalence of fibrotic disease in children, large gaps exist in our understanding of the mechanisms that lead to fibrosis, and there are currently no therapies to treat or reverse it. We previously observed that castration significantly reduces fibrosis in the bladders of male mice that have been partially obstructed. Here, we investigated if the expression of androgen response genes were altered in mouse bladders after partial bladder outlet obstruction (PO). Using a QPCR microarray and QRTPCR we found that PO was sufficient to increase expression of the androgen response gene *Nkx3.1*. Consistent with this was an increase in the expression of NKX3.1 protein. Immunofluorescent antibody localization demonstrated nuclear NKX3.1 in most bladder cells after PO. We tested if genetic deletion of *Nkx3.1* alters remodeling of the bladder wall after PO. After PO, $Nkx3.1^{KO/KO}$ bladders underwent remodeling, demonstrating smaller bladder area, thickness, and bladder: body weight ratios than obstructed, wild type controls. Remarkably, $Nkx3.1^{KO/KO}$ specifically affected histological parameters of fibrosis, including reduced collagen to muscle ratio. Loss of *Nkx3.1* altered collagen and smooth muscle cytoskeletal gene expression following PO which supported our histologic findings. Together these findings indicated that after PO, *Nkx3.1* expression is induced in the bladder and that it mediates important pathways that lead to tissue fibrosis. *As Nkx3.1* is an androgen response gene, our data suggest a possible mechanism by which fibrosis is mediated in male mice and opens the possibility of a molecular pathway mediated by NKX3.1 that could explain sexual dimorphism in bladder fibrosis.

Keywords: Nkx3.1, bladder outlet obstruction, testosterone, androgen receptor, tissue fibrosis

INTRODUCTION

Muscle hypertrophy and tissue fibrosis are common irreversible responses to obstruction seen in diseases affecting various organs such as the bladder, heart, blood vessels, lungs, and intestines. In part, this response is adaptive to allow the obstructed organ to overcome increased pressure. However, it becomes maladaptive over time with worsening fibrosis and organ dysfunction. There are significant gaps in our knowledge of the molecular and cellular processes that lead to muscle hypertrophy and fibrosis in obstructed systems. One emerging paradigm for fibrosis includes sexual dimorphism in which severity, depending on the type of fibrosis, varies based on sex. For example, in pulmonary arterial hypertension, females are more impacted than males (1). Fibrotic chronic kidney disease such as polycystic kidney disease, IgA nephropathy, and membranous glomerulopathy progress more slowly in pre-menopausal women than in age-matched men (2). Liver fibrosis associated with human immunodeficiency virus and hepatitis C virus coinfection is less severe in women than men (3). Other forms of liver injury and fibrosis through non-alcoholic fatty liver disease are known to have a predilection for men over women (4). Thus, sex is likely a key confounding factor in human fibrotic diseases.

Animal models have also implicated sexual dimorphism in the fibrotic process. For example, in a model that measured baboon intrauterine growth restriction, male fetuses responded to undernutrition with more cardiac fibrosis compared to females (5). In a sterile peritonitis model after oophorectomy in mice, loss of KLF11 reduced fibrosis in females with the response restored after administration of progesterone (6). Similarly, castration was found to be protective against fibrosis in male mice in the model, with testosterone replacement ultimately reversing this effect. Similar observations have been made in models of cardiac, kidney, and bladder fibrosis, indicating that sex alters tissue fibrosis in experimental animals (7–9).

In the urinary tract specifically, PO is a common urologic problem causing fibrosis that results from a number of pathologies. Posterior urethral valves (PUV) is a common congenital etiology in young boys that leads to bladder fibrosis with a prevalence of 2.1 per 10,000 live male births (10). Benign prostatic hyperplasia (BPH), which affects older men, is the most common cause of PO and leads to fibrotic remodeling of the bladder wall. Bladder remodeling can also happen in females after long-term bladder outlet obstruction secondary to pelvic organ prolapse (11). For BPH and pelvic organ prolapse, many treatment options are available, which prevents maladaptive remodeling from occurring if addressed early on. However, despite early treatment of the obstructing tissue in PUV, children often develop fibrotic bladders with poor function (12). Frequently, this progression worsens during early adolescence, suggesting that increased androgen levels during puberty could affect the development of fibrosis.

Mouse models of PO have demonstrated increased fibrosis with hypertrophy-thickened bladders (13). In a castrated PO model, however, bladders were noted to have an attenuated fibrotic response with preserved contractility (9). Upon testosterone replacement, the deleterious effects in the non-castrate model were ultimately restored, implicating testosterone as an essential contributor to maladaptive remodeling. Despite this connection, the mechanism of action by which androgens affect the bladder's response to obstruction is not well-understood. The androgen receptor (AR) pathway has been studied extensively and found to control a number of genes (14). We aimed to identify androgen response genes altered in the bladder specifically in PO and determine their function in bladder remodeling. We identified the AR response gene, *Nkx3.1*, as a gene upregulated after PO. Here, we present our results with genetic deletion of *Nkx3.1* in the context of PO in mice.

Abbreviations: PO, partial bladder outlet obstruction; CPO, partial bladder outlet obstruction in a castrated male mouse; AR, androgen receptor; PUV, posterior urethral valves; BPH, benign prostatic hyperplasia; VSOP, voiding stain on paper; H&E, hematoxylin and eosin staining; MT, Masson's trichrome staining; ANOVA, analysis of variance; WT, wild type; mVV, mean voided volumes.

MATERIALS AND METHODS

Experimental Animals

This study was carried out in accordance with the recommendations of surgical guidelines, Animal Care and Use Committee. The protocol was approved by the Institutional Animal Care and Use Committee of Northwestern University (protocol no. IS00003975). Initial experiments with DNA microarray and validation were performed on C57BL/6J mice (000664, Jackson). $Nkx3.1^{KO/KO}$ mice were obtained from the laboratory of Dr. Sarki Abdulkadir and were reported to be in the C57BL/6 background. Generation of this mouse strain and primers used to genotype these mice are described in Abdulkadir et al. (15). Initial surgeries on $Nkx3.1^{KO/KO}$ mice resulted in lethality near the time of recovery from surgery. This included mice subjected to sham laparotomy suggesting that lethality was a response to isoflurane anesthesia. Since we had not observed this either in C57BL/6 or CD-1 wild type mice, we reasoned that the $Nkx3.1^{KO/KO}$ line may carry a secondary allele that rendered them susceptible to recovery from isoflurane anesthesia. To cross away this putative allele and to generate a genetic background similar to the one we used in Flum et al. (9) we backcrossed $Nkx3.1^{KO/KO}$ mice to CD1 for three generations, and then bred back into the C57 BL/6 background for at least three generations. In each generation we selected mice both positive and negative for the $Nkx3.1^{KO}$ allele by PCR. This allowed us to create two "backbred" lines that were in a highly similar genetic background: $Nkx3.1^{KO/+}$ and wild type ($Nkx3.1^{+/+}$). After each backcross, mice were subjected to sham surgery and it was determined if the lethal response to anesthesia was still present. Once it was determined that this response no longer remained, we interbred $Nkx3.1^{KO/+}$ mice and selected for $Nkx3.1^{KO/KO}$ males and females. Then these mice were interbred to establish the new *Nkx3.1* null line in the mixed CD1/C57 BL/6 background. Backbred wild type mice were used as controls in our study.

Partial Bladder Outlet Obstruction Model

Male mice at 6–8 weeks of age were randomly assigned to sham operation, partial bladder outlet obstruction (PO), or surgical castration and partial bladder outlet obstruction (CPO). All surgeries were done as described previously described (9). Deviations from this protocol include the use of 6–0 Prolene around the bladder neck, 4–0 Vicryl for closure of the abdominal wall, and subcutaneous buprenorphine (0.05 mg/kg) for post-operative pain control. All mice were sacrificed at 1 or 4 weeks postoperatively by CO_2 asphyxia followed by cervical dislocation. Bladders were dissected out at the level of the ligature, emptied of urine, and weighed prior to subsequent experiments.

Void Stain on Paper (VSOP)

VSOP was performed and analyzed as previously described in Tassone et al. (16).

RNA Expression QPCR Microarray

Bladders were harvested from mice 1 week after sham, PO, and CPO surgeries and placed in Trizol (Thermo Fisher). Tissue was homogenized in Trizol using a Bead Bug homogenizer and 3 mm zirconium beads. Total RNA was extracted from homogenized bladders using the RNeasy Plus Mini Kit. RNA was converted into cDNA using a High-Capacity cDNA kit (Applied Biosystems, Catalog #4368814). Differential expression was analyzed using an AR RT^2 Profiler™ Array (Qiagen 330231 PAMM-142ZA) to profile the expression of 84 genes that mediated signal transduction in cells responsive to male sex hormone.

Quantitative PCR

Reverse transcriptase qPCR was performed as previously described (17). Validated primers were purchased from Applied Biosciences. Relative expression was quantified using the ΔΔCt method (18). In all cases GAPDH served as the internal control and expression under each condition was assessed relative to this gene. As ΔΔCt values are not paired, statistical significance was determined using ΔCt values, which are paired. Standard error of the mean was calculated using error propagation (19). This method takes into account experimental variation at each step in the analysis: Ct values, ΔCt values, and ΔΔCt values.

Western Blot

Mouse bladders were homogenized in RIPA buffer (Thermo Fisher Scientific, Catalog #89900) containing 100X Halt Protease Cocktail Inhibitor (Thermo Fisher Scientific, Catalog #78440). The insoluble fraction was pelleted at 13,000 RPM for 20 min at 4°C. Soluble protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Catalog #23227) and absorbances were read using a BMG Labtech Clariostar microplate reader. Protein samples were diluted in 2X Laemmli sample buffer with 5% 2-mercaptoethanol. Proteins were resolved on Bolt™ 4–12% Bis-Tris Plus pre-cast gels. Gels were transferred using an iBlot 2 (Life Technologies) at 20 V for 7 min. Membranes were blocked and antibodies diluted in Tris buffered saline containing milk (5% w/v). Blots were developed using a Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific, Cat: 34577) and imaged using a ChemiDoc MP Imager (BioRad). Densitometry was performed using ImageJ. The rabbit anti-mouse IgG, affinity purified antibody to NKX3.1 was from Athena Enzyme Systems (Catalog #0315).

Histologic Analysis

Bladders were dissected at the base of the neck and urine expressed. Bladders were equilibrated in physiologic buffer (Dulbecco's PBS), fixed in 10% formalin for 24 h and sent to Northwestern's Mouse Histology and Phenotyping Laboratory for paraffin embedding, sectioning, and staining. Bladders were cut into 4 μm sections in the sagittal orientation inclusive of the bladder neck, dome, and body. They were stained with hematoxylin and eosin (H&E) as well as Masson's trichrome (MT). Sections were imaged at 5x and 40x magnification using a Leica upright microscope (DM R). Sections were randomized during the acquisition of the images. Bladder circumference, thickness, and area were computed using ImageJ (National Institutes of Health, Bethesda, MD, USA), and collagen: smooth muscle ratio and muscle content were quantified using Photoshop (Adobe) and averaged from 10 or more 40x images of each bladder. In the investigation of smooth muscle, regions of urothelium and stroma were excluded from the analysis. The method to use Photoshop to measure collagen accumulation as assessed by MT stain is described in detail in Dahab et al. (20). Here, red pixels (acid fuchsin and xylidine ponceau stained) are used to measure muscle in the detrusor and blue pixels (methyl blue stained) are used to measure fibrous collagen.

Immunofluorescence Analysis

Bladders or prostates were dissected as above, equilibrated in physiologic buffer (Dulbecco's PBS) and fixed 12–18 h in 4% formaldehyde made from 16% paraformaldehyde in Dulbecco's PBS. Fixed tissue was washed in Dulbecco's PBS several times and equilibrated in sucrose (20% and 30% w/v). Tissue was placed in OCT compound (Tissue-Tek) and frozen on dry ice. Ten micrometer mid-coronal sections were cut and placed on Super Frost Plus glass slides (Thermo Fisher). Sections were blocked in Dulbecco's PBS with Tween-20 (0.1% v/v) and bovine serum albumin (0.1% w/v). Sections were probed with rabbit anti-mouse NKX3.1 (Athena Enzyme Systems), washed and then with donkey anti-rabbit IgG coupled with Alexa Fluor 568 (Thermo Fisher). Sections were counterstained with the 4′,6-diamidino-2-phenylindole (DAPI) nuclear stain. Sections were imaged on a Zeiss LSM 880 microscope using confocal optics.

Statistical Analysis

Statistical analysis was performed using One- and Two-Way ANOVA (Prism 7.0e). Multiple comparisons with One- and Two-Way ANOVA followed Tukey Test parameters with family-wise significance and confidence level set to 0.05 (95% confidence interval).

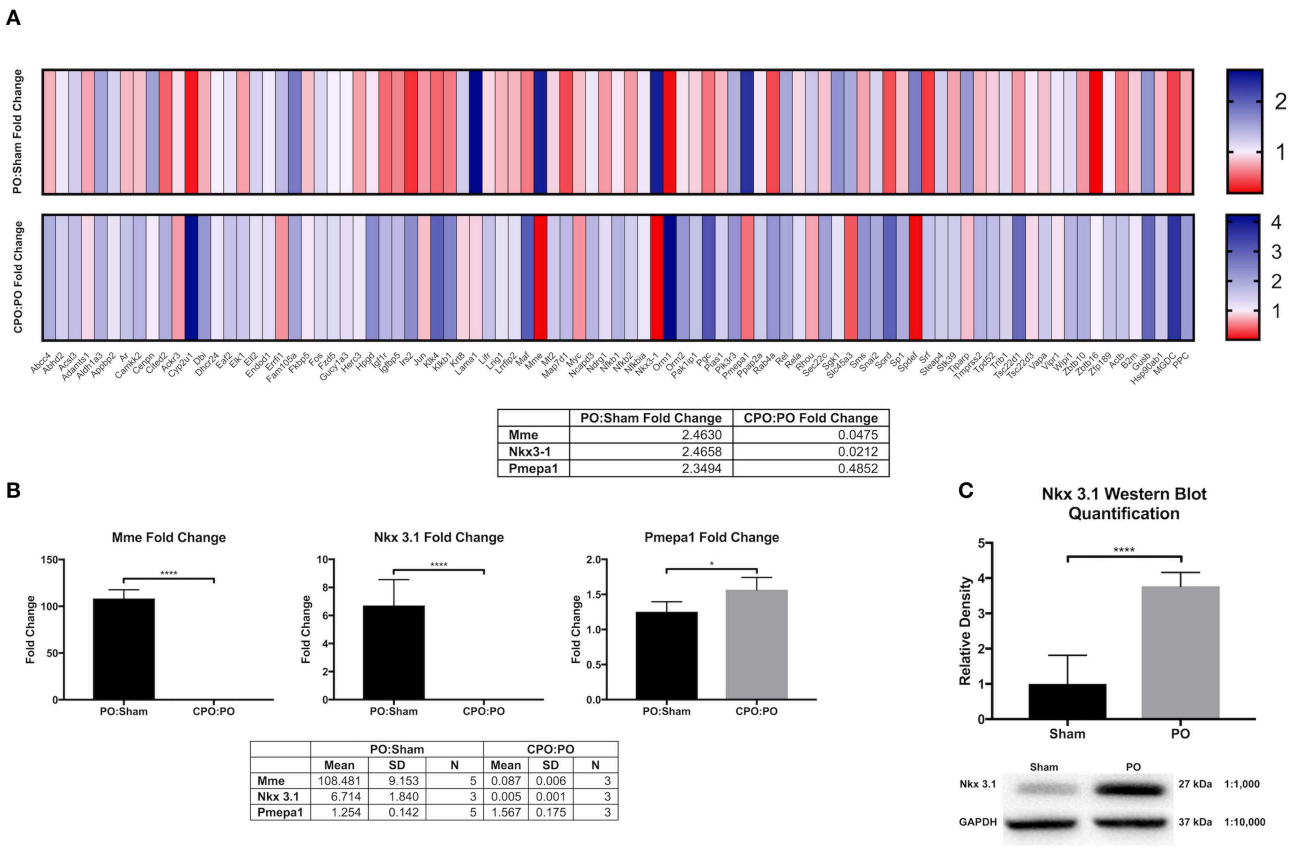
RESULTS

The Androgen Response Gene *NKX3.1* Is Upregulated in the Bladder After PO

In hormonally intact mice, we observed that nuclear localized androgen receptor (AR) is increased in bladder cells suggesting

that an androgen response is activated by PO (Firmiss and Gong, **Supplementary Materials** (doi: 10.6084/m9.figshare.9899312). Therefore, we hypothesized that AR response genes were transcriptionally altered following PO. To test this hypothesis, we interrogated a QPCR microarray of genes responsive to male sex hormone. We also tested a group of bladders taken from castrated male mice subjected to PO (CPO) to determine if change were affected by androgen levels. We specifically looked to identify genes that were increased after PO compared to sham and that were unchanged or downregulated in CPO compared to PO mice. **Figure 1A** shows the microarray heatmap demonstrating the transcriptional changes observed in PO and CPO mice. Here three genes met our criteria: *Mme*, which encodes neprilysin or membrane metallo-endopeptidase; *Nkx3.1*, which encodes NK3 homeobox 1 and *Pmepa1,* which encodes prostate membrane protein 1 androgen induced. The data for the QPCR array can be found here (doi: 10.6084/m9.figshare.9887408).

To validate these findings, we performed RT-qPCR in independently generated bladders (**Figure 1B**). Here we found that that the upregulation and downregulation was consistent for *Mme* and *Nkx3.1*, however, *Pmepa1* did not follow the same expression pattern. *Pmepa1* was found to be upregulated in both the PO and CPO models suggesting it may be activated in the setting of obstruction outside of the AR pathway. Meanwhile, both *Mme* and *Nkx3.1* seemed to be regulated in an androgen dependent fashion and, therefore, may function as regulators of bladder remodeling after obstruction. $Nkx3.1^{KO/KO}$ mice were readily available for our use through partnership with the Abdulkadir Laboratory (Northwestern Feinberg School of Medicine, Department of Urology), and therefore, *Nkx3.1* was chosen as the gene of choice for further investigation. Prior to assessment of the effects of *Nkx3.1* deletion, we first attempted to further validate the finding of *Nkx3.1* expression in the bladder, as it was previously not reported to be active in this organ. We tested if the *Nkx3.1* protein product was increased after PO (**Figure 1C**). Here we observed minimal NKX3.1 protein in the sham group with much higher expression in the PO group. To localize this expression in bladders we stained sections from the sham operated and PO groups. Here we observed very little positive staining in bladders from the sham group (**Figures 2A,B**). Positive staining was observed in



	PO:Sham Fold Change	CPO:PO Fold Change
Mme	2.4630	0.0475
Nkx3-1	2.4658	0.0212
Pmepa1	2.3494	0.4852

	PO:Sham			CPO:PO		
	Mean	SD	N	Mean	SD	N
Mme	108.481	9.153	5	0.087	0.006	3
Nkx 3.1	6.714	1.840	3	0.005	0.001	3
Pmepa1	1.254	0.142	5	1.567	0.175	3

FIGURE 1 | Bladder AR target gene analysis and validation. **(A)** Heat maps comparing androgen receptor target gene upregulation (blue) and downregulation (red) in PO:sham and CPO:PO groups ($N = 3$ for each surgical group). **(B)** RT-qPCR validation of the genes identified by the DNA microarray as upregulated in response to obstruction and downregulated in response to PO and CPO ($N = 3$ for each surgical group). **(C)** Western blot quantification of NKX3.1 in sham ($N = 3$) and PO ($N = 3$) bladders. Error bars in all graphs are represented as a standard deviation with family-wise significance and confidence level calculated at a 95% confidence interval (n.s., $^*P < 0.05$ or $^{****}P < 0.0001$).

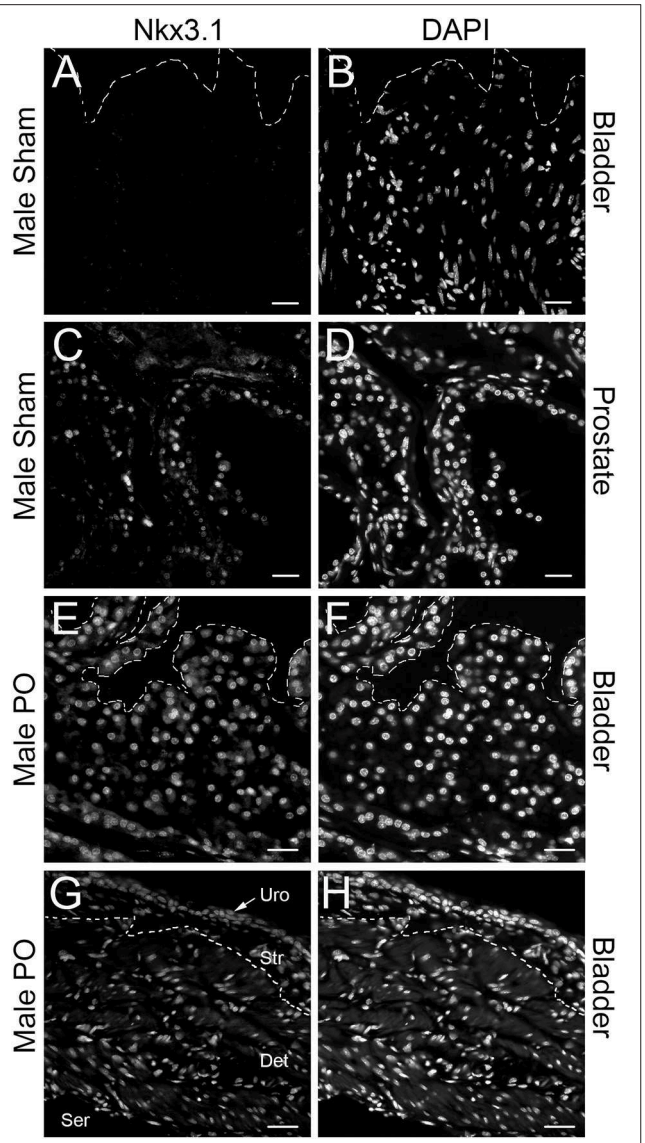


FIGURE 2 | Immunolocalization of NKX3.1. Sham bladder stained with anti-NKX3.1 **(A)** and DAPI **(B)**; prostate from sham animal stained with anti-NKX3.1 **(C)** and DAPI **(D)**; PO bladder stained with anti-NKX3.1 **(E)** and DAPI **(F)**; separate PO bladder stained with anti-NKX3.1 **(E)** and DAPI **(F)** showing lower magnification with urothelium (Uro), stroma (Str), detrusor (Det) and serosal (Ser) layers. The orientation of each bladder is presented with the urothelium at the top of each panel and indicated by a dotted line in **(A,B,E,F)**. In **(G,H)** the dotted line marks the approximate border between stroma and detrusor. Magnification bars are 40 μm in **(A–F)** and 60 μm in **(G,H)**.

nuclei of prostate epithelial cells, indicating antibody activity on our sections (**Figures 2C,D**). Positive staining was observed in PO bladders (**Figures 2E–H**). This staining was nuclear and was surprisingly throughout the bladder. This included positive reaction in urothelium, stroma (**Figure 2E**) and detrusor muscle (**Figures 2E,H**). Thus, our results indicated that PO is sufficient to induce both *Nkx3.1* RNA and protein expression in the male mouse bladder. Localization of the protein indicated that multiple different cell-types induce nuclear expression of NKX3.1 in response to PO.

Loss of Nkx3.1 Does Not Significantly Alter Bladder Development and Function

To test if NKX3.1 has functions in the intact bladder at baseline, we analyzed bladder function and histology in $Nkx3.1^{KO/KO}$ male mice. Previous studies have not implicated any role for the gene in the bladder (21). However, since we observed low levels of NKX3.1 protein in sham bladders (**Figure 1C**), there was the possibility that *Nkx3.1* bladder functions had been overlooked in previous studies. If present, such defects could alter our interpretation of bladder function after obstruction. Non-operated WT and $Nkx3.1^{KO/KO}$ mice were assessed by voiding stain on paper (VSOP) and bladders were harvested after 4 weeks for weight. **Figure 3A** shows non-significantly different mean voided volumes (mVV) and bladder: body weight ratios for both groups. Similarly, bladder area and circumference were equal between WT and $Nkx3.1^{KO/KO}$ mice (**Figure 3B**). One difference we found was that $Nkx3.1^{KO/KO}$ bladders were 6.7% thinner at baseline (299.05 μm ± 3 vs. 279 ± 4 μm, $P < 0.05$). Another important indicator of normal bladder development is muscle content which is altered in the presence of obstruction. Bladders from non-operated WT and $Nkx3.1^{KO/KO}$ mice were analyzed by MT staining (**Figure 3C**). Here we found that muscle content (81.52% ± 0.7% vs. 83.0% ± 0.6%, $P < 0.05$) was increased at baseline in $Nkx3.1^{KO/KO}$ bladders. This was supported by the observation that collagen: smooth muscle ratio (0.2321 ± 0.011 vs. 0.207 ± 0.008, $P < 0.05$) was decreased in $Nkx3.1^{KO/KO}$ bladders. Thus, loss of *Nkx3.1* alone had small but significant effects on structure indicating that NKX3.1 functions in the uninjured bladder.

Nkx3.1 Deletion Alters Bladder Remodeling After PO

To test if alterations to bladder remodeling after PO involve *Nkx3.1*, we investigated whether $Nkx3.1^{KO/KO}$ mice undergoing PO could replicate our previous findings in castrated male mice. We compared functional and histologic data for $Nkx3.1^{KO/KO}$ mice undergoing sham and PO surgeries. **Figure 4** shows a lower mVV for $Nkx3.1^{KO/KO}$ PO mice compared to $Nkx3.1^{KO/KO}$ sham. Additionally, bladder: body weight ratios were significantly higher between PO and sham as expected. These findings are similar to the changes seen in WT after PO and sham, however, we found that bladder: body weight ratios for $Nkx3.1^{KO/KO}$ PO were significantly lower than WT PO, indicating an altered remodeling process. Morphologically, $Nkx3.1^{KO/KO}$ bladders had a similar area and circumference but significantly lower bladder thickness after PO compared to sham (**Figure 5**). This was a slightly different response than observed for WT, which showed a higher bladder area after PO compared to sham but non-significant changes in circumference and thickness. When examining collagen: smooth muscle ratio and muscle content, sham and PO bladders were also similar for $Nkx3.1^{KO/KO}$ bladders, unlike the response seen in WT where collagen: smooth muscle ratio is increased and muscle

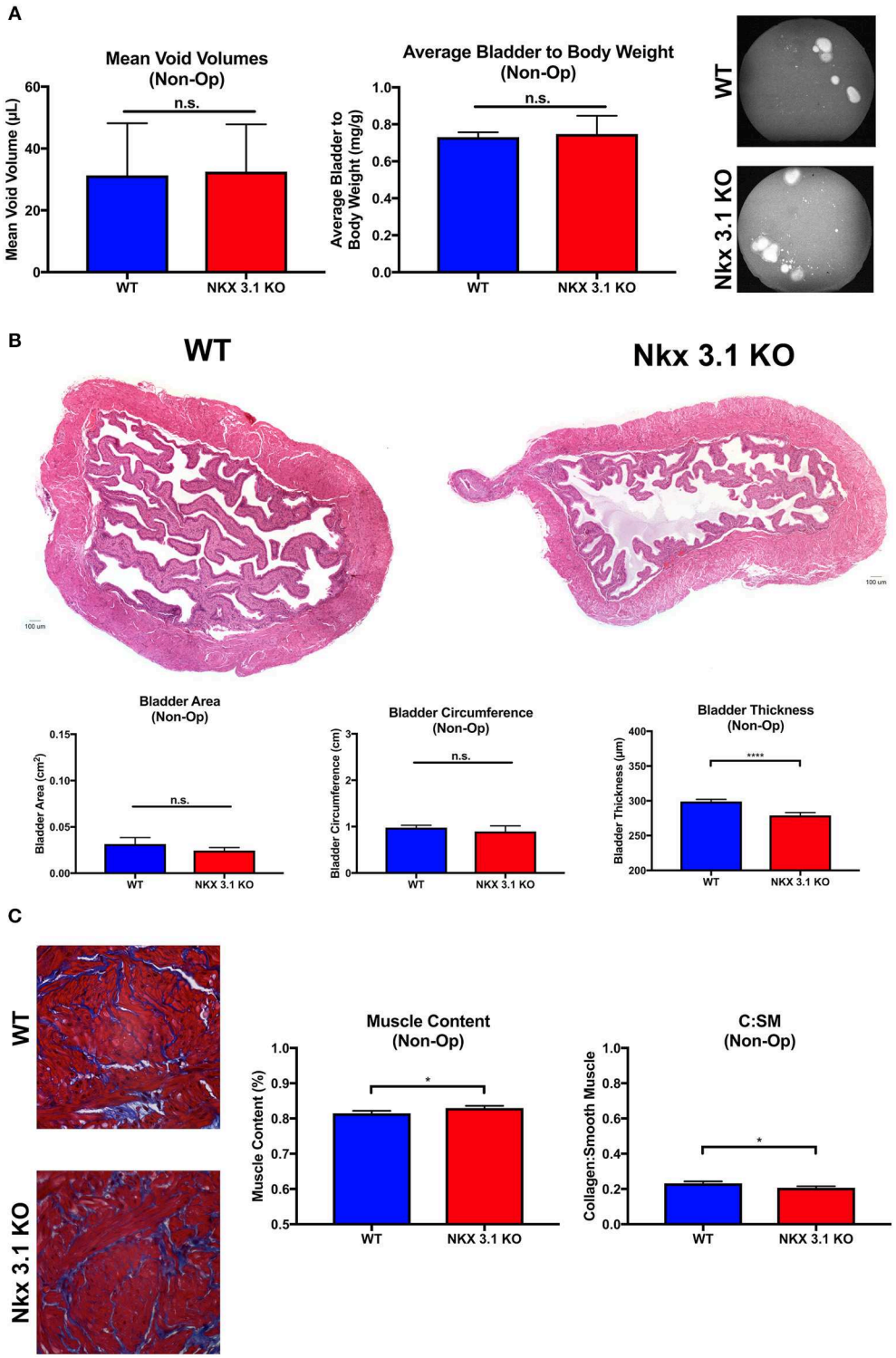


FIGURE 3 | Functional and histologic analysis of unoperated WT and *Nkx3.1*$^{KO/KO}$ bladders. **(A)** Comparison of mVV (WT, $N = 4$; *Nkx3.1*$^{KO/KO}$, $N = 4$), bladder: body weight ratios (WT, $N = 4$; *Nkx3.1*$^{KO/KO}$, $N = 4$), and representative VSOP images. **(B)** Histologic analysis of HandE images with basic bladder metrics such as bladder area, circumference, and thickness (WT, $N = 4$; *Nkx3.1*$^{KO/KO}$, $N = 4$). **(C)** MT images, muscle content, and collagen: smooth muscle ratio (WT, $N = 4$; *Nkx3.1*$^{KO/KO}$, $N = 4$). Error bars in all graphs are represented as a standard deviation with family-wise significance and confidence level calculated at a 95% confidence interval (n.s., $^{*}P < 0.05$ or $^{****}P < 0.0001$).

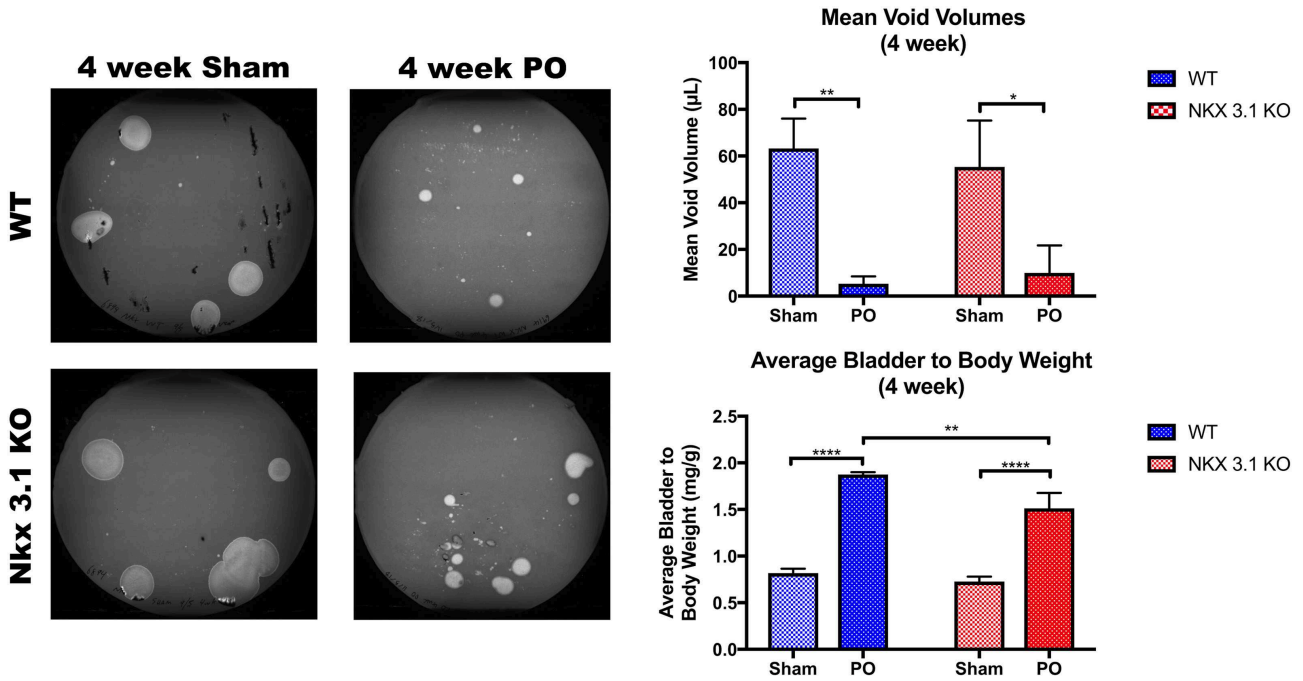


FIGURE 4 | Functional analysis of WT and *Nkx3.1*$^{KO/KO}$ mice after sham and PO. VSOPs demonstrated voiding patterns with mVV and bladder: body weight ratios for WT (Sham, $N = 5$; PO, $N = 3$) and *Nkx3.1*$^{KO/KO}$ mice (Sham, $N = 4$; PO, $N = 5$). Error bars in all graphs are represented as a standard deviation with family-wise significance and confidence level calculated at a 95% confidence interval (n.s., $^{*}P < 0.05$, $^{**}P < 0.01$, or $^{****}P < 0.0001$).

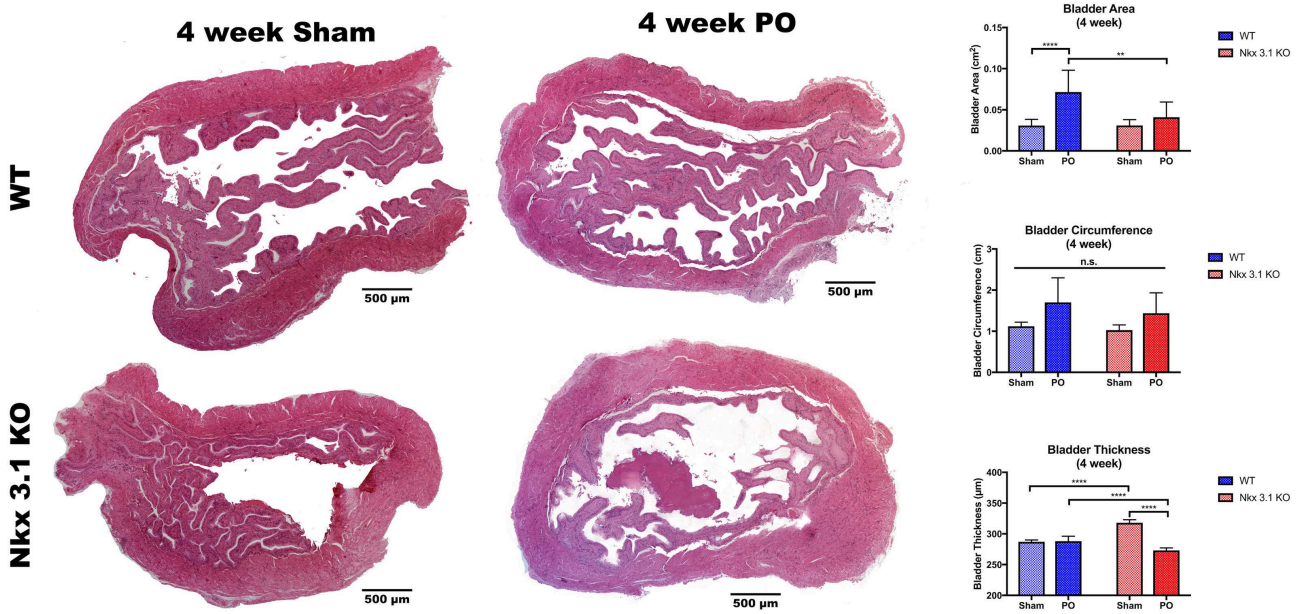


FIGURE 5 | Morphologic analysis of WT and *Nkx3.1*$^{KO/KO}$ mice after sham and PO. Representative whole bladder images and comparisons of bladder area, circumference, and thickness between WT (Sham, $N = 5$; PO, $N = 3$) and *Nkx3.1*$^{KO/KO}$ (Sham, $N = 4$; PO, $N = 5$) mice. Error bars in all graphs are represented as a standard deviation with family-wise significance and confidence level calculated at a 95% confidence interval (n.s., $^{**}P < 0.01$ or $^{****}P < 0.0001$).

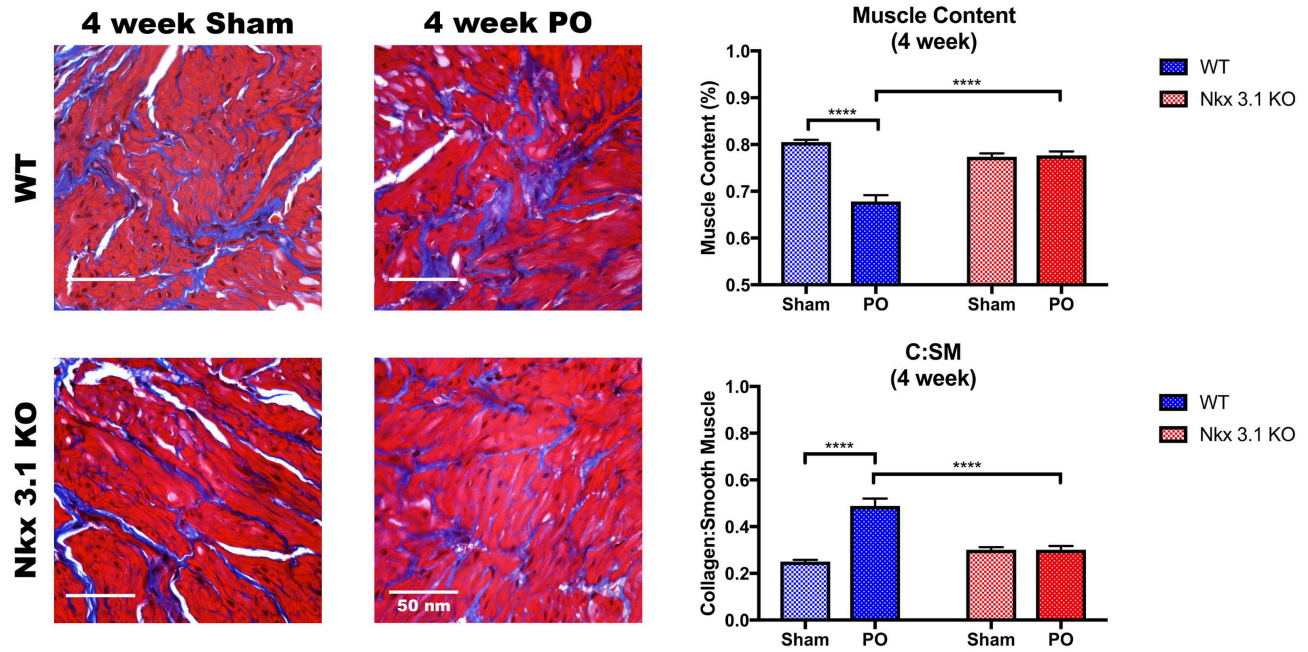


FIGURE 6 | Histologic analysis comparing WT and *Nkx3.1*$^{KO/KO}$ after sham and PO. Representative MT images and comparisons of muscle content and collagen: smooth muscle ratio between WT (Sham, $N = 5$; PO, $N = 9$) and *Nkx3.1*$^{KO/KO}$ (Sham, $N = 4$; PO, $N = 5$) mice. Error bars in all graphs are represented as a standard deviation with family-wise significance and confidence level calculated at a 95% confidence interval (n.s., $^{****}P < 0.0001$).

content is decreased (**Figure 6**). Despite the absence of *Nkx3.1*, these bladders showed a response to obstruction. However, our data indicated that *Nkx3.1* null male bladders remodel differently than either hormonally intact WT or castrated mice. This supported the idea that *Nkx3.1* function represents a smaller process than is altered by castration in the context of bladder PO.

Nkx3.1 Deletion Alters Collagen and Smooth Muscle Gene Expression in the Bladder

To investigate how NKX3.1 affects collagen matrix deposition and loss of muscle, we studied expression of collagen and smooth muscle genes in non-operated, sham and PO bladders. We expected there to be no difference in collagen gene expression in non-operated bladders comparing *Nkx3.1*$^{KO/KO}$ and WT. However, here we found that multiple collagens were slightly altered in expression; most notably, *Col7α1* had significantly increased expression in *Nkx3.1*$^{KO/KO}$ mice at baseline compared to WT (**Figure 7A**). This was surprising because it demonstrated that Nkx3.1 regulates collagen gene expression in male adult bladders. When comparing collagen gene expression after PO relative to sham, WT bladders demonstrated an increase in all collagens, except *Col7α1*, while *Nkx3.1*$^{KO/KO}$ bladders showed an increase in each collagen evaluated (**Figure 7C**). When comparing the increases in collagen gene expression between PO and Sham, WT showed a significantly higher difference compared to KO for two collagens (*Col3α1* and *Col8α1*), consistent with our prior histologic findings of greater fibrosis in WT bladders after PO. Interestingly, *Col7α1* followed a different pattern. WT bladders showed an equivalent expression of *Col7α1* after PO and sham. However, KO bladders showed a significant induction in *Col7α1* expression after PO compared to sham. While *Nkx3.1* seems to globally affect the transcriptional response of certain collagens, it seems to be particularly important as a negative regulator of *Col7α1* at baseline and in response to obstruction.

Loss of *Nkx3.1* appeared to preserve muscle in PO bladders based on MT stain. We therefore tested if smooth muscle gene expression was maintained in *Nkx3.1*$^{KO/KO}$ bladders after PO. In non-operated mice, all smooth muscle genes other than *Cald1* are slightly increased in expression at baseline in *Nkx3.1*$^{KO/KO}$ compared WT, although these differences are not as pronounced as the upregulation in *Col7α1* (**Figure 7B**). Given our previous findings of preserved muscle histologically in *Nkx3.1*$^{KO/KO}$ bladders after PO, we expected *Nkx3.1*$^{KO/KO}$ bladders to have preserved smooth muscle gene expression compared to WT. When comparing smooth muscle gene expression after PO relative to sham, both WT and *Nkx3.1*$^{KO/KO}$ bladders had decreased levels of all muscle markers except for *Cnn2*, which increased (**Figure 7D**). While WT bladders showed a significantly greater decrease in *Myh11* and *Cald1* expression, *Nkx3.1*$^{KO/KO}$ bladders showed a significantly greater decrease in *Srf* and *Acta2*. Although these findings are not what we initially expected, it indicates that perhaps the differences in muscle content percentages between WT

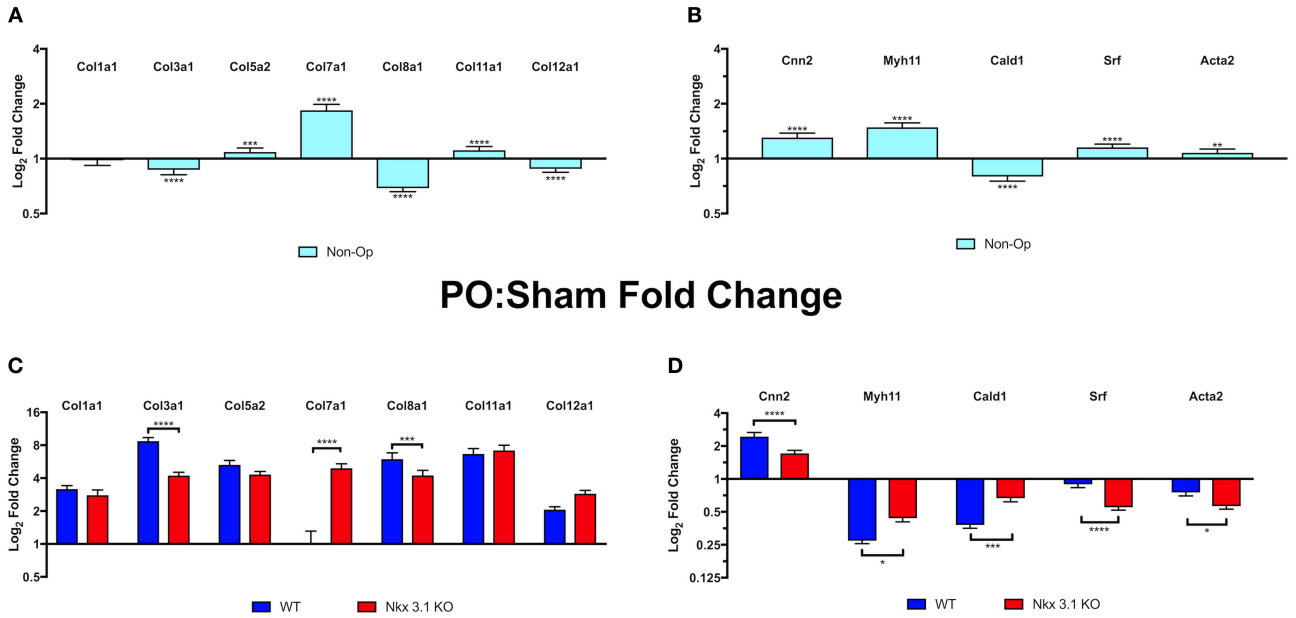


FIGURE 7 | Quantitative PCR comparison of collagen and smooth muscle transcripts in WT and *Nkx3.1*$^{KO/KO}$. **(A)** Collagen gene expression in non-operated *Nkx3.1*$^{KO/KO}$ bladders ($N > 3$) relative to non-operated WT bladders ($N > 3$). Horizontal line indicates point where the relative level of expression is the same in *Nkx3.1*$^{KO/KO}$ and WT bladders. Bars above the line indicate increased expression in *Nkx3.1*$^{KO/KO}$. **(B)** Smooth muscle gene expression in non-operated *Nkx3.1*$^{KO/KO}$ bladders ($N > 3$) relative to WT ($N > 3$). **(C)** Collagen gene expression in sham operated (blue bars) and PO operated (red bars) *Nkx3.1*$^{KO/KO}$ bladders relative to WT. **(D)** Smooth muscle gene expression in sham operated (blue bars) and PO operated (red bars) *Nkx3.1*$^{KO/KO}$ bladders ($N > 3$) relative to WT ($N > 3$). In all graphs horizontal lines indicate a value of 1 which is no change in gene expression. Values above the line indicate increased gene expression in *Nkx3.1*$^{KO/KO}$ bladders. Values below the line indicate decreased gene expression in *Nkx3.1*$^{KO/KO}$ bladders. The Y axis indicates $\log_2$ fold change as calculated using the $\Delta\Delta$Ct method. The Y axis is presented using a logarithmic scale. Error bars in all graphs are represented as a standard deviation with family-wise significance and confidence level calculated at a 95% confidence interval (n.s., $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, or $^{****}P < 0.0001$).

and *Nkx3.1*$^{KO/KO}$ are in fact a result of the differences in collagen content.

DISCUSSION

Although little research has focused on the effects of androgens on the bladder itself rather than the effects of the prostate on the bladder outlet, it has been previously suggested that there is a correlation between androgen activity and bladder dysfunction (22). Little is known, however, about the functional importance of the androgen pathway in bladder physiology after embryonic development, specifically in the setting of outlet obstruction. Our lab has previously shown that castrate, partially-obstructed male mice who undergo testosterone replacement have higher bladder weights and higher collagen: smooth muscle ratios than intact animals or those without testosterone replacement (9). Although the exact mechanism of action of the androgen pathway on the bladder is yet to be determined, it is clear that it does indeed play a role.

To further underscore this, our results have now identified *Nkx3.1*, as a critical mediator of the fibrotic response. Future experiments such as demonstrating that AR binds to the *Nkx3.1* promoter in response to PO will need to be done to cement the role of *Nkx3.1* as a direct response to AR. Nonetheless, our work here shows that partial bladder obstruction is sufficient to induce *Nkx3.1* expression in the bladder and that loss of *Nkx3.1* has significant effects on the remodeling that occurs after PO. Functionally, however, these bladders were similar to WT in mVV, in contrast to our previous findings with castration. As *Nkx3.1* is only one of a large number of genes involved in the intricate androgen pathway, it is likely there are additional pathways responsible for the other effects noted in the castration model.

Nkx3.1 was initially found as an androgen regulated homeobox gene located mainly in the prostate, and was extensively studied as a tumor suppressor in prostate cancer (23, 24). Multiple studies, however, have since linked it to other tissue types and downregulation to their associated malignancies including the breast, oral mucosa, and liver (25–28). There is scarce literature describing *Nkx3.1* expression specifically in the bladder. In fact, NKX3.1 positivity has been shown to be a useful adjunct marker in differentiating prostate and urothelial carcinomas (29, 30). At baseline, we observed low levels of *Nkx3.1* expression in the bladder and surprisingly an effect

on *Col7α1* expression in the knockout. This indicates that *Nkx3.1* may indeed have some functions in the normal bladder. Remarkably this effect was on a collagen gene that has not been implicated as a target of NKX3.1. This and our other findings involving collagen genes merit further study into how NKX3.1 regulates these genes as well as its involvement in modifying collagen architecture.

Importantly, bladder development and function are preserved in *Nkx3.1* null mice. In the prostate, *Nkx3.1* deletion has been shown to result in defects in prostate ductal morphogenesis and secretory protein production (21). Although our results cannot exclude a role for *Nkx3.1* in bladder development, they do demonstrate that the bladder is able to develop with normal voiding function and histologic features in its absence with small but significant differences in collagen: smooth muscle ratio, and bladder thickness.

Despite identifying a downstream androgen receptor target in bladder remodeling after obstruction, it remains unclear how NKX3.1 ultimately affects this process. The overall response to injury from PO is likely a multifactorial process, with NKX3.1 playing a small but critical role. NKX3.1 was shown to have an interactive relationship with the Myc oncoprotein (31). In fact, the authors of this study proposed that NKX3.1 and Myc both bind to a number of target genes and had opposing effects on the expression of these genes. In this model, NKX3.1 and Myc had dynamic and potentially opposing functions. Myc has been implicated in regulating smooth muscle cell proliferation and survival (32, 33). Thus, NKX3.1 could be induced in the bladder in response to obstruction to modify pathways controlled by the Myc oncoprotein.

Nkx3.1 loss has previously been shown to be related to increased expression of VEGF-C in prostate cancer (34). If NKX3.1 represses VEGF-C in obstruction, we might expect to see alterations to blood vessels. Deletion of *Nkx3.1* in the context of PO would lead to increased neoangiogenesis which may improve healing and remodeling after obstruction. pBOO has been shown to cause tissue hypoxia and upregulation of the HIF1 pathway and subsequent studies have shown that inhibition of this pathway with 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) after PO improved bladder function and collagen: smooth muscle ratio (35–37). Although these results seem to contradict our findings, VEGF remained relatively similar in their PO + placebo and PO + 17-DMAG group, even though they showed improvement in bladder remodeling and voiding function. This may indicate that perhaps the benefits of blocking the HIF1 pathway are from alternative pathways outlined by Iguchi et al. (38).

Additionally, *Nkx3.1* may mediate its function through regulation of stem cell differentiation. The murine bladder has previously been shown to support a population of mesenchymal stem cells, which *Nkx3.1* may modulate (39). MicroRNAs have been shown to have a critical role in keratinocyte differentiation through silencing of *Nkx3.1* (40). Similarly, *Nkx3.1* may play a critical role in bladder remodeling by inhibiting stem cell response to obstruction. Future studies will attempt to clarify the stem cell response to PO and the role of *Nkx3.1* in this process.

PO is a common urologic pathology seen in young boys with PUV, males with BPH, and females with pelvic organ prolapse. The long-term effects of obstruction on the bladder are irreversible and, therefore, early treatment is imperative. Despite prompt treatment in PUV, these patients often progress to poorly functioning fibrotic bladders. Identifying the molecular mechanisms of this process will provide potential therapeutic targets to prevent this maladaptive remodeling process from occurring. Our findings identify *Nkx3.1* as a key modulator of fibrosis. As *Nkx3.1* has mainly been studied in the realm of prostate cancer, it's role in organ fibrosis has not previously been described. As it clearly plays a role in bladder fibrosis, its effect on other organs and fibrotic remodeling will warrant further investigation. The clinical implications of our study are that there is likely a molecular basis for differences in bladder fibrosis observed between males and females and that pharmacologic approaches to modulate NKX3.1 in prostate cancer could be extended to treat obstructive uropathies.

CONCLUSION

We have identified *Nkx3.1* as an important mediator of bladder fibrosis in bladder outlet obstruction. Although the bladder seems to be morphologically and functionally intact, fibrosis is attenuated in the absence of *Nkx3.1*. Maladaptive changes to collagen and smooth muscle specific gene expression after PO were ameliorated by loss of *Nkx3.1*. Our results provide evidence that *Nkx3.1* plays a role in both bladder homeostasis and fibrosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of surgical guidelines, Animal Care and Use Committee. The protocol was approved by the Institutional Animal Care and Use Committee of Northwestern University (protocol no. IS00003975).

AUTHOR CONTRIBUTIONS

MP, DB, and NT contributed to data collection, analysis, conception, and design. MP and RD wrote the first draft of the manuscript. NT performed the statistical analysis. AG, KK, PF, NK, MD, and BL contributed to data collection and analysis. EG and RD contributed to analysis, conception, and design. MP, NT, AG, EG, and RD contributed to manuscript revisions, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2019.00446/full#supplementary-material

Supplemental Table 1 | Raw data from AR target gene qRT-PCR microarray. Control group is RNA from sham operated bladders. Group 1 is RNA from PO operated bladders. Position is the position on the microarray plate. Symbol is the gene abbreviation. AVG Ct is the average of three biological replicates. Standard deviation is the standard deviation of the biological replicates.

Supplemental Table 2 | Raw data from AR target gene qRT-PCR microarray. Control group is RNA from PO operated bladders. Group 1 is RNA from CPO (PO in castrated mice) operated bladders. Position is the position on the microarray plate. Symbol is the gene abbreviation. AVG Ct is the average of three biological replicates. Standard deviation is the standard deviation of the biological replicates.

Supplemental Table 3 | RT2 Profiler PCR Array Key. This is the key to the plates for which data is shown in **Supplemental Tables 1**, **2**.

Supplemental Figure 1 | Accumulation of nuclear localized androgen receptor after partial bladder outlet obstruction. Top: Bar graph showing quantification of nuclear localized AR in the bladder after (left to right) sham, partial obstruction in a normal male mouse (PO), partial obstruction in an orchiectomized mouse (CPO) partial obstruction in an orchiectomized mouse administered testosterone (CPOT) and partial obstruction in an orchiectomized mouse administered dihydrotestosterone (CPOD). Percent nuclear stain indicates the percent of red pixels (AR) that are colocalized with blue (DAPI) pixels. Sample sizes are: sham (5), PO (5), CPO (6), CPOT (4), CPOD (4). Error bars indicate standard error of the mean. Significance is indicated by lines extending between bars. Bottom are representative images used during the quantification. Blue represents DAPI nuclear stain and red indicates staining with anti-androgen receptor antibody.

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ORIGINAL RESEARCH
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The Engagement of the Pelvic Floor Muscles to the Urethra, Does Variation in Point of Action Exist?

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Purpose: Lower urinary tract dysfunction (LUTD) occurs frequently in girls and may display a spinning top urethra (STU) on voiding cysto-urethrogram (VCUG) in case of dysfunctional voiding. A STU presents as a narrowing of the urethra caused by a lack of relaxation of the pelvic floor musculature during micturition and may vary in length between the proximal and the distal urethra. Although a STU has been recognized since 1960 as a pathological entity on VCUG, no reports exist on the different levels of engagement of the pelvic floor muscles to the urethra as expressed by the varying length of the phenomenon. The aim of our study is to demonstrate the wide anatomical variation in the level of engagement of the pelvic floor musculature to the urethra.

Materials and Methods: Dynamic ultrasound videos of pelvic floor musculature of 40 girls with LUTD were reassessed by three observers, looking for the level of engagement of the puborectalis muscle (PRM) to the urethra during coughing, Valsalva and hold-up maneuver. Three levels were defined, for the level of engagement of the pelvic floor to the urethra, proximal, mid, and distal. Intra- and inter-rater variability was analyzed using Cohen's kappa statistics.

Results: A wide range of points of action was found on the assessed ultrasound videos. Intra- and inter-rater agreement showed different levels of conformity, varying over a wide spectrum (intra-rater kappa 0.145–0.546; inter-rater kappa −0.1030.724). Throughout the assessed videos, all not-corresponding intra-rater observations differed maximal one category. Of the not-corresponding inter-rater observations, 90% differed maximal one category.

Conclusion: An anatomical variation in levels of engagement of the PRM to the urethra does exist. The clinical value of this finding, whether the point of engagement influences symptomatology or treatment success of LUTD, is currently being studied.

Keywords: pelvic floor anatomy, puborectalis muscle, urethra, dynamic ultrasound, anatomical variation, female

INTRODUCTION

From pediatric and radiologic literature on VCUG we have learned that a spinning top urethra (STU) may be seen in girls with LUTD (1–3), diagnosed as dysfunctional voiding, who are not able to properly relax their pelvic floor during micturition (3–5). This widely used term reflects a marked dilatation of the proximal part of the urethra (3–5), up to the level of engagement of the puborectalis muscle (PRM), a finding that has been recognized as a pathological entity during VCUG for nearly five decades now (6). Firstly, the narrowing of the urethra was considered to be an anatomical obstruction with abundant literature to cure this by dilatation or urethrotomy (7). The dilatation and internal urethrotomy that had been advised was later named as a barbaric procedure (8). During the eighties of the last century literature describing non-neurogenic functional problems of the lower urinary tract came up culminating in the first standardization report in 1998 (9). It has learned us that the constriction of the urethra in the VCUG with STU does not represent a membrane but a functional constriction by the pelvic floor musculature, with the PRM in direct contact with the rectum, vagina, and urethra (1–4, 10).

From experience, we know that the length of the STU on VCUG may vary between the proximal and distal urethra, an observation that is suggestive for the existence of different levels of point of action of the pelvic floor musculature to the urethra (**Figure 1**). Literature on this phenomenon is very sparse (7, 10, 11).

Over years, we have assessed the function of the pelvic floor musculature (PFM) and the urethra by perineal dynamic ultrasound (US) of the pelvic floor during coughing, straining (Valsalva) and when performing a hold-up maneuver (try to hold back your micturition by contracting your pelvic floor muscles). During contraction of the PFM and sphincter muscles a lengthening and anterior displacement of the urethra and compression of the vagina and rectum can be observed (10, 12, 13). A comprehensive overview of the anatomy and dynamic function of the pelvic floor musculature is given by Chamie et al. (10). **Figure 2** is a schematic drawing of the PRM in relationship to the rectum, vagina, and urethra.

The aim of our study is to demonstrate the existence of a wide range of points of action considering the level of engagement of the PRM to the urethra. **Figure 2A** gives an overview of the aspect of the pictures that are observed; **Figures 2B–E** show the difference between a distal and a proximal engagement of the PRM in relaxed and contracted state. Two corresponding videos showing contraction of the PFM in a case of proximal engagement and a case of distal engagement are uploaded as proximal and distal (**Supplementary Videos 1**, **2**). **Figure 3** is a schematic drawing of the anatomy.

Abbreviations: PRM, puborectalis muscle; PFM, pelvic floor muscles; STU, spinning top urethra; VCUG, voiding cysto-urethrogram; LUTD, lower urinary tract dysfunction; US, ultrasound.

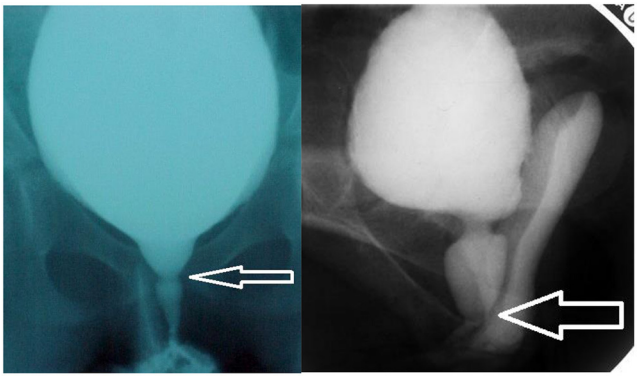

FIGURE 1 | (A) (left) proximal spinning top urethra and **(B)** distal STU. Two examples of VCUG pictures with spinning top urethra (STU), one with a short STU with proximal constriction of the pelvic floor musculature, one with a distal constriction and a long STU.

MATERIALS AND METHODS

In our 3rd line referral center for children with LUTD refractory to treatment in the second line, as part of our standard protocol, a perineal US is performed to assess the pelvic floor function in children. Urethra, bladder neck, and pelvic floor muscle function are routinely observed while resting, during coughing, when performing Valsalva and during a hold-up maneuver (12, 13). When making a dynamic video the US stores 140 pictures in 3 s. The pictures and videos are stored in the electronic patient data system and can be retrieved for review at any time.

We retrospectively re-assessed dynamic ultrasound videos of 40 school age girls with refractory LUTD that were referred to our pediatric incontinence university clinic between the years 2010–2016. Age at US assessment was between 9 and 15 years. They all had earlier unsuccessful urotherapy and pharmacotherapy in outpatient programs in general hospitals, for LUTD diagnosed as dysfunctional voiding and/or overactive bladder. The vast majority (33) was at prepubertal age and menstrual status has not been recorded routinely. Inclusion was determined by the availability of a complete set of US pictures and at least 3 videos of sufficient quality for reassessment.

The level of engagement of the PRM upon the urethra was assessed on the existing US videos. Contraction of the PFM on the videos can be observed during hold-up maneuver, Valsalva, and coughing. Three different observers (a pediatric urologist with abundant US experience and two non-specialists MD) classified independently the level of engagement of the PRM to the urethra. US videos were reviewed in randomized order and assessed during two reading sessions with unlimited reading time available, a minimum of 14 days existing in-between readings.

US was done by placing a 7 Mhz convex probe, covered by a protective sleeve, directly on the urethral meatus with the patient in supine position. A Philips HD11XE® US system has been used.

Prior to the independent video assessment, the pediatric urologist trained the other observers in reviewing dynamic US videos. Points of action were predefined in three thirds; proximal-, mid-, and distal urethra, indicating the level of engagement of the PRM to the urethra. During

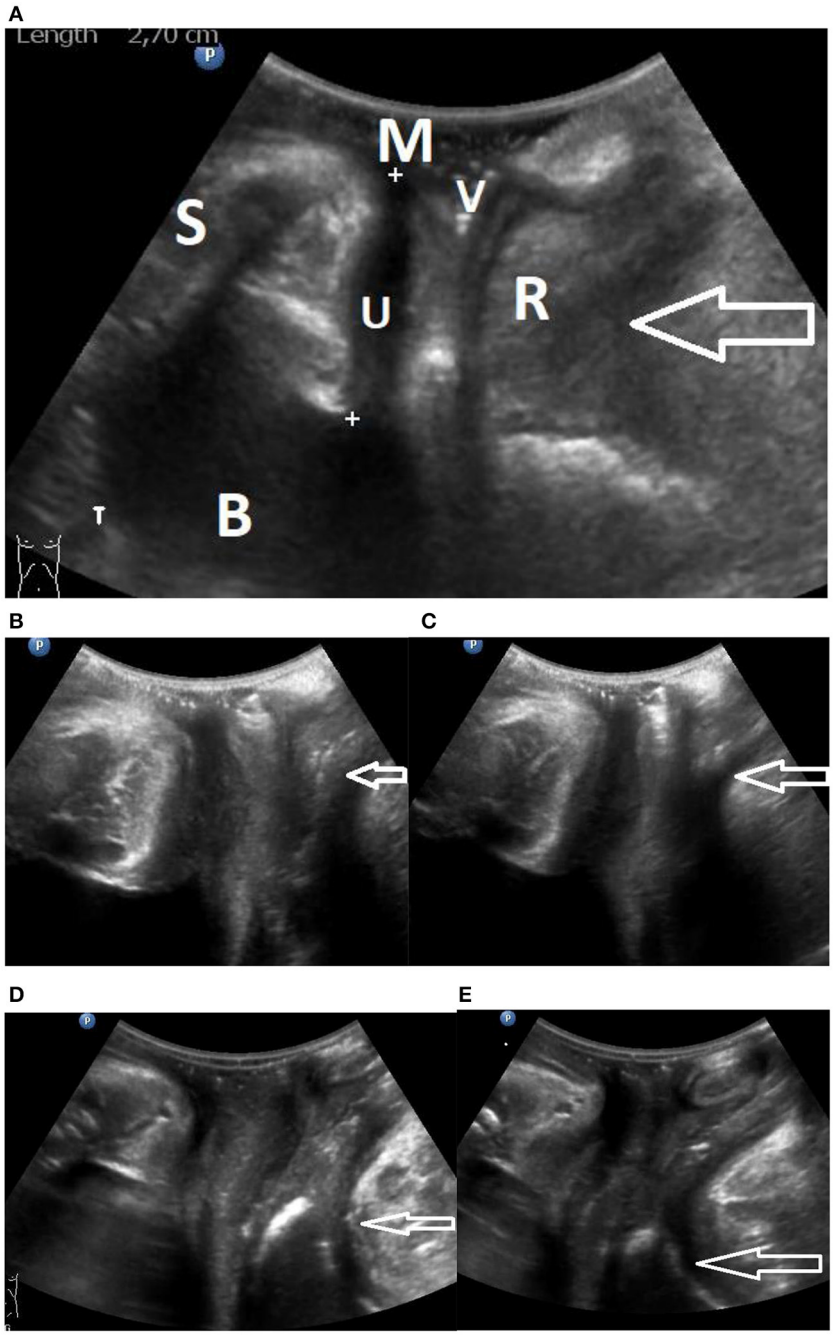

FIGURE 2 | (A) Gives an overview of a perineal ultrasound image. B, bladder; U, urethra; V, vagina; R, rectum; M, meatus; S, symphysis. The point of the arrow is in the puborectalis muscle. **(B,C)** Snapshots from a video relaxed and contracted. Distal engagement of the PRM, **(B)** relaxed and **(C)** contracted state, arrows point at PRM engagement. Videos are uploaded as proximal video and distal video. **(D,E)** Snapshots from a video relaxed and contracted. Proximal engagement of the PRM, **(D)** relaxed state, **(E)** contracted state. Arrows point at PRM engagement.

contraction of the pelvic floor and sphincteric muscles an elongation and compression of the urethra is to be expected. The level of compression of the urethra is the point of engagement of the PRM and can be seen varying from the bladder neck to the distal urethra near the meatus.

OUTCOME MEASURES

Primary end-point was the existence of variability in the level of engagement of the PRM to the urethra. Intra- and inter-rater variability were analyzed using Cohen's kappa statistics.

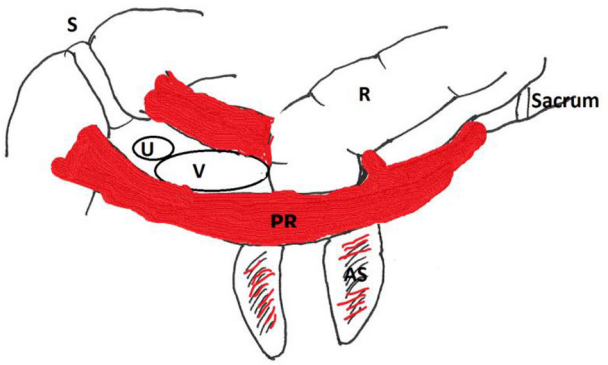

FIGURE 3 | Schematic drawing. S, Symphysis; R, rectum; PR, puborectalis muscle; V, vagina; U, urethra. AS, anal sphincter.

TABLE 1 | Variation is points of action classification.

N = 40	Proximal engagement	Mid engagement	Distal engagement
Observer 1; reading 1 (Pct. of total)	*N* = 6 (15%)	*N* = 28 (70%)	*N* = 6 (15%)
Observer 1; reading 2 (Pct. of total)	*N* = 23 (57.5%)	*N* = 11 (27.5%)	*N* = 6 (15%)
Observer 2; reading 1 (Pct. of total)	*N* = 9 (22.5%)	*N* = 20 (50%)	*N* = 11 (27.5%)
Observer 2; reading 2 (Pct. of total)	*N* = 8 (20%)	*N* = 24 (60%)	*N* = 8 (20%)
Observer 3; reading 1 (Pct. of total)	*N* = 18 (45%)	*N* = 16 (40%)	*N* = 6 (15%)
Observer 3; reading 2 (Pct. of total)	*N* = 14 (35%)	*N* = 20 (50%)	*N* = 6 (15%)

Kappa values were defined as follows: slight: 0.00–0.20; fair: 0.21–0.40; moderate: 0.41–0.60; substantial: 0.61–0.80; and almost perfect alignment: 0.81–1.00 (14). Statistical analyses have been performed using IBM Statistical Package for Social Sciences software (version 21, SPSS).

The Institutional Ethical Committee consented with the followed procedure (reference number WAG/mb/19/020335).

RESULTS

Results on the dynamic ultrasound video assessment of the 40 included patients are presented in **Table 1**. All three predefined categories of points of action were independently visualized by all observers, although in different frequencies. Results show the distribution of variation in level of engagement on the urethra, throughout the different readings by different observers.

TABLE 2 | Intra-rater agreement.

N = 40	Observer 1	Observer 2	Observer 3
No. of agreement (Pct. of total)	*N* = 16 (40%)	*N* = 29 (72.5%)	*N* = 22 (55%)
Cohen's kappa	0.145	0.546	0.274

TABLE 3 | Inter-rater agreement.

N=40		Observer 3		Observer 2	
		Reading 1	Reading 2	Reading 1	Reading 2
Observer 1	Reading 1	32.5%	62.5%	77.5%	85%
	(Cohen's kappa)	(−0.61)	(0.358)	(0.612)	(0.205)
	Reading 2	65%	60%	45%	52.5%
	(Cohen's kappa)	(0.425)	(0.452)	(0.205)	(0.315)
Observer 2	Reading 1	27.5%	55%	X	X
	(Cohen's kappa)	(−0.103)	(0.286)		
	Reading 2	37.5%	57.5%	X	X
	(Cohen's kappa)	(0.033)	(0.302)		

Cohen's kappa was utilized to determine intra- and inter-rater agreement. **Table 2** shows the outcome for all individual observers when the different reading sessions are compared to each other. Intra-rater conformity between the two different sessions varies from 40% (*kappa* 0.145) to 72.5% (*kappa* 0.546). Inter-rater variability through comparison of the different reading sessions performed by the observers is presented in **Table 3**. Results are displayed as agreement percentage with coordinating Cohen's kappa. Herein a wide range in conformity is found, with values varying from 27.5% (*kappa* –0.103) to 85% (*kappa* 0.724).

To determine the scope of the non-conforming observations, we subsequently assessed our data. We hereby determined that 90% (n = 36) of the non-conforming observations differed maximal one category when classified points of action were compared. This implies that an observation could change from proximal into mid or mid into distal, not from proximal into distal.

DISCUSSION

Our results show that the PRM have different points of engagement to the urethra. All observers reported independently on the existence of different points of action. We found that the level of agreement differed between the various observers and reading sessions. The diverse levels of agreement suggests that assessing the exact level of engagement is challenging, but underscores the finding that different levels of engagement do exist. In all intra-rater cases and in the vast majority (90%)

of inter-rater cases scoring differences were to the utmost 1 category, that is proximal instead of mid or mid instead of distal.

To our best knowledge, this is, after a histological suggestion done in 1986, the first report on the existence of anatomical variation with different points of actions of the PRM on the urethra (11). Therefore, no reference standard was available for assessing the levels of engagement of the PRM onto the urethra. We acknowledge this as one of our shortcomings as it may have resulted in an intra- and inter-rater variability.

The clinical relevance to do dynamic ultrasound of the lower urinary tract lies in the fact that the vast majority of our refractory patients did have earlier pelvic floor physical therapy. By looking at the conscious command of the PFM we can detect those subjects with apraxia of the PFM in need for specific physical therapy with anal balloon biofeedback. Of course, one can discuss whether this study is focussed specifically on the PRM or that we should call it a study on the pelvic floor musculature, since the PRM is part of the levator ani and pelvic floor musculature as a whole. It is not exactly possible to discriminate, by US, between the puborectalis and the pubococcygeus muscle. Chamie et al. (10), published an elegant study with, other than US, also MRI pictures and videos that may be used as a control study and justifies the use of the term PRM in our report.

Although we report on the existence of different points of action, our result do not provide an answer to the clinical relevance of this observation in children with LUTD. We tried to demonstrate the wide variability, but did not assess the difference in symptomatology or treatment success. The study has been initiated by the fact that we had an impression that those girls with a distal engagement of the PRM performed worse in urotherapy. This impression could not be confirmed in this relatively small group of patients.

Limitations of the study are the fact that it has been a retrospective study and that 2 of 3 reviewers of the US reassessment were relatively unexperienced MD's.

CONCLUSION

The results of this study have demonstrated that different points of action of the PRM can be observed on dynamic ultrasound videos. Therefore, we conclude that anatomical variation with different levels of engagement on the urethra does exist. The clinical value of this finding, whether the point of engagement influences symptomatology or treatment success of LUTD, is subject of an on-going study. The relatively poor inter- and intra-observer levels of agreement illustrate that correct interpretation of the pictures is difficult, but the different observations vary maximal one level, thus not influencing the final conclusion.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical committee University Medical Center Utrecht. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TJ: design of the study, instruction of co-authors to assess ultrasound videos, and editing of the manuscript. F-JG: assessment of ultrasound video's, statistic evaluation, and preparation and writing the manuscript. HJ: assessment of ultrasound videos and preparation and writing the manuscript. KM: collection of patients and patient material, codesigner of the study, and literature search.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2019.00522/full#supplementary-material

Supplementary Video 1 | Shows a contraction of a distal engagement of the PRM.

Supplementary Video 2 | Shows a contraction of a proximal engaged PRM.

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ORIGINAL RESEARCH
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Diagnosis and Treatment of Hypospadias With Megameatus Intact Prepuce

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Purpose: To evaluate the diagnosis and treatment methods of hypospadias with megameatus intact prepuce (MIP).

Materials and Methods: A retrospective analysis was performed in 27 MIP children, 13 of whom underwent tubularized incised plate urethroplasty (TIP procedure), 7 underwent the Duplay procedure, 5 underwent the Mathieu procedure, 1 underwent meatal advancement and glanuloplasty (MAGPI procedure), and 1 underwent the glans approximation procedure (GAP). The patients were followed for 6–36 months to evaluate the surgical outcomes by the Pediatric Penile Perception Score (PPPS).

Results: A total of 27 patients with a mean age of 8.12 ± 3.0 years were enrolled in this study, and 25 cases (25/27, 92.6%) were accidentally discovered during the first visit for phimosis. The patients had a formed urethra of 0.5 to 1.5 cm. Complications occurred in 4 of the 27 patients (14.81%): 2 patients with urethral fistula and 2 patients with meatal stenosis. One patient had a case of self-healed urethral fistula, and the remaining 3 patients underwent reoperation. The post-operative effect was satisfactory in all patients, and the urinary flow and stream during urination were normal. The overall average PPPS score of non-operative surgeons and parents was satisfactory. There were no significant differences in meatus appearance, glans appearance, skin appearance, and general appearance PPPS score among the Mathieu, TIP, and Duplay surgical procedures.

Conclusions: MIP clinical manifestations are concealed and usually noted when circumcision is attempted. The suitable procedure for each patient should be tailored according to the anatomic features, and several techniques can be used with good functional and cosmetic results.

Keywords: hypospadias, megameatus, intact prepuce, urethral plate, PPPS

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INTRODUCTION

Megameatus intact prepuce (MIP) is a unique variant of hypospadias and is a clinically rare condition, with an incidence of ∼1/10,000, accounting for 1–3% of the incidence of hypospadias (1, 2). MIP was first reported in detail by Duckett and Keating (3), and the discovery rate of MIP is also rising with the increasing popularity of health examinations.

In recent years, an increasing number of articles have focused on this rare hypospadias variant, and the awareness of and surgical procedures for MIP continue to evolve (4, 5). Due to the anatomical particularity of MIP, it is necessary for clinicians to design a suitable surgical method taking into account the development of the glans, the width of the urethral plate, the depth of the urethral groove, and the shape and position of the urethral opening, to achieve good therapeutic effects (3, 6, 7). No single urethroplasty method provides a universal solution for all patients. In the current study, we reviewed the clinical data of 27 MIP children from our hospital to better characterize the diagnosis of and treatment methods for MIP.

MATERIALS AND METHODS

Institutional review board approval for this study was obtained from the Ethics Committee and Institutional Review Board of the Hospital of Shantou University Medical College (No. 2018-39). For the protection of privacy, the identities of the patients and physicians were scrambled in accordance with the Personal Electronic Data Protection Law. A retrospective analysis was performed for 27 children with MIP who were admitted to our hospital from January 2010 to January 2018. The ages ranged from 2 to 13 years, with an average age of 8.12 ± 3.0 years. All children with diagnosis of MIP were included. Exclusion criteria were as follows: (i) patients that have previously underwent urethroplasty/circumcision; (ii) MIP diagnosed in adulthood; (iii) Non-MIP patients with hypospadias. The first visit of the children to the hospital was as follows: 2 cases were due to abnormal position of the urethra, and the other 25 cases were discovered with phimosis during the examination before post-hetomy or exposure of the balanus during post-hetomy. Of the 27 MIP patients, the urethral opening was located in the coronary sulcus in 15 cases, the distal penis in 10 cases and the glans in 2 cases (**Figure 1**). Thirteen patients underwent one-stage tubularized incised plate urethroplasty (TIP procedure), 7 patients underwent one-stage penile skin tube urethroplasty (Duplay procedure), 5 patients underwent the Mathieu procedure, 1 patient underwent the glans approximation procedure (GAP), and 1 patient underwent meatal advancement and glanuloplasty (MAGPI) (**Table 1**).

The same surgical team performed the surgeries in all cases. The urethra was sutured with 6-0 absorbable thread, and proper pressure was applied to the penis with a self-adhesive elastic bandage. Antibiotics were used to prevent infection after surgery. Urethral catheters were in place for 12–14 days, and the patients were followed for 6–36 months.

The surgical results were evaluated according to the non-operative surgeons and parents' satisfaction with the appearance of the penis after surgery and the urination of the child. Satisfaction with the appearance of the penis after surgery was assessed with reference to the Pediatric Penile Perception Score (PPPS) (8, 9). The specific scoring items include the appearance of the meatus, the appearance of the glans, the appearance of the skin, and the general appearance. Each item is scored according to the subjective satisfaction of the subject: very satisfied (3 points), satisfied (2 points), dissatisfied (1 point), and very dissatisfied (0 points) (8, 9). The outcomes were compared in the different groups. One-way analyses of variance (ANOVA) were adopted for statistical analysis, assuming $p < 0.05$ as significant.

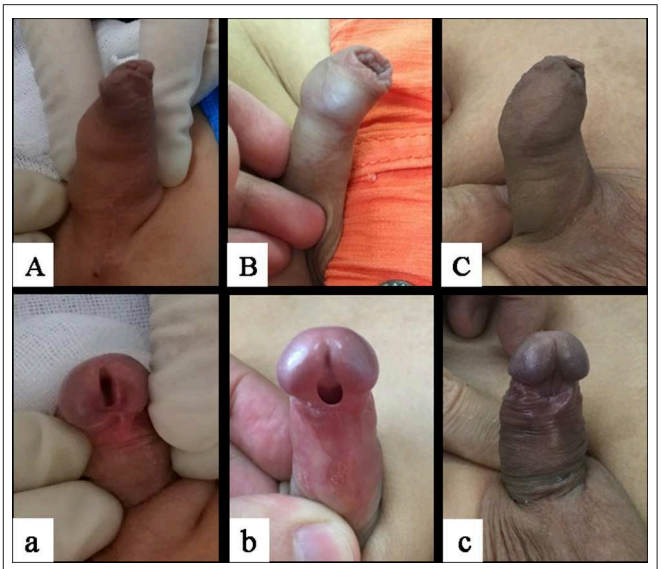

FIGURE 1 | Types of megameatus intact prepuce. (**A**a) Glans, (**B**b) coronary sulcus, (**C**c) distal penile; **(A–C)** intact foreskin, (a–c) abnormal urinary meatus. The images were published with the written informed consent of the parents.

TABLE 1 | The types of MIP and the surgical techniques for the study group.

Meatal location	Patients	TIP	Duplay	Mathieu	MAGPI	GAP
Glans	2	0	0	0	1	1
Coronary sulcus	15	10	0	5	0	0
Distal penis	10	3	7	0	0	0

RESULTS

The urethras formed in the 27 MIP patients ranged from 0.5 to 1.5 cm (**Figure 2**). One patient underwent MAGPI, one underwent the GAP, 5 underwent the Mathieu procedure, 13 underwent the TIP procedure, and 7 underwent the Duplay procedure. Complications occurred in 4 of the 27 patients (14.81%): 2 patients with urethral fistula and 2 patients with meatal stenosis. Urinary fistula occurred as early as 2 weeks after surgery, and meatal stenosis occurred 48 weeks after surgery. There was 1 case of self-healed urethral fistula, and the remaining 3 patients underwent reoperation (**Table 2**).

The post-operative effect was satisfactory in all patients, and the urinary flow and stream during urination were normal. There was no urinary fine line or dysuria, no urine flow spray, and no urinary fistula or other complications (**Figure 3**). According to the PPPS score, non-operative surgeons and parents had a satisfaction score for the meatus appearance, glans appearance, skin appearance, and general appearance. One-way ANOVA was

used to compare the statistical differences of these PPPS score among the Mathieu, TIP, and Duplay surgical procedures, and the results showed no significant differences ($P > 0.05$) (**Table 3**).

DISCUSSION

The embryologic pathogenesis of MIP still remains unclear. Duckett and Keating (3) has suggested that the foreskin and urethra develop independently and are unrelated. Due to excessive division of the glans, the distal urethra that has already formed is split to form a large urethral opening, while the foreskin develops normally. Nonomura et al. (10) speculated that ischemia and compression necrosis may occur after formation of a normal urethra, causing MIP. Stephens and Fortune (11) considered that the ingrowth of the epithelium on the top of the glans leads to delayed connection or failed fusion with the proximal urethra, which results in temporal high-pressure blockage of the distal urethra, thus forming MIP. It has been theorized that hypospadias results from incomplete fusion of the urethral folds, resulting in an incomplete urethra and incomplete or hooded foreskin. In the MIP variant of hypospadias, glanular urethra forms from ectodermal pit at glans tip and open end of urethral groove. Maldevelopment of glanular epithelial infolding would appear to be the abnormal process responsible for MIP formation. Complete closure of urethral fold and prepuce, but canalization of glanular plate is incomplete that leads to megameatus intact prepuce (5, 12, 13). Until now, all of these theories have failed to explain the embryology of MIP, and the specific reasons need further exploration.

The MIP lesion is hidden and difficult to find; most patients are often misdiagnosed early as having phimosis because the foreskin is intact. Some cases of MIP are found during circumcision in neonates or infancy in Europe or America (14). In the current study, 25 cases were accidentally discovered during a visit for phimosis, while the family members were unaware of any urinary tract abnormalities. Abnormalities were found during circumcision, which was then changed to surgery for hypospadias. The actual incidence of MIP might be much higher than what is reported because a significant number of children with MIP are not detected or are untreated after diagnosis.

The distinct anatomic features of MIP have led it to differ from other typical hypospadias. The anatomic characteristics of MIP are as follows: an intact foreskin, wide and fish-like urethral opening, wide and shovel-like glans, deep navicular fossa, and no ventral downward curvature or just slight dorsal bending of the penile body (7). MIP can be divided into glans, coronary sulcus and distal penis types according to the position of the urethral opening (7). For the particularity of MIP anatomical morphology, it is necessary for clinicians to design a suitable surgical method taking into account the development of the glans, the width of the urethral plate, the depth of the urethral

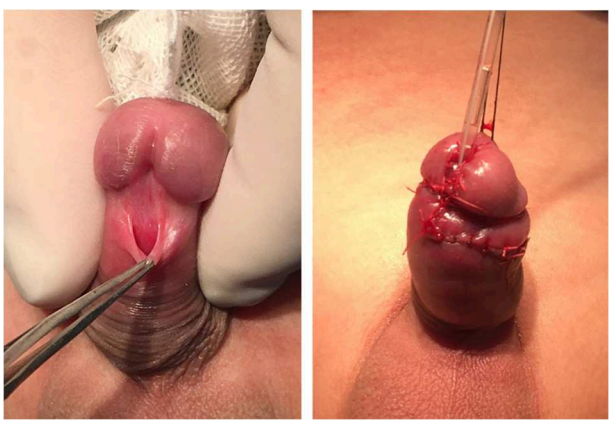

FIGURE 2 | The giant urethral opening was reduced and translocated to the middle of the glans. The images were published with the written informed consent of the parents. The patient is the same case as **Figure 1C**,c (**Supplementary Figure 1**).

FIGURE 3 | Postoperative follow-up showed satisfactory appearance and normal urinary flow and stream. The images were published with the written informed consent of the parents.

TABLE 2 | The complications of the surgical procedures.

Surgical procedures	Patients	Complications	Cure rate	Fistula	Meatal stenosis	Diverticulum	Infection	Reoperation
TIP	13	2	11/13	1	1	0	0	1
Duplay	7	1	6/7	0	1	0	0	1
Mathieu	5	1	4/5	1	0	0	0	1
MAGPI	1	0	1/1	0	0	0	0	0
GAP	1	0	1/1	0	0	0	0	0

TABLE 3 | PPPS scores of urinary surgeon and parents of children with MIP among different surgical procedures*.

Surgical procedures	Meatus appearance		Glans appearance		Skin appearance		General appearance	
	Parent's score	Urinary surgeon's score	Parent's score	Urinary surgeon's score	Parent's score	Urinary surgeon's score	Parent's score	Urinary surgeon's score
Mathieu	2.60 ± 0.89	2.80 ± 0.45	2.40 ± 0.89	2.80 ± 0.45	2.60 ± 0.55	2.80 ± 0.45	2.40 ± 0.89	2.80 ± 0.45
TIP	2.23 ± 0.44	2.31 ± 0.48	2.23 ± 0.73	2.62 ± 0.51	2.15 ± 0.55	2.54 ± 0.52	2.31 ± 0.63	2.46 ± 0.52
Duplay	2.29 ± 0.63	2.43 ± 0.53	2.43 ± 0.79	2.71 ± 0.49	2.14 ± 0.69	2.43 ± 0.53	2.43 ± 0.98	2.43 ± 0.53
F	0.62	1.83	1.80	0.28	1.15	0.79	0.61	0.95
P	0.55	0.19	0.84	0.76	0.34	0.46	0.94	0.40

**Excludes the procedures with only one patients data (MAGPI and GAP procedures).*
Scores correspond to satisfaction: very satisfied (3 points), satisfied (2 points), dissatisfied (1 point), and very dissatisfied (0 points).

groove, and the shape and position of the urethral opening to achieve good therapeutic effects (6, 15).

It still remains controversial whether patients with partial MIP, but with normal micturition and an unobstructed sexual life and those whose daily life is unaffected must undergo surgical intervention. These patients can choose instead a conservative treatment (12). However, surgical correction of MIP in the era of increased cosmetic awareness is justified. The purpose behind MIP treatment is to reduce the giant urethral opening and move the urethral opening to the middle of the glans.

For the glans type of MIP, MAGPI or the GAP procedure can achieve good surgical results in order to restore the morphology and function (6, 16). These procedures can overcome the challenges of a wide, deep glanular groove and a non-compliant fish mouth. In our series, two patients with glanular MIP underwent MAGPI and the GAP procedure. The post-operative results were satisfactory, and there were no complications. For the coronary sulcus or distal penis type of MIP, the urethral plate should be retained during correction because of the lack of an obvious penile curvature, and the Mathieu, Duplay or TIP procedures can be performed. The Mathieu technique uses a reverse proximal urethral flap to match the urethral orifice, retaining the distal urethral plate, with no distal or proximal urethral anastomoses, thus reducing the occurrence of urethral stricture (12). The patients who underwent the Mathieu procedure no meatal stenosis occurred except for one case of urinary fistula.

The Duplay procedure is suited for patients with a wider urethral plate that can be directly rolled up to complete the urethral formation (7). Because the urethral plate retains a good blood supply and there is no annular anastomosis, the occurrence of a urethral stricture or urinary fistula is significantly reduced. The patients in our study who underwent the Duplay procedure had a fascia flap, with a rich blood supply, applied to cover the formed urethra, further reducing the incidence of post-operative urinary fistula. One of the patients who underwent the Duplay procedure developed a meatal stenosis, which may be associated with urethral scar hyperplasia and contracture.

If the patient has a general wide or a usual urethral plate, the TIP procedure seems to be a good alternative treatment. The TIP procedure can result in a good penile appearance and functional effects for the treatment of distal hypospadias, as well as a very low complication rate (17, 18). In our study, 13 patients underwent the TIP procedure, one of whom needed reoperation for repair. The success rate of the one-time TIP procedure was 92.6% (12/13) (19). To better reduce the occurrence of a urinary fistula in the TIP procedure, we found that the U-shaped incision of the urethral plate around the urethral opening should be over the urethral cavernosum surface to avoid damage to the cavernosum and bleeding. The membranous urethra and the urethral plate peripheral skin had to be fully resected to facilitate healing of the sewn urethra. The midline incision of the urethral plate should be over the corpus cavernosum surface to prevent bleeding, and the urethral plate should be fully expanded to ensure that the urethral suture is tension-free (20). In addition, we used the subcutaneous fascia to cover the urethra, which significantly reduces the incidence of urinary fistula (21, 22).

Our satisfaction score results show that there were no significant differences in meatus appearance, glans appearance, skin appearance, and general appearance PPPS score among the Mathieu, TIP, and Duplay surgical procedures. These procedures have good therapeutic effects on MIP. The Mathieu procedure is suitable for cases where the urethral plate is not wide enough and the formed urethra is short. The Duplay procedure can be used in patients with wider urethral plates that can be directly rolled up for complete urethroplasty. The TIP procedure can be used for a general wide or a usual urethral plate MIP and is also suitable for cases where initial surgery fails and there is a need for reoperation (23).

According to the PPPS score (9), the overall average PPPS score of non-operative surgeons and parents was satisfactory. The post-operative effect was satisfactory in all patients, and the urinary flow and stream during urination were normal. The high success rate of these operations may be due to the normal blood supply of the urethral plate and the urethral covering of the fascia; the flat urethral plate supported by the sponge can provide a fixed platform for the newly formed urethra. Histological studies of the urethral plate have found that the urethral plate, with a rich blood flow supported by the corpus cavernosum, contains abundant vascular smooth muscle, glands and nerves, and its smooth muscle and connective tissue have strong extensibility. These characteristics are very beneficial for urethral reconstruction (23, 24). The MIP anatomical features of

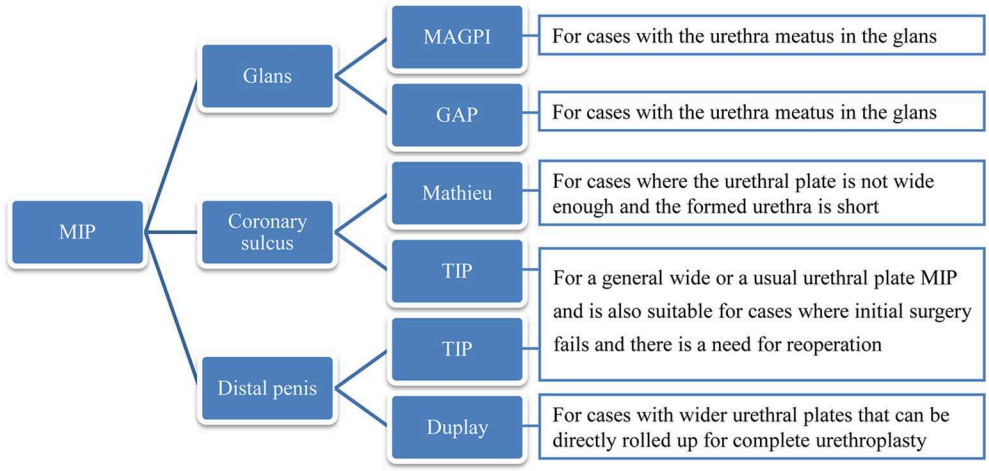

FIGURE 4 | The detailed flowchart of the decision can be driven according to the anatomy of the specific case.

a wide urethral plate, deep navicular fossa, and large glans create favorable conditions for these three surgical methods.

CONCLUSION

In conclusion, MIP clinical manifestations are concealed and usually noted when circumcision is attempted. The suitable procedure for each patient should be tailored according to the anatomic features, and several techniques can achieve good cosmetic and functional results. For the glans type of MIP, both MAGPI and GAP procedures can achieve good surgical results; for the coronary sulcus or distal penis type of MIP, the Mathieu, Duplay, or TIP procedures can be performed. The choice of a particular technique can follow the process as presented in the flowchart (**Figure 4**), taking into consideration the anatomy of the specific case to drive the decision-making process.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available because the patients' files are publicly available but information was collected for scientific purposes. Requests to access the datasets should be directed to Shouxing Duan, duanshouxing@126.com.

ETHICS STATEMENT

Ethical approval for this retrospective study was obtained from the Ethics Committee and Institutional Review Board of the Hospital of Shantou University Medical College (No. 2018-39).

AUTHOR CONTRIBUTIONS

SD: data collection, wrote, and corrected the manuscript. SD and LZ: data analysis and manuscript preparation. JL, XJ, XZ, WO, MF, and KC: performed surgery and data collection. LZ and SM: study idea and revised the manuscript. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00128/full#supplementary-material

Supplementary Figure 1 | The patient of **Figure 2** is the same case as **Figure 1C**,c.

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ORIGINAL RESEARCH
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The Application of External Ureteral Catheters in Children With Acute Kidney Injury Caused by Ceftriaxone-Induced Urolithiasis

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Objective: To evaluate our use of external ureteral catheters in children with acute kidney injury (AKI) resulting from ceftriaxone-induced urolithiasis.

Methods: From July 2010 to June 2015, a series of 15 children, including 12 males and 3 females, were referred to our department. All of them were diagnosed of post-renal AKI and underwent emergent hospitalization. Evaluation of serum electrolytes, creatinine (Cr), blood urea nitrogen (BUN), complete blood count, and blood gas analysis were completed in each child both before they were admitted, and again after surgery. Bilateral externalized ureteral catheters were placed cystoscopically in each of these patients. The composition of collected calculi was analyzed by infrared spectrography.

Results: Bilateral externalized ureteral catheters were placed successfully in all patients. There were no procedure-related complications. Two days after catheter placement, the levels of serum Cr and BUN had improved in all patients, and these levels were noted to be significantly lower than before catheterization ($P < 0.001$). Infrared spectrography demonstrated that the primary composition of all calculi collected was ceftriaxone. No recurrent AKI or renal deterioration was detected during the follow-up which ranged from 3 to 8 years.

Conclusions: These results show that short-term external ureteral catheters can be effectively utilized in children with AKI caused by ceftriaxone-induced urolithiasis. We recommend this procedure as a viable replacement to indwelling stents in patients with bilateral ureteral stones.

Keywords: external ureteral catheter, acute kidney injury, ceftriaxone, urolithiasis, children

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INTRODUCTION

One of the most common causes of urinary tract obstruction and postrenal acute kidney injury (AKI) is ureteral calculi. In a previous manuscript we reported a series of children who had developed AKI after developing bilateral ureteral obstruction from ceftriaxone calculi (1). Emergent intervention is, of course, required for such children. Ureteroscopy (URS) is the most common endoscopic method for treating ureteral stones but can be difficult, and sometimes impossible, at the time of the first intervention. It is therefore often necessary in children, to first place

double J ureteral stents for a period of time, allowing for passive ureteral dilation, prior to proceeding to URS and stone removal. Many pediatric urologists will then leave double J ureteral stents in place again after URS, for a period of time, to allow procedural trauma and edema to heal. Since children often cannot tolerate office-based cystoscopy and stent removal, final stent removal may be performed in the operating room with general anesthesia. So, some children will require a total of three separate general anesthesia-based procedures to treat the ureteral stone. While the use of double J ureteral stents has been shown to be safe and effective by various studies (2–5), this treatment protocol can be rather lengthy and difficult for patients. In addition, it can be difficult to place double-J stents for some children. Additionally, there are a variety of complications related to the use of double-J ureteral stents. In this article, we describe our experience using external ureteral catheters instead of double-J stents, as a viable option for relieving bilateral ureteral obstruction in children who suffer from AKI caused by ceftriaxone-associated urolithiasis.

METHODS

Patients and Symptoms

We performed a retrospective review of 15 children that suffered from AKI. From July 2010 to March 2015, 15 patients (12 male and 3 female) were referred to our department, the Pediatric Urology Department at Xin Hua Hospital, affiliated with Shanghai Jiaotong University School of Medicine. The patients' mean age was 3.76 ± 2.74 years (range 5 months to 11 years). Within several days of presentation, all children had received ceftriaxone therapy at a dose of 1 g once daily, which is equivalent to the adult dose. No other risk factors of stone formation could be identified. Neither urinary tract abnormality nor a family history of urolithiasis was found in any of the patients.

The main clinical manifestation of anuria was present in nine patients for ∼10 h to 3 days. Six patients had a history of oliguria for ∼1–7 days; oliguria is defined as a urine output <1 mL/kg/h in infants or <0.5 mL/kg/h in children. We estimated the urine output volume of the children based on the description of the parents.

Examinations

An abrupt reduction in kidney function is currently defined as a ≥50% increase in serum creatinine or the occurrence of oliguria (5). But under our emergency situation, we didn't have time to repeat the examination before surgery. So our diagnosis of AKI was based on sudden onset of anuria or oliguria associated with elevated serum creatinine in a child who had no past history of urolithiasis. Serum electrolytes, serum creatinine, blood urea nitrogen (BUN), a complete blood counts, and blood gases were evaluated in every child before and after surgery. Urinalysis and urine culture were performed after the operation; preoperative urinalysis was not performed because of insufficient urine volume. All patients underwent ultrasound before and after surgery. Kidney, ureter and bladder (KUB) X-rays and CT were performed in some patients. All stones, which were collected intraoperatively, were analyzed by infrared spectrometer (Tensor 27, Bruker AXS company, Germany). Single photon emission computed tomography (SPECT) was used to evaluate the effective renal plasma flow (ERPF) and split renal function of bilateral kidneys during follow-up.

External Ureteral Catheterization Procedure

After the diagnosis was established, emergency intervention was carried out without delay. General anesthesia was employed in all patients during the procedure. Under cystoscopy, we inserted a 4-Fr or 5-Fr ureteral catheter at the opening of one ureter and gradually entered it until we felt resistance. We gently increased the pushing force and let the catheter move forward. Then we would feel the resistance suddenly disappear and a cloud of bloody mixture poured from the opening of the ureter (**Video S1**). At that time, we checked the end of the catheter, if you see a continuous urine outflow (**Video S2**), it meant that the tube had passed through the obstruction zone, demonstrating relief of the obstruction. The same way we inserted another catheter on the other side. Then we fixated the external stents to an urethral catheter so that it would not dislocate. As a result, the children had limited mobility. We attached a bag to each catheter in order to observe the drainage of each catheter. The urinary sediment was collected from the urine bags, and the stones were analyzed. None of the patients underwent continuous renal replacement therapy (CRRT), PCNL, ureteroscopy, or double–J stent insertion.

Removal of External Ureteral Catheter

Successful calculi eradication was defined as complete clearance of stones as evidenced by ultrasonography. Patients were also considered stone-free if residual stones after treatment were smaller than 2 mm, defined as clinically insignificant residual fragments. We removed the external ureteral catheters when the ultrasound confirmed the stone-free, usually 2–3 days after catheterization.

Statistical Analysis

Statistical analysis was performed using SPSS, version 19.0. Data are presented as mean ± standard deviation; differences were analyzed using the Student's *t*-test. A $p < 0.05$ was considered statistically significant.

RESULTS

Multiple calculi in the upper urinary tract was identified with all patients. Two patients were diagnosed with bilateral renal calculi. Three of them had bilateral ureteral stones and bilateral renal calculi. Nine of them had bilateral ureteral stones, and one of them had unilateral ureteral calculi and contralateral renal calculi. Mild to moderate hydronephrosis was found bilaterally in all patients.

Acoustic shadows at the rear and trailing edge were detectable by ultrasonography. KUB X-ray of all examined patients showed no stone-like shadows. The CT values of stones of all examined patients were relatively low (mean, 144.09 ± 50.09 Hounsfield Units).

TABLE 1 | The results of blood biochemical indexes before and after treatment.

Time	K (mmol/L)	Na (mmol/L)	BUN (mmol/L)	Cr (umol/L)
Before treatment	5.22 ± 1.10	134.92 ± 3.50	22.72 ± 10.77	355.46 ± 193.05
Two days after treatment	3.98 ± 0.28	135.08 ± 3.55	4.61 ± 2.32	38.00 ± 18.02
P-value	<0.001	0.91	<0.001	<0.001

All of the patients had successful external ureteral catheterization procedures. Two days after treatment, the levels of serum creatinine and BUN showed clear improvement and were significantly lower than preoperative levels ($p < 0.001$). In addition, serum potassium (K+) had also significantly improved relative to pre-treatment values ($p < 0.05$); these parameters are shown in **Table 1**. The urine cultures of all patients were negative.

Most of the calculi was flushed away during the catheterization, so we could only collect four samples of urinary calculi. The results of infrared spectrometry showed that the main calculus composition was ceftriaxone. No severe intraoperative complications, such as ureteral perforation, catheter breakage, or mucosal avulsion, occurred. All of the external ureteral catheters were removed when ultrasound examination could confirm stone-free after treatment.

No renal deterioration was detected among our group of discharge. All patients were followed up for 3–8 years. No recurrent AKI was found, and ultrasonography showed that none of the patients had urolithiasis or hydronephrosis. Renal isotope scans (SPECT) were performed to assess patients' excretory function and split renal function of their follow-ups. All patients showed good excretory function and normal split renal function 6 months after hospital discharge.

DISCUSSION

A recent assessment of the incidence of urolithiasis in children reported 50 cases per 100,000 children (6). Moreover, the incidence of pediatric urolithiasis is increasing in China. The majority of these stones are composed of calcium—predominantly calcium oxalate but also, to a lesser extent, calcium phosphate. Much less commonly, calculi is composed of urate, cysteine, or struvite (7). Although it is rare that the stones are composed of ceftriaxone calcium, ceftriaxone has been associated with renal, and ureteral calculi in adult and pediatric populations, as demonstrated by our previous report and other studies (1, 8–10).

Our recent study also revealed that this type of stone tends to obstruct the ureters bilaterally, which rapidly leads to AKI. Undoubtedly, AKI is an emergency condition that can threaten the lives of patients. Thus, we prefer relieving the obstruction or urinary diversion surgically, using techniques such as placing indwelling double-J stents by cystoscopy, ureteroscopy, percutaneous nephrolithotomy, or open surgery, rather than administering dialysis (e.g., CRRT). The placement of indwelling double-J stents is most often chosen to relieve bilateral ureteral obstruction because it is a fast and convenient procedure that induces minimal damage. However, ureteroscopy, percutaneous nephrolithotomy, or even open surgery are necessary if the insertion of double-J stents via cystoscopy fails. Additionally, indwelling double-J stents remained *in situ* for several weeks postoperatively, which necessitates a second operation and can cause duration-dependent complications like stent colic and hematuria. In our population parents are relucatnat to subject children to successive anesthetic sessions, therefor neccesitating several weeks of indwelling time for internal stent. If we choose DJ stents with strings, the major risk of leaving the string of the stent are accidental dislodgement, and early removal of the stents by the patients. Gonen et al. showed that external ureteral catheters were more comfortable with the adult patients in a short time while being as reliable and safe as an indwelling stent (11). Vikram et al. also reported that external open-ended ureteral catheter drainage was equally effective and better tolerated than routine stenting following the uncomplicated ureteroscopic removal of stones (12). In this study, we demonstrate that the placement of external ureteral catheters instead of double-J stents, is a viable option to relieve bilateral ureteral obstruction and provide short-term urine drainage.

Most of the stones were spontaneously discharged through the bilateral catheters because of the distinctive loose and sand-like texture of ceftriaxone-associated calculi. No additional cystoscopy procedures were required after the ureteral catheters were removed.

Our study found this technique to be highly effective; moreover, there was no need for additional follow-up related to stent-related symptoms or stent removal (no lost or retained stents). This is particularly beneficial to a developing country like China, where the removal of an indwelling stent constitutes an additional procedure that is both a physical and also a financial burden on patients.

CONCLUSION

In conclusion, our results showed that the application of external ureteral catheters in children with AKI caused by ceftriaxone-induced urolithiasis was effective and reliable. We recommend this procedure as a viable replacement of indwelling stents in patients with bilateral ureteral stones. However, further studies are necessary to better define the role of this method, focusing on the evaluation of symptoms and complications.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shanghai xinhua hospital ethics committee. Written

informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LH: conceived and designed the experiments. HG, MX, GX, XF, and LH: performed the experiments. MX and HL: analyzed the data. LH and GX: video making. MX: manuscript writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00200/full#supplementary-material

Video S1 | External ureteral catheterization procedure. The ureteral opening was identified using the cystoscope, and the external stent was inserted. After entering the ureter, there was a sense of resistance; after the thrust was appropriately increased, the stent continued to enter. At this time, the ureteral opening contained urine and turbid stones.

Video S2 | Urine continued to flow from the end of the stent, confirming that the obstruction was relieved.

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ORIGINAL RESEARCH
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Severity-Dependent Profile of the Metabolome in Hypospadias

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Background & Objective: Hypospadias, characterized by the displacement of the opening of the urethra at any point in the medial-ventral side of the penis, is classified upon severity as mild (Type I) and severe (Type II and Type III) hypospadias. Hypospadias' etiology is idiopathic in the majority of cases, and underlying causes seem of multifactorial origin. Studies regarding genetic variants support this notion. It is unknown whether downstream gene products fit this profile. This study evaluated the metabolome of hypospadias by using the emerging technology of metabolomics in the search for distinct cellular processes associated with hypospadias' etiology according to the severity of this congenital urogenital condition.

Methods: Foreskin samples were collected during urethroplasty from boys with Type I, II, and III hypospadias or undergoing elective circumcision ($N = 28$) between 5 and 28 months of age. Samples were processed and submitted to gas chromatography-mass spectrometry (GC/MS). MetaboloAnalyst (http://www.metaboanalyst.ca/) online platform was used for bioinformatic analyses.

Results: Thirty-five metabolites across experimental groups were identified by GC/MS. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) showed that the metabolome of Type II and Type III hypospadias patients differs from the metabolome of Type I hypospadias and control patients. Of those 35, 10 amino acids were found in significantly low concentrations in severe hypospadias: aspartate, glutamate, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, and tyrosine. A high concentration of the amino acid lysine was detected in mild hypospadias.

Conclusions: The observed downregulation of specific amino acids in severe hypospadias provides alternative routes for future research aiming to identify disrupted networks and pathways while considering the severity of hypospadias.

Keywords: hypospadias, severity, etiology, metabolites, metabolome, metabolomics

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INTRODUCTION

Hypospadias is characterized by the opening of the urethra at any point in the ventral side of the penis rather than at its usual position at the tip of the glans. It is classified depending upon severity as: mild (Type I), moderate (Type II), and severe (Type III) hypospadias. It is further grouped as mild (Type I) and severe (Type II & III) hypospadias (1, 2). Approximately 70% of cases are mild hypospadias, and 30% are severe cases (3–7). Given the marked differences in the prevalence of each subtype, a working hypothesis is that hypospadias' etiology may be severity-dependent. Moreover, molecular studies support this emergent scenario in hypospadiology.

Clinical studies assessing genetic variants (single nucleotide polymorphisms; SNPs) show differences between hypospadias' sub-types. Carmichael and colleagues (8) found an increased risk association for several diacylglycerol kinase kappa (*DGKK*) gene SNPs for mild to moderate hypospadias but not for severe hypospadias. In addition, significant associations of *DGKK* SNPs variants were found by Xie and colleagues (9) for mild and moderate hypospadias when compared to controls, but not for severe hypospadias when compared to controls. Another study found that the distribution frequency of CC/CT/TT genotypes in the ryanodine receptor 1 (*RYR1*) gene was significantly different between patients with mild hypospadias in comparison to patients with moderate to severe hypospadias (10). Differences in SNPs variants have been found in genes related to sex hormone biosynthesis and metabolism. SNPs in the hydroxysteroid (17-beta) dehydrogenase 3 (*HSD17B3*), hydroxy-delta-5-steroid dehydrogenase, 3 beta-and steroid delta-isomerase 1 (*HSD3B1*), and stAR-related lipid transfer domain containing 3 (*STARD3*) genes were associated to an increased hypospadias risk for moderate and severe hypospadias but not for mild hypospadias (11). Another study found a significant association of an SNP in the androgen receptor (*AR*) gene with an increased risk for severe hypospadias; this association was not found in mild cases of hypospadias (12). At the protein level, findings also differ between hypospadias severities. Protein expression level of c-Jun N-terminal kinase 2 (*JNK2*), involved in cell migration, was found significantly increased in penile skin tissue from boys with severe hypospadias in comparison to boys with mild hypospadias (13). In addition, *AR* protein expression was found at higher levels in preputial tissue from subjects with severe hypospadias than those with mild hypospadias and control subjects (14). Altogether, this evidence supports a severity-dependent etiology for hypospadias. However, it came to our attention that not many studies have assessed whether these severity-dependent differences are observed in downstream gene products.

The metabolome is the final downstream product of all cellular changes that occur at the genome, transcriptome, and proteome level (15–17). Metabolomics is a high throughput approach that provides the identification and quantification of the metabolome from a variety of sample types (18). Metabolomics is employed to assess congenital conditions such as congenital heart defects and congenital glaucoma (19, 20) in search of novel biological candidates underlying these conditions.

We studied the metabolome from foreskin samples obtained from boys undergoing elective circumcision and boys undergoing hypospadias I-, II-, or III- repair with the hypothesis that each group will express a distinct profile of the metabolome. The goal was to determine whether a metabolomics approach could provide a first level of analysis pointing at distinct and novel cellular processes associated with hypospadias.

MATERIALS AND METHODS

Human Samples

This study was approved by the Institutional Review Board (IRB) under the Human Research Subjects Protection Office (HRSPO) at the University of Puerto Rico, Medical Sciences Campus. Parents of children undergoing urethroplasty and boys scheduled for elective circumcision were recruited at the HIMA San Pablo Hospital, Caguas, Puerto Rico. Hypospadias severity was assessed by Marcos Raymond Pérez-Brayfield, MD (pediatric urologist, Faculty in Surgery, Urology Section, School of Medicine, UPR). Informed consent was obtained from legal parents or guardians. Foreskin samples were collected from boys with Type I, II, & III hypospadias as well as control boys between the ages of 5–28 months of age**.** The sample was obtained from available inner foreskin, which is largely comprised of mucous membrane. Both lateral and dorsal inner foreskin were present in most samples. Boys with suggestive clinical changes of balanitis xerotica obliterans (*BXO*) were excluded from the study. Hypospadias was the only clinical presentation for patients in the hypospadias group.

The population used in this study were boys born with hypospadias and/or undergoing elective circumcision in Puerto Rico. From June 2017 to June 2019, the estimated population of Puerto Rico within the time of sample collection was ∼3 million people (21). The 2017 Annual Report of the Surveillance System of Congenital Defects from the Department of Health, Commonwealth of Puerto Rico, estimated that the prevalence of hypospadias in Puerto Rico was 24.86/10000 live births. Sample size estimation for our study included the following criteria: population size (for finite population correction factor or fpc) (N): 3000000; Hypothesized % frequency of outcome factor in the population (p): 0.25%+/−5; confidence limits as % of 100 (absolute +/– %)(d): 5%; Design effect (for cluster surveys-DEFF): 3. According to the Sample Size for Frequency in a Population formula (22) [sample size n = [DEFF*Np(1-p)]/[(d2/Z21-α/2*(N-1)+p*(1-p)], this study has an 80% confidence level with a total of 28 patients. These include seven patients with Type I hypospadias, seven patients with Type II hypospadias, six patients with Type III hypospadias, and eight patients undergoing circumcision as control subjects. The specific anatomical location of the urethral meatus was confirmed during the urethroplasty procedure after penile degloving by the pediatric urologist of the research team (MRP-B). **Table 1** shows the average age of patients (in months) for each experimental group. **Figure 1** shows the anatomical location of the urethral meatus for hypospadias patients that were

TABLE 1 | Average age (in months) at the time of urethroplasty.

Groups	(Avg. ± SD)
Controls	18.3 (9.11)
Type I	10.9 (7.56)
Type II	6.71 (1.98)
Type III	6.00 (0.926)

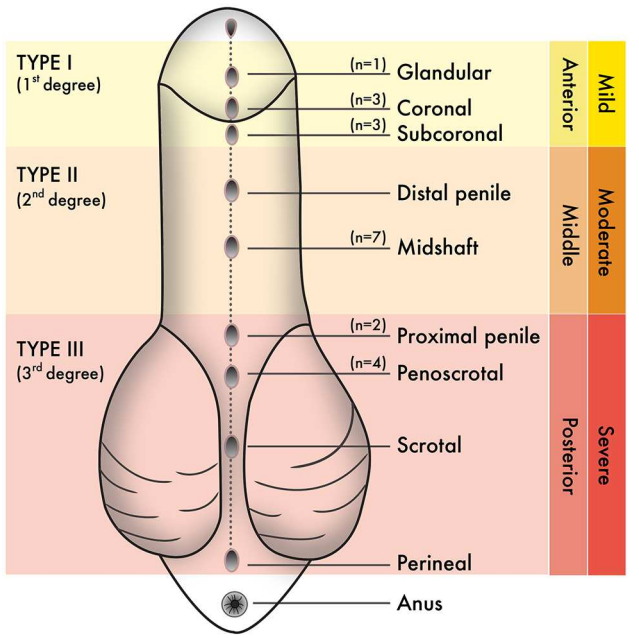

FIGURE 1 | Staging or classification systems that describe the anatomical position of the urethral meatus in hypospadias have been created over the years. From these, "Type I–Type III" terminology is used in this study. *N* values correspond to the number of hypospadias cases reported hereafter penile degloving and chordee release during each urethroplasty procedure.

included in this study according to various staging/classification systems. For reporting purposes, this study uses Type I-III terminology (1–2). Tissues were immediately frozen in dry ice and stored at −80°C until homogenization and processing for GC/MS.

Homogenization and Sample Preparation for Extraction of Metabolites

Fifty milligrams per sample were weighed and homogenized in 1 ml of chloroform/methanol/water (2:5:2), HPLC grade, by 15 s (3x) of sonication (on ice). Homogenates were shaken at 4°C for 15 min and centrifuged at 13,000 × 10 min at 4°C. The supernatant was completely evaporated. After evaporation, metabolites were derivatized by methoxyamination by adding 50 µl of 20 mg/ml solution of methoxyamine hydrochloride (Sigma-Aldrich, Catalog #: 226904) in pyridine (Sigma-Aldrich, Catalog #: 270407) and incubated at 37°C for 2 h. Trimethylsilylation was subsequently performed by adding 50 µl of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA+1% TMCS, Sigma-Aldrich; Catalog #: 375934) and incubated for 1 h at 65°C. Samples were centrifuged at 13,000 rpm for 10 min at RT. Supernatants were transferred to glass vials. Twenty microliters per sample were added to glass vials with inserts followed by the addition of 1 mM 2-Fluobiphenyl (Sigma-Aldrich, Catalog #: 102741) as an internal standard. Samples were processed by gas chromatography-mass spectrometry GC/MS-QP2010 (Shimadzu, Inc.) using analytical conditions as previously described (23).

Data Processing and Bioinformatics

Raw chromatography data were obtained and processed in GCMS Solution Postrun Analysis software (Shimadzu) equipped with NIST14/2014/EPA/NIH database. After peak integrations for all metabolites and extensive mass spectral library searches of the major chromatographic peaks resulted in a final data set consisting of 35 metabolic features selected for the metabolomics analysis. Reproducibility of metabolite recovery, the performance of sample extraction, derivatization, and instrumentation were validated by the utilization of several blank samples, including a system suitability blank, extraction processing blank, and derivatization processing blank. To investigate the reproducibility of the metabolic features, a pooled composite sample was prepared from each experimental sample, aliquoted and processed similar to experimental samples as quality control (QC) ($n = 3$). To evaluate analytical accuracy and precision, an external quality assessment was performed using 2-Fluobiphenyl spiked into derivatization blank samples before running on the GC/MS ($n = 6$). The percent of relative standard deviation (%RSD) of 2-Fluobiphenyl peak abundances accounted for 7.3%, which demonstrates good reproducibility of the method. To mitigate systematic bias, we performed the randomization of the sample analysis order. Blanks and QC samples were spaced evenly among the injections to monitor instrument stability. Identified metabolites were transferred to a data matrix alongside retention time, peak area, and reference ions for each metabolite. Quantitative analysis of metabolic feature's concentrations in each sample was performed by calculation of a response factor using the internal standard. A table containing metabolite concentrations for each sample was uploaded in comma-delimited (*.csv) format to the MetaboAnalyst (24) online platform. MetaboAnalyst was used for data processing and statistical analyses that included data normalization, multivariate-, and univariate-analyses. Samples were normalized by sample weight and range scaling. Multivariate analyses consisted of principal component analysis (PCA) to assess variance between samples and partial least squares-discriminant analysis (PLS-DA) to identify separation between groups. Quality and reliability were assessed by cross-validation by using the parameters R2 and Q2, where R2 measures the degree of goodness in fit of the data, and Q2 measures quality assessment (17). PLS-DA significance was assessed by Permutation Test, where $p \leq 0.05$ was considered statistically significant. Univariate analyses between Type I, II, & III hypospadias and control groups was

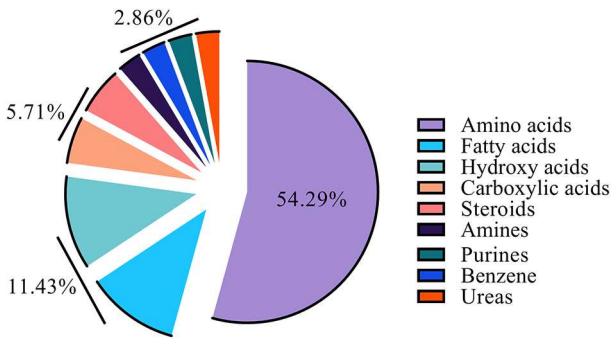

FIGURE 2 | Constituent ratio of the 35 metabolites identified by class.

assessed by one-way ANOVA with Fisher's LSD *post hoc* test. A $p \leq 0.05$ was considered statistically significant. Significant values were submitted to Bonferroni correction ($p < 0.05/14$). PCA, PLS-DA, and Heatmap plots were generated in the MetaboAnalyst platform.

RESULTS

Identification of Metabolites

Gas chromatography-mass spectrometry (GC-MS) was employed to identify and to quantify metabolites from foreskin samples as boys underwent elective circumcision or a urethroplasty procedure to manage hypospadias I-, II-, or III. A total of 35 metabolites were identified and classified as: amino acids (19); fatty acids (4), hydroxy acids (4), carboxylic acids (2), steroids (2), amines (1), purines (1), benzene (1), and urea (1) (**Figure 2**). Identified metabolites and their concentrations are appraised in **Figure 3**.

Metabolic Differences Between Hypospadias Severities and Control Samples

Multivariate analyses revealed differences in metabolic profiles between Type II and III hypospadias when compared to Type I hypospadias and control group. These metabolic differences were submitted to principal component analysis (PCA) to calculate variance between samples. PCA displayed a total of 61.0% variance, attributed to PC1 (50.7%) and PC2 (10.3%) components. **Figure 4A** depicts the 95% confidence ellipses for these groups. By using PCA score plots, we found that Type II and Type III hypospadias samples are more similar to each other and produce less variation than Type I hypospadias and control groups.

Furthermore, partial least squares-discriminant analysis (PLS-DA) displayed a total variance of 59.7%. PLS1 carried 49.2% of the total variance, whereas PLS2 contributed 10.5%. Type II and Type III hypospadias displayed satisfactory separation between Type I hypospadias and the control group, with a minor overlap of the 95% confidence ellipses (**Figure 4B**). On the contrary, Type I hypospadias and the control group showed no visible separation between them. PLS-DA was characterized by the following parameters: R2 = 0.359 and Q2 = 0.206. Permutation test revealed that separation between groups was statistically significant ($p = 0.0265$).

Univariate analysis was performed to evaluate whether there were statistically significant differences in metabolite concentrations between Type I, II, and III hypospadias and control groups. A one-way ANOVA followed by *post hoc* analysis revealed that 14 metabolites had statistically significant differences in metabolite concentrations between groups (**Figure 5**). After applying the Bonferroni correction, ten metabolites were identified: aspartate, glutamate, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, and tyrosine. Specifically, in comparison to the control group, Type I had one metabolite with higher concentrations, Type II had four metabolites with lower concentrations, and Type III had nine metabolites with lower concentrations. In comparison to Type I, ten metabolites were found with significantly lower concentrations in Type II and Type III hypospadias. These significant differences in metabolite concentrations between all experimental groups per metabolite are detailed in **Table 2**.

DISCUSSION

To the best of our knowledge, this is the first study that assesses the metabolome of hypospadias severities by using human foreskin samples collected during urethroplasty. Our results demonstrate a tangible difference in the metabolome between mild and severe types of hypospadias, which further supports the notion based on previous genetics research work of plausible severity-dependent etiologies for hypospadias.

We were able to make a positive identification of 35 metabolites from inner foreskin samples through GC/MS analysis. The metabolome of Type II and Type III hypospadias displayed variance and separation from Type I hypospadias and control groups. Ten metabolites had significantly lower concentrations in Type II and Type III hypospadias in comparison to Type I hypospadias and control samples. These metabolites comprised the amino acids aspartate, glutamate, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, and tyrosine. While our study aimed to identify and quantify the metabolome of foreskin sample from hypospadiac boys, we speculate that the etiology of severe hypospadias might not just be the result of upstream dysregulations, such as genetic variants, protein synthesis or modifications, as it may be the result of reduced availability of downstream products that serve as metabolic intermediates and signaling molecules, such as amino acids. Amino acids have many functions, besides serving as structural units of proteins. Some of these functions include synthesis of hormones and neurotransmitters, serving as intermediates in signaling pathways, and as major metabolic intermediates for energy production such as in the citric acid cycle. Hence, our data supports the tenet that hypospadias' etiology is multifactorial, and that scarcity of amino acids may play an important role. In addition, although Type I hypospadias displayed an apparent upregulation of metabolites, only lysine had a significantly higher concentration from control samples.

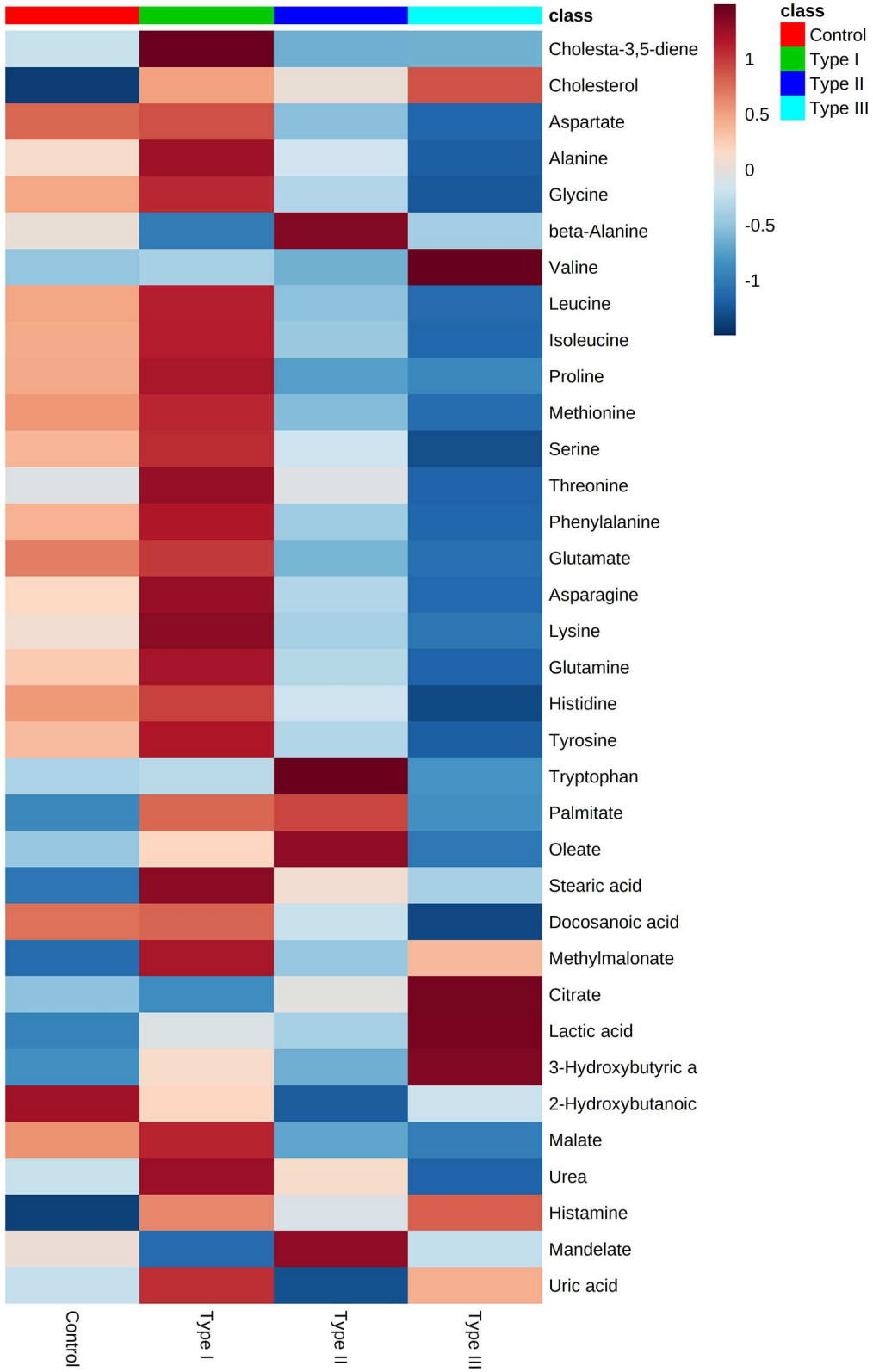

FIGURE 3 | Metabolome of Type I, II, and III hypospadias, and controls. Heatmap displays average metabolites concentrations for each group. Control: $n = 8$; Type I hypospadias: $n = 7$; Type II hypospadias: $n = 7$; Type III hypospadias: $n = 6$. Blue represents low concentration; red represents high concentration.

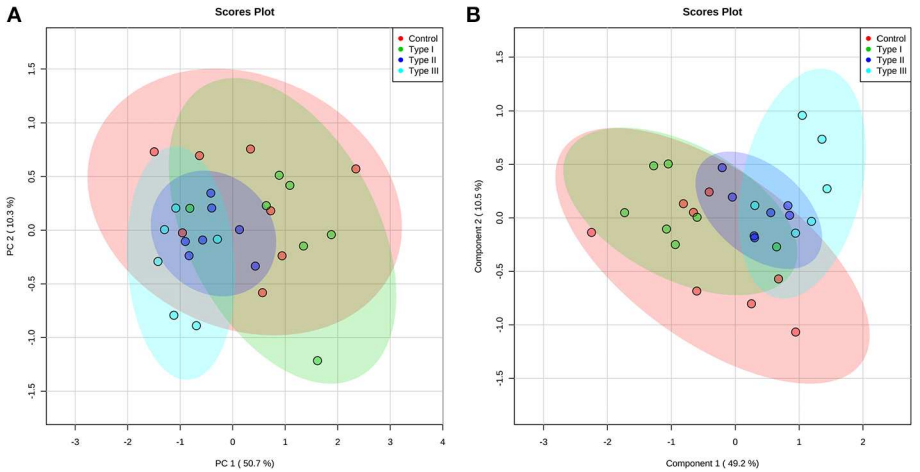

FIGURE 4 | Type II and Type III hypospadias metabolome differs from Type I hypospadias and controls. **(A)** Principal component analysis (PCA) and **(B)** partial least squares-discriminant analysis (PLS-DA) analysis scores plots display variance and separation for Type II and Type III hypospadias between Type I hypospadias and control groups. The ellipses of the score plots illustrate 95% confidence region of the groups. Control: $n = 8$; Type I hypospadias: $n = 7$; Type II hypospadias: $n = 7$; Type III hypospadias: $n = 6$.

Therefore, the metabolomics profile in mild hypospadias is intriguing as it suggests a homeostatic mechanism to compensate for an altered developmental program. Further research is needed to address this hypothesis.

Aspartate is of particular interest as it was detected in lower concentrations in Type II and Type III hypospadias. Aspartate is a non-essential amino acid that is made from glutamine by enzymes using vitamin B6. This amino acid has essential roles in the urea cycle, DNA metabolism, and steroid hormone synthesis. In the rat model, it has been shown that aspartate induces the release of testosterone, luteinizing hormone, progesterone, and growth hormone release (25–28); entering the cell and increasing steroidogenesis in Leydig cells in the rat testis (26, 29). In humans, an oral dose of aspartate for 12 days increased testosterone and luteinizing hormone levels in serum in comparison to basal levels in men (30). Moreover, one study showed that aspartate upregulates the expression of androgen receptor protein levels in rat testis (28). We speculate that low concentrations of aspartate in Type II and III hypospadias might be related to circulating testosterone levels. This idea is consistent with previous work on genetic variants related to sex hormone biosynthesis and metabolism, as these have been associated with an increased risk for moderate and severe hypospadias but not for mild hypospadias (11). It is important to note that Type I hypospadias had similar aspartate concentrations to control samples. Therefore, from an embryological point of view, the etiology of Type I hypospadias may not be heavily influenced by androgen-dependent pathways.

Glutamate, isoleucine, leucine, phenylalanine, and proline are metabolites that are involved in energy metabolic processes. These metabolites were found in significantly lower concentrations in Type II and III hypospadias in comparison to Type I hypospadias and controls. Proline is a non-essential amino acid that is synthesized from glutamate. Glutamate is converted to alpha-ketoglutarate, one of the substrates involved in the rate-limiting step of the citric acid cycle. Phenylalanine, as well, is part of the citric acid cycle, as it is converted to acetoacetate and fumarate. Isoleucine and leucine are branched-chain amino acids which play critical roles in energy metabolism. These metabolites have been reported to participate in lipolysis, lipogenesis, glucose metabolism, among other energetic functions (31). Most importantly, branched-chain amino acids have been found to regulate mammary function and embryo development through the activation of the mechanistic target of rapamycin (*mTOR*) signaling pathway (31). The downregulation of these metabolites and their relationship to energy metabolic processes, including the citric acid cycle, lead us to speculate that the measured lower amino acid concentrations in severe hypospadias may be related to basic cellular processes that are essential for the differentiation of the male reproductive system.

Glutamate, methionine, glycine, isoleucine, leucine, lysine, and phenylalanine were found in significantly lower concentrations in severe hypospadias. These metabolites are associated with inborn errors of metabolism (32–38). Some examples of inborn errors of metabolism diseases are phenylketonuria, maple syrup urine disease, tyrosinemia type I, glycine encephalopathy, which involve the deficiency or accumulation of these amino acids (32). Moreover, Carmichael and colleagues (38) investigated whether hypospadias is associated with maternal dietary intake of nutrients related to one-carbon metabolism, including methionine. Data suggested that increased intake of methionine, choline, and vitamin B12 was associated with reduced risk of hypospadias. Thus, it deems vital that future studies investigate the plausible correlation between hypospadias risk and methionine intake as well as these newly identified metabolites.

There are a number of limitations of this study that warrants further research. First, aside from the atypical anatomical

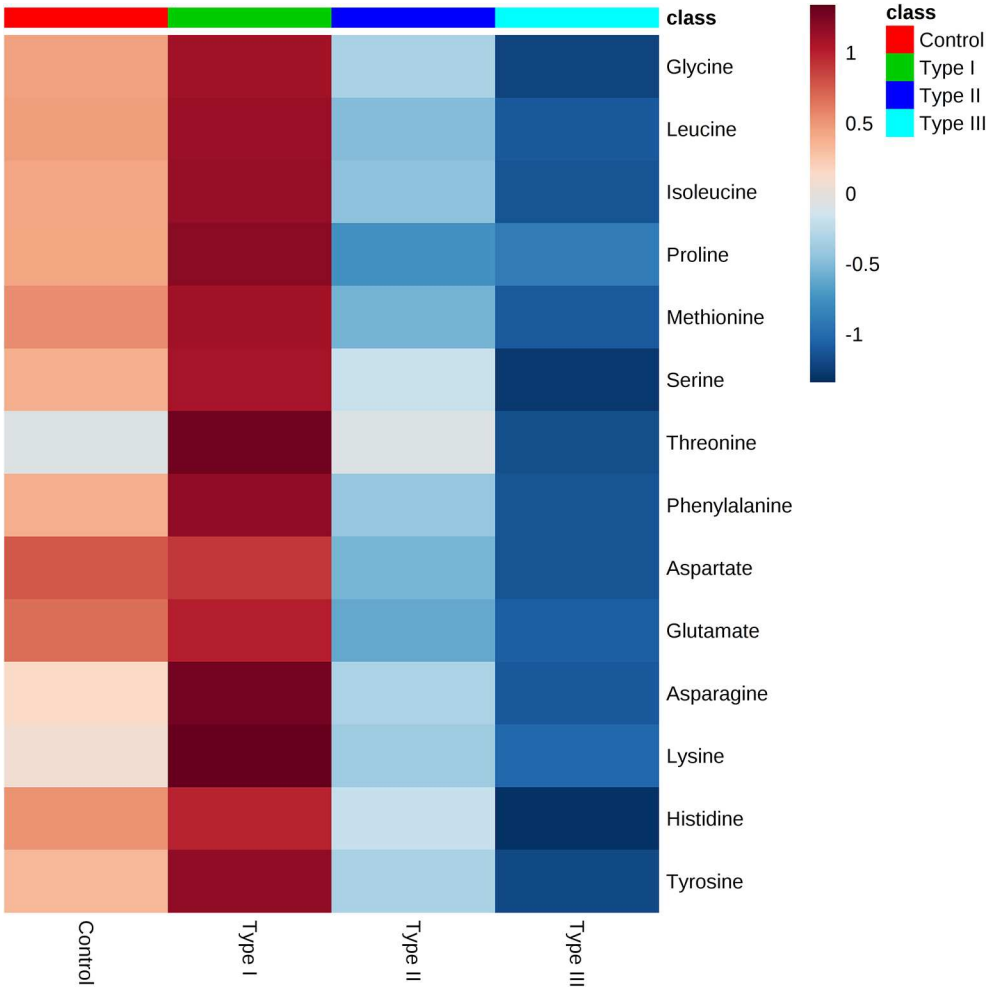

FIGURE 5 | Significant downregulation of amino acids in Type II and Type III hypospadias. Heatmap represents metabolite concentrations (mM). Statistical analyses revealed that 14 amino acids had statistically significant lower concentrations in Type II and Type III hypospadias in comparison to Type I hypospadias and control groups. $p < 0.05$. Control: $n = 8$; Type I hypospadias: $n = 7$; Type II hypospadias: $n = 7$; Type III hypospadias: $n = 6$. Blue represents low concentration; red represents high concentration.

location of the urethral meatus, hypospadias is also characterized by atypical formation of the foreskin. Therefore, in search of hypospadias' etiology, the scientific rationale has been to study foreskin as a proxy measure of atypical location of the urethral meatus largely because it is absolutely unacceptable to collect urethral tissues for research purposes. The second one is related to the embryology of the urogenital systems, as it is not possible to research tissue samples of human urethral plates during embryonic and fetal development. Third, even though our study has an 80% confidence level, future studies with a larger sample size should confirm our findings. Fourth, analytical platforms for metabolomics other than the ones employed here could expand significant findings.

The age of the patient at the time of tissue collection and the type of tissue sample are inherent biases of this first study of the metabolome in hypospadias. Even though differences in age between experimental groups were dictated by best practices in the care of children born with hypospadias, this study assumes that such differences at the time of the urethroplasty procedure do not affect the metabolome. To further assess the reported differences seen here, it would be advantageous to submit to metabolomics analyses blood and/or urine samples along with inner foreskin samples from the same patient; both as an internal control and as an experimental strategy to address the question of whether age differences within infancy affect metabolomics profiles in hypospadias. In addition, this study employed inner foreskin tissue samples that would otherwise be discarded after the urethroplasty procedure. Available tissues, in most cases, included both lateral and dorsal inner foreskin tissues. Nevertheless, it is unknown whether specific histological features around the inner preputial coronal-shaft junction (39) differentially affect the metabolome in hypospadias. These inherent biases deserve further examination.

TABLE 2 | Significant differences in metabolite concentrations between groups.

Metabolite	Difference between groups	*f*-value	*p*-value*	FDR
Aspartate	Control vs. Type II & III; Type I vs. Type II & III	7.306	0.001	0.011
Glutamate	Control vs. Type II & III; Type I vs. Type II & III	6.370	0.002	0.013
Glycine	Control vs. Type III; Type I vs. Type II & III	5.677	0.004	0.015
Isoleucine	Control vs. Type III; Type I vs. Type II & III	8.074	0.001	0.009
Leucine	Control vs. Type II & III; Type I vs. Type II & III	8.113	0.001	0.009
Lysine	Control vs. Type I; Type I vs. Type II & III	5.833	0.004	0.015
Methionine	Control vs. Type III; Type I vs. Type II & III	6.152	0.003	0.013
Phenylalanine	Control vs. Type III; Type I vs. Type II & III	7.973	0.001	0.009
Proline	Control vs. Type II & III; Type I vs. Type II & III	6.325	0.003	0.013
Tyrosine	Control vs. Type III; Type I vs. Type II & III	7.004	0.002	0.011

**Significant values after Bonferroni correction.*

In conclusion, the metabolomics profile of Type II and Type III hypospadias patients differs from Type I hypospadias and control patients. The observed downregulation of specific amino acids in severe hypospadias provides alternative routes for future research aiming to identify disrupted networks and pathways while considering the severity of hypospadias. Our findings are consistent with the emergent working hypothesis in hypospadiology that favors the idea of severity-dependent etiologies underlying the atypical phenotypes of the male phallus at birth. Prenatal identification of these metabolites could lead to the development of biomarkers for hypospadias.

DATA AVAILABILITY STATEMENT

This data is available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) website, the Metabolomics Workbench, https://www.metabolomicsworkbench.org where it has been assigned Project ID (PR000887). The data can be accessed directly via it's Project (doi: 10.21228/M81M59). This work is supported by NIH grant U2C-DK119886.

ETHICS STATEMENT

This study was approved by the Institutional Review Board (IRB) under the Human Research Subjects Protection Office (HRSPO) at the University of Puerto Rico, Medical Sciences Campus. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MP-B performed all surgical procedures and collected all tissue samples. CP-R assisted in sample collection and processed all tissue samples for metabolomics. CP-R conducted experiments under the guidance and expert advice of NC. NC evaluated the quality and reproducibility of data. JJ designed experimental protocol and cross-checked data for accuracy and supervised data analyses. MP-B and JJ provided advice during data collection. Depiction of data and writing of the first draft were by CP-R and JJ. All authors participated in the drafting and approval of the final version of the manuscript.

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Treatment Strategies and Outcome of the Exstrophy–Epispadias Complex in Germany: Data From the German CURE-Net

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Introduction: To evaluate the impact of reconstructive strategies and post-operative management on short- and long-term surgical outcome and complications of classical bladder exstrophy (CBE) patients' comprehensive data of the multicenter German-wide Network for Congenital Uro-Rectal malformations (CURE-Net) were analyzed.

Methods: Descriptive analyses were performed between 34 prospectively collected CBE patients born since 2009, median 3 months old [interquartile range (IQR), 2–4 months], and 113 cross-sectional patients, median 12 years old (IQR, 6–21 years).

Results: The majority of included individuals were males (67%). Sixty-eight percent of the prospectively observed and 53% of the cross-sectional patients were reconstructed using a staged approach ($p = 0.17$). Although prospectively observed patients were operated on at a younger age, the post-operative management did not significantly change in the years before and after 2009. Solely, in prospectively observed patients, peridural catheters were used significantly more often ($p = 0.017$). Blood transfusions were significantly more frequent in males ($p = 0.002$). Only half of all CBE individuals underwent inguinal hernia repair. Cross-sectional patients after single-stage reconstructions showed more direct post-operative complications such as upper urinary tract dilatations ($p = 0.0021$) or urinary tract infections ($p = 0.023$), but not more frequent renal function impairment compared to patients after the staged

approach ($p = 0.42$). Continence outcomes were not significantly different between the concepts ($p = 0.51$). Self-reported continence data showed that the majority of the included CBE patients was intermittent or continuous incontinent. Furthermore, subsequent consecutive augmentations and catheterizable stomata did not significantly differ between the two operative approaches. Urinary diversions were only reported after the staged concept.

Conclusions: In this German multicenter study, a trend toward the staged concept was observed. While single-stage approaches tended to have initially more complications such as renal dilatation or urinary tract infections, additional surgery such as augmentations and stomata appeared to be similar after staged and single-stage reconstructions in the long term.

Keywords: exstrophy–epispadias complex (EEC), operative outcome, outcome assessment, treatment strategies, staged approach, single-stage approach, post-operative complications

INTRODUCTION

Worldwide, enormous efforts have been made to improve operative techniques of the exstrophy–epispadias complex (EEC) (1–7). For a considerable long period, however, major reconstructive continence concepts in classical bladder exstrophy (CBE) remained unchanged, although long-term results in regard of bladder function and achievable continence vary significantly. There is still no consensus about the best operative strategy in CBE in respect of future bladder and upper tract outcome or the need for consecutive operations. This was the cornerstone for initiation of the multicenter German-wide population-based Network for Congenital Uro-REctal malformations (CURE-Net) with its comprehensive national data collection on EEC patients. The aim of this current study was to analyze CBE treatment practice over a long-term period with a focus on operative treatment strategies, their early and late post-operative complications, and consecutive operations to achieve continence. This study is the first to compare a prospectively observed with a cross-sectional cohort of CBE patients in a nationwide survey.

PATIENTS AND METHODS

Study Population

In CURE-Net, individuals with EEC are identified and recruited through participating departments of pediatric urology and pediatric surgery throughout Germany and the two German self-help organizations Blasenekstrophie/Epispadie e.V. and Kloakenekstrophie e.V. Since 2009, the centralized database comprises clinical data of a prospectively observed cohort (infant baby, ≤1 year old at the time of data acquisition) and a cross-sectional cohort (>1 year at the time of data acquisition) of EEC individuals throughout Germany. Data of the exstrophy group since 2009 were collected prospectively, starting at the time of reconstruction through the treating physician and still ongoing by recontacting EEC patients over time. Patients older than 18 years and parents of affected minors were personally interviewed by a physician using a standardized questionnaire. Additionally, cross-sectional patients' data were also retrieved from hospital letters and charts if available. Written informed consent was obtained from all subjects or their legal guardians in case of minors. This study was approved by each participating center's Institutional Ethics Committee (e.g., University of Regensburg No. 09/053, University of Ulm No. 425/13).

Structure of Data Analysis

From May 2009 to December 2016, 59 prospectively observed and 178 cross-sectional EEC patients were enrolled in the complete CURE-Net cohort. Current analysis focused exclusively on CBE patients, including 34 prospectively observed infant babies from 2009 onward [median, 3 months; interquartile range (IQR), 2–4 months; 10 females (29%), 24 males (71%); male-to-female ratio 2.4:1] and 113 cross-sectional patients [median, 12 years (IQR, 6–21 years); 39 females (35%), 74 males (65%); male-to-female ratio 1.9:1]. Further 19 cross-sectional CBE patients (3 females, 16 males) were excluded because of operative technique (primary urinary diversion).

Operative techniques were differentiated between a single-stage approach with bladder closure obligatory including a bladder neck reconstruction, pelvic ring adaptation, genital reconstruction, and optional with ureterocystoneostomy (in Germany mostly "Erlangen technique" (8) or CPRE) and a staged approach with postnatal bladder closure alone and further procedures to reconstruct the bladder neck and genitalia at a later time in life. In both approaches, pelvic ring adaptation technique with a simple traction bandage and intraoperative absorbable polydioxanone sutures of the pubic bones (9) was used for most exstrophy patients. After immobilization with a mummy wrap for about 10 days, a substantial connective tissue bridge is formed (9).

Expert consensus defined perioperative management strategies as parts of a standardized perioperative treatment recommendation (**Table 1**) and perioperative and post-operative complications (**Tables 1**, **2**). Continence definition was adapted to the International Children's Continence Society (ICCS) terminology, referring to continuous urinary continence, intermittent, and continuous urinary incontinence (10).

TABLE 1 | Perioperative management and complications according to initial operative technique.

	Prospectively observed patient group ($n = 34$)			Cross-sectional patient group ($n = 113$)		
	Staged approach ($n = 23$)	**Single-stage approach ($n = 11$)**	**P^{Δ}**	**Staged approach ($n = 60$)**	**Single-stage approach ($n = 53$)**	**P^{Δ}**
Postoperative intensive care observation			1.0			0.29
Yes	21 (90%)	8 (73%)		51 (84%)	42 (80%)	
No	0	0		7 (13%)	2 (6%)	
Missing data	2 (7%)	3 (20%)		2 (3%)	9 (14%)	
Peridural catheter (PDC)			**0.009**			0.84
Yes	18 (64%)	2 (33%)		22 (39%)	20 (37%)	
No	3 (29%)	5 (40%)		30 (43%)	23 (41%)	
Missing data	2 (7%)	4 (27%)		8 (19%)	10 (22%)	
Wound and tissue infection			0.36			0.43
Yes	4 (13%)	3 (33%)		22 (32%)	11 (22%)	
No	17 (81%)	5 (47%)		32 (60%)	32 (58%)	
Missing data	2 (7%)	3 (20%)		6 (8%)	10 (20%)	
Blood transfusion			0.68			0.19
Yes	13 (45%)	4 (40%)		23 (32%)	13 (26%)	
No	8 (48%)	4 (40%)		31 (60%)	30 (49%)	
Missing data	2 (7%)	3 (20%)		6 (8%)	10 (25%)	
Anticholinergic medication			0.27			0.59
Yes	18 (71%)	5 (53%)		36 (64%)	24 (48%)	
No	2 (19%)	2 (20%)		9 (13%)	9 (17%)	
Missing data	3 (10%)	4 (27%)		15 (23%)	20 (35%)	
Low-dose antibiotic prophylaxis			1.0			1.0
Yes	21 (90%)	7 (73%)		55 (87%)	43 (78%)	
No	0	0		0	0	
Missing data	2 (%)	4 (27%)		5 (13%)	10 (22%)	
Transurethral catheter in place (days)			$0.53^{\Delta\Delta}$			$0.93^{\Delta\Delta}$
Median (IQR)	15.5 (14–19); Min 6, Max 28	20 (14–31); Min 0, Max 40		21 (19–29); Min 0, Max 80	21 (19–32); Min 0, Max 49	
Discharge (days)			$0.11^{\Delta\Delta}$			$0.17^{\Delta\Delta}$
Median (IQR)	21 (18–23); Min 10, Max 36	39.5 (22–46); Min 14, Max 57		27 (24–30); Min 17, Max 50	28.5 (23–42); Min 19, Max 150	

$^{\Delta}$*Calculated by the Fisher's exact test.*

$^{\Delta\Delta}$*Calculated by the t-test.*

IQR, interquartile range. Bold values are the significant ones $p < 0.05$.

Statistical Analysis

Descriptive data of the study population, operative treatment strategies, and post-operative management are presented in absolute and relative frequencies. To assess possible differences between the prospectively observed and cross-sectional patient group, as well as between female and male patients, Fisher's exact test was used, and the *t*-test to calculate possible differences in symphysis width, length of transurethral catheter placement, and hospital stay. Statistical significance was defined by $p < 0.05$. Analyses were performed by the statistics software SAS©, version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Operative Procedures

Operative approaches were performed almost in an equal proportion in the cross-sectional cohort (staged approach 53%, single-stage approach 47%). Twenty-three (68%) prospectively observed patients had a staged and 11 (32%) a single-stage procedure. Proportions of operative approaches between both cohorts were not statistically significant ($p = 0.17$). Mean age for primary reconstructions was 0.8 year in the cross-sectional and 0.3 year in the prospectively observed patient group. Subgroup analysis showed that single-stage approaches were done at a mean age of 0.4 year in the cross-sectional and of 0.6 year in the prospectively observed patient group. Interestingly, the average age of bladder closure within the staged approach was 0.2 year in the prospectively observed and 1.3 year in the cross-sectional cohort.

Inguinal hernia repair was reported in 44% of the prospectively observed and 52% of the cross-sectional patients. Thirty-seven percent of all included CBE patients did not undergo herniotomy during any primary or secondary procedure. In the prospectively observed group, primary hernia

TABLE 2 | Postoperative complications after primary reconstruction.

Complications	Prospectively observed patient group ($n = 34$)			Cross-sectional patient group ($n = 113$)		
	Staged approach ($n = 23$)	Single-stage approach ($n = 11$)	P^{Δ}	Staged approach ($n = 60$)	Single-stage approach ($n = 53$)	P^{Δ}
Urinary tract dilatation after reconstruction			0.71			**0.0021**
Yes	11 (48%)	6 (55%)		11 (18%)	24 (45%)	
No	12 (52%)	5 (45%)		18 (30%)	19 (36%)	
Missing data	0	0		31 (52%)	10 (19%)	
UTI after reconstruction			**0.020**			**0.023**
Yes	7 (30%)	8 (73%)		26 (43%)	35 (66%)	
No	16 (70%)	2 (18%)		11 (18%)	10 (19%)	
Missing data	0	1 (9%)		23 (38%)	8 (15%)	
Renal deterioration after reconstruction			1.0			0.42
Yes	0	0		2 (3%)	4 (7%)	
No	21 (91%)	10 (91%)		28 (47%)	37 (70%)	
Missing data	2 (9%)	1 (9%)		30 (50%)	12 (23%)	
Unilateral nephrectomy			1.0			0.50
Yes	2 (9%)	0		2 (3%)	0	
No	21 (91%)	10 (91%)		58 (97%)	49 (93%)	
Missing data	0	1 (9%)		0	4 (7%)	
Disturbed bladder function (diagnosed by urodynamic findings)			1.0			**0.025**
Yes	0	0		1 (2%)	7 (13%)	
No	20 (87%)	9 (82%)		22 (37%)	27 (51%)	
Missing data	3 (13%)	2 (18%)		37 (62%)	19 (36%)	
Epidydimitis during follow-up*			1.0			0.10
Yes	0	0		8 (22%)	4 (11%)	
No	18 (100%)	5 (83%)		14 (38%)	23 (62%)	
Missing data	0	1 (17%)		15 (40%)	10 (27%)	

**Only in male patients.*

ΔCalculated by the Fisher's exact test.

UTI, urinary tract infection. Bold values are the significant ones $p < 0.05$.

repair was performed almost equally in girls (40%) and boys (46%), with a bilateral repair in 87%. In the cross-sectional patient group, hernia repair was significantly more often done in males than in females (26% females, 66% males, $p < 0.0001$; bilateral repair 76%). Redo surgery for recurrent hernias was necessary in five cross-sectional patients (4%). Symphysis diastasis with inguinal hernia compared to those with no inguinal hernia was significantly different neither in prospectively observed ($p = 0.76$) nor in cross-sectional patients ($p = 0.06$). Furthermore, no differences were observed between both groups ($p = 0.23$).

Symphysis adaptation was documented in 101 of 147 patients [69%; prospectively observed patients: $n = 24$ (71%), cross-sectional patients: $n = 77$ (68%)]. An osteotomy was performed in 7 of 113 cross-sectional patients [6%; 4 (4%) with staged approach, 3 (3%) with a single-stage approach] and in 3 of 34 prospectively observed patients [9%; 2 (6%) with staged approach, 1 (3%) with a single-stage approach]. The most common osteotomy type performed was posterior osteotomy; only two cross-sectional patients underwent an anterior osteotomy. From operative reports, median intraoperative symphysis width achieved at primary closure for CBE patients was 1 cm ($n = 18$; IQR, 0.5–1.2 cm) in the cross-sectional and 0 cm ($n = 20$; IQR, 0–1 cm) in the prospectively observed patient group.

Post-Operative Management

When comparing perioperative management strategies as a part of a standardized perioperative treatment recommendation and possible perioperative complications, no significant differences were found between either the two operative techniques or both cohorts (**Table 1**). Only peridural catheters (PDCs) were inserted nearly twice as often in staged than in single-stage approaches in the prospectively observed group. When comparing both patient groups in general, a significantly higher frequency of PDCs was found in the prospectively observed than in the cross-sectional cohort ($p = 0.017$). Stratification for sex showed no differences between both patient groups regarding the incidence of post-operative intensive care observation ($p = 0.49$), PDC use ($p = 0.55$), wound infection ($p = 1.0$), and

medication such as anticholinergic drugs ($p = 1.0$) or antibiotic prophylaxis ($p = 0.25$). In contrast, blood transfusions were found significantly more often in males ($p = 0.002$). Additionally, blood transfusions were predominant in males after a single-stage approach ($p = 0.004$) compared to a staged approach ($p = 0.22$). These cross-sectional patients with a blood transfusion after a single-stage approach did not have more frequently an osteotomy done, compared to those who had no blood transfusion ($p = 0.95$). There were no age differences in the prospectively observed cohort who needed a blood transfusion in regard of gender ($p = 0.68$) or operative approaches ($p = 1.0$).

Postoperatively, a transurethral catheter remained in place for a median of 16 days (IQR, 14–20 days) in the prospectively observed and for a median of 21 days (IQR, 19–35 days) in the cross-sectional patient group ($p = 0.0013$).

Although the proportion of the reconstruction methods varied between the prospectively observed and cross-sectional patients, there was no significant difference between the duration of catheter placement between the single-stage and the staged approach (prospectively observed cohort $p = 0.53$, cross-sectional cohort $p = 0.93$). Additionally, neither male nor female gender did influence the time of having a catheter in place (prospectively observed cohort $p = 0.64$, cross-sectional cohort $p = 0.89$). The prospectively observed patients stayed significantly shorter in hospital than did patients of the cross-sectional group [median, 21.5 days (IQR, 17–30 days) vs. median, 28 days (IQR, 24–35 days)] ($p = 0.019$). There were no differences in regard to either the mode of reconstruction (**Table 1**) or gender (prospectively observed group $p = 0.83$, cross-sectional group $p = 0.28$).

Post-Operative Complications and Following Operations

In the cross-sectional cohort, urinary tract infection (UTI) and upper tract dilatation occurred significantly more often after single-stage than after staged approach (**Table 2**). In the prospectively observed group, UTIs were quite common after single-stage procedures and therefore significantly more often than after staged approaches ($p = 0.02$) (**Table 2**). However, subsequent impairment of renal function did not differ between the two groups ($p = 0.42$) or the prospectively observed and cross-sectional cohort. Renal function impairment was confirmed in two patients after staged approach by a renal scan with no further surgical therapy. Further two patients with a staged approach underwent nephrectomy. No patients with single-stage approach had a nephrectomy done; however, in four patients, renal function impairment was reported after sonography; all of them needed therapy such as ureterocystoneostomy or percutaneous kidney drainage. The significant frequency in disturbed bladder function must be treated with caution because of very small numbers and a high rate of missing data.

Augmentations with ileum ($p = 0.67$) and catheterizable stomata according the Mitrofanoff principle (Appendix $p = 0.84$, Monti $p = 0.59$) were necessary in about the same extent after the staged and the single-stage approach in cross-sectional

TABLE 3 | Following operations to increase bladder capacity, improve bladder emptying, or gain urinary continence (bladder neck procedure) in the long-term follow-up.

Following operations	Cross-sectional patient group ($n = 113$)		
	Staged approach ($n = 60$)	Single-stage approach ($n = 53$)	P^{Δ}
Bladder augmentation with			
1. Ileum			0.67
Yes	15 (25%)	15 (28%)	
No	45 (75%)	36 (68%)	
Missing data	0	2 (4%)	
2. Colon			0.60
Yes	1 (2%)	2 (4%)	
No	59 (98%)	50 (94%)	
Missing data	0	1 (2%)	
3. Foreign material (such as SIS)			0.46
Yes	0	1 (2%)	
No	60 (100%)	51 (96%)	
Missing data	0	1 (2%)	
Catherizable stoma according the Mitrofanoff principle			
1. Appendix			0.84
Yes	20 (33%)	18 (34%)	
No	40 (67%)	32 (60%)	
Missing data	0	3 (6%)	
2. Monti			0.59
Yes	1 (2%)	2 (4%)	
No	59 (98%)	48 (90%)	
Missing data	0	3 (6%)	
Urinary diversion			
1. Rectosigmoid pouch			**0.030**
Yes	6 (10%)	0	
No	54 (90%)	50 (94%)	
Missing data	0	3 (6%)	
2. Colon conduit			0.50
Yes	2 (3%)	0	
No	58 (97%)	51 (96%)	
Missing data	0	2 (4%)	
3. Ileum conduit			1.0
Yes	1 (2%)	0	
No	59 (98%)	52 (98%)	
Missing data	0	1 (2%)	
(Redo) bladder neck plasty			0.83
Yes	15 (25%)	14 (26%)	
No	45 (75%)	38 (72%)	
Missing data	0	1 (2%)	
Redo-ureterocystoneostomy			0.15
Yes	8 (13%)	13 (25%)	
No	50 (83%)	38 (72%)	
Missing data	2 (3%)	2 (4%)	

$^{\Delta}$*Calculated by the Fisher's exact test.*

SIS, small intestinal submucosa. Bold values are the significant ones $p < 0.05$.

TABLE 4 | Following operations for genital reconstruction or complications in the long-term follow-up.

Operations due to complications	Cross-sectional patient group ($n = 113$)		
	Staged approach ($n = 60$)	Single-stage approach ($n = 53$)	P^{Δ}
Closure of urethral penile fistula			0.64
Yes	11 (18%)	12 (23%)	
No	49 (82%)	39 (73%)	
Missing data	0	2 (4%)	
Scar correction			0.38
Yes	17 (28%)	10 (19%)	
No	43 (72%)	39 (73%)	
Missing data	0	4 (8%)	
Vaginal introitus plasty*			0.73
Yes	6 (38%)	6 (27%)	
No	10 (62%)	16 (73%)	
Missing data	0	0	
Hysterectomy*			1.0
Yes	0	0	
No	16 (100%)	22 (100%)	
Missing data	0	0	
Uterine sacropexy*			1.0
Yes	0	1 (5%)	
No	16 (100%)	21 (95%)	
Missing data	0	0	
Penile deflexion**			0.07
Yes	22 (58%)	13 (35%)	
No	14 (37%)	22 (60%)	
Missing data	2 (5%)	2 (5%)	

$^{\Delta}$*Calculated by the Fisher's exact test.*

**Only in female patients.*

*** Only in male patients.*

patients (**Table 3**). Augmentations and catheterizable stomata were performed approximately at the same age after both reconstruction concepts ($p = 0.57$) [staged approach: median age, 9 years (IQR, 7–12 years); single-stage approach: median age, 9 years (IQR, 6–12 years)]. One patient received small intestinal submucosa (SIS) for augmentation with unfavorable outcome published elsewhere (11). Urinary diversions preferably by a rectosigmoid pouch or a conduit were reported as salvage procedures only after the staged approach (**Table 3**). A (redo) bladder neck plasty was performed in 15 patients (25%) with a staged concept as a regular reconstruction part at the median age of 6 years (IQR, 5–7 years). However, 26% of the single-stage reconstructed patients needed a true redo bladder neck plasty to improve continence, which was performed at school age [median, 6 years (IQR, 6–7 years)].

In the cross-sectional group with a median age of 12 years, no significant differences occurred in regard to additional surgery between the two operative approaches (**Table 4**).

Self-reported continence data were available from 20 of the 36 CBE patients reconstructed with a single-stage approach without further surgery [56%; median, 8 years (IQR, 5–10)]. Five (25%) of these were continuous continent, eight (40%) were intermittent incontinent, and three (15%) were continuous incontinent. In four patients (20%), data were missing. Four of 22 patients after a staged approach (14%); median, 10 years (IQR, 5–15) were voiding spontaneously after a bladder neck plasty as a regular part of the staged concept. None was continent; one (33%) CBE patient each reported to be intermittent incontinent and continuous incontinent. In one further patient (33%), data were missing.

DISCUSSION

To improve knowledge about the best care for CBE, the comprehensive EEC database of the CURE-Net was reviewed in respect of epidemiology, perioperative management, and early and long-term post-operative outcome.

Although in both sexes an increasing awareness of the high rate of inguinal hernias was observed, a considerable amount of children with CBE still do not undergo initial inguinal hernia closure at primary reconstruction. One reason might be that approximately only a quarter of hernias are clinically apparent before initial bladder closure (12). However, surgeons intraoperatively commonly find wide gaping processus vaginales; a short, steep inguinal channel; and a wide internal inguinal ring in CBE males. Additionally, one might not wish to get into an emergency situation in an EEC patient. Recent data suggested that an osteotomy might be preventive in terms of *de novo* or recurrent inguinal hernias by remodeling the anterior pelvic ring shape (13). In our cohort, neither a larger symphysis diastasis (>4 cm) ($p = 0.45$) nor the fact of having undergone osteotomy itself ($p = 0.74$) was correlated with a likelihood of an inguinal hernia. To prohibit emergency situations for inguinal hernia incarceration, concomitant inguinal herniotomy was recently recommended as a safe and effective preventive procedure during initial bladder closure (14).

Nowadays, a straightforward management with a "detailed and regimented plan" is postulated in primary and delayed closure to prevent complications and additionally an increase in costs (15–17). Although not statistically significant, prospectively observed patients were operated on more often with the staged approach at an earlier age. The reason for this decision making remains unclear. Historical considerations and experts' personal experiences might have anticipated this trend. The group from Great Ormond Street confirmed that the use of a PDC has a major impact on a successful CBE reconstruction, even being the same or more important than osteotomy (18). Considering that PDC is nowadays a standard in modern perioperative care, a rate of 59% of PDC use in the prospectively observed cohort seems to be low. Peridural catheter, however, was inserted significantly more often in the prospectively observed than in the cross-sectional cohort.

The main advantage of PDC use in Great Ormond Street for the affected babies was that intensive care unit treatment was not needed. However, more than 80% of the here analyzed German

cases in both cohorts independently went postoperatively to an intensive care unit.

In this study, 36% of all CBE patients needed blood transfusions, with a significant predominance in males ($p = 0.002$). Neither age at reconstruction nor the need for osteotomy or the reconstruction technique itself did influence the necessity of blood transfusions. In the "Hopkins cohort," blood transfusions were needed in ∼58% of newborn closures (19). The authors noted no differences in terms of age and gender; however, blood transfusion was more likely with the need for osteotomy (19). This fact seems negligible in the current CURE-Net cohort, as only a vast minority had an osteotomy done. Others reported a lower transfusion rate as well; the reason for this remains unclear (20). Although patients' age at initial treatment decreased, no other fundamental changes in the perioperative management could be observed comparing the years before and after 2009, with the exception of a modest increased PDC use. Either we might conclude that treatment strategies are already optimal, or we impute that reasonable changes have sadly not been brought to daily praxis so far.

The length of urethral catheter placement after any urethral and any bladder neck reconstruction has always been a matter of debate and has a direct impact on the length of hospital stay. After primary bladder closure, the Great Ormond patients stayed in hospital significantly shorter with PDC than without (18). Seven urethral strictures occurred in the whole cohort (9.5%), leading to a bladder rupture in one case (18). Schaeffer et al. (15) reported 2% posterior bladder outlet obstruction requiring intermittent catheterization. Duration of catheter placement, however, was not reported in this series (15). Any post-operative infravesical obstruction can be disastrous for the future bladder and the upper tract development and may therefore endanger reconstruction itself (21). Although transurethral catheters remained in place significantly shorter in the prospectively observed than in the cross-sectional group, catheters in the current CURE-Net series remained longer than reported in literature before. Because of insufficient post-operative micturition, three cystoscopies were performed in the prospectively observed group (9%), detecting an anatomical stenosis in one (3%). In the cross-sectional patients, 15 cystoscopies (13%) were undertaken with eight urethral stenoses found (7%). Although there is a recent trend to earlier hospital discharge in Germany, CBE patients stay considerably longer in hospital than in other European centers (18).

Regarding the long-term outcome, complications such as upper tract dilatations seem to occur significantly more often after the single-stage procedures in the cross-sectional cohort. Urinary tract infections were generally more common after single-stage procedures in the prospectively observed and the cross-sectional cohort. Fortunately, from these data, the renal function impairment with or without the consecutive need for nephrectomy is rare in both cohorts. However, no systematic renal function assessment tests were performed in both cohorts. Urinary diversions were only reported after the staged approach. This observation is almost impossible to explain because of the retrospective data retrieval and the multicenter setting. Presumably, the operative staging is a mandatory reaction to intraoperative findings, such as a small and not compliant bladder. Therefore, it is very astonishing that long-term secondary operations, such as augmentations or catheterizable stomata, were equally distributed in the cross-sectional cohort after both primary reconstruction methods. The time periods from the initial reconstruction and the augmentations and stomata were fairly equal between the two approaches [$p = 0.57$; staged approach median, 9 years (IQR, 7–12 years); single-stage median, 9 years (IQR, 6–12 years)]. Although it must be taken in consideration that initially significantly more complications occur after single-stage reconstruction, long-term consequences are presumably the same. Additionally, a quarter of patients treated with a so-called "single-stage" approach needed further bladder neck procedures to gain continence during follow-up. This important fact confirmed by results of the CPRE concept in literature (22) should clearly be addressed during parental discussion. To gain continence is a major aim in reconstruction of the EEC. However, patients' individual outcome perspective cannot be clearly determined. In literature, continence definition and pediatric continence rates—mostly from small single-center series—vary widely from ∼10 to 80%. In a systematic review on urinary continence including more than 2,500 EEC patients, only 68% of the included studies used a concise continence definition, such as "dryness with voiding or catheterization at 3-h intervals" (23). Continence according to this definition was achieved in only 51% (23). In this current evaluation, we used the current ICCS terminology (10). Comparable to a German-wide survey of 100 EEC patients aged median 13 years (IQR, 7–18 years), 33% of the patients or their families declared themselves as continuous continent, 29% described themselves as intermittent incontinent, and 28% as continuous incontinent (24). There was no significant difference in the reported continence outcome between the single-stage and the staged approach ($p = 0.51$). However, the numbers of the included patients after staged approach were substantially low. Therefore, we plan to contact all included CBE patients again to reevaluate continence status during follow-up. These independent outcome data advocate the urgent necessity to improve urinary continence status in patients with EEC at any age. A standardized definition of continence including objective and subjective criteria would be desirable.

This study has several limitations including the fact that there is no mandatory reporting of congenital malformations to a centralized birth register in Germany. Because of voluntary participation, case completeness is limited. Data retrieval was retrospective in the large amount of cross-sectional participants, so missing or inconsistent data did occur. Multicenter treatment always implies a diversity of decision making, treatment accuracy, or operative skills and disease management most probably affecting the outcome. The strengths of this study include the large multicenter and German-wide recruitment of EEC patients in general and the integrative help of the German self-help organizations and treating physicians. Personal interviews of patients and their families by specially trained physicians guarantied

an extensive data collection based on clinical data and medical records.

CONCLUSION

The CURE-Net data provide a trend in Germany toward the staged concept. Although prospectively observed patients were operated on at a younger age, standardized perioperative management did not significantly change in years before and after 2009. Single-stage approaches tended to have initially more post-operative complications; however, this did not result in either an increase of renal function impairment or an increase of secondary operations, such as augmentations or catheterizable stomata in the long term. Furthermore, continence outcome—although difficult to assess—did not differ between the groups and was generally lower than the reported single-center data. The results of this study suggest that following secondary operations in the long term does not differ between the different staging concepts in CBE treatment.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study involving human participants was reviewed and approved by each participating center's Institutional Ethics Committee (e.g., University of Regensburg No. 09/053, University of Ulm No. 425/13). Written informed consent to participate in this study was provided by the participants' legal guardian.

AUTHOR CONTRIBUTIONS

A-KE and NZ were the major and equally contributors in writing the manuscript. A-KE, NZ, and WR analyzed and interpreted the patient data. All authors read and approved the final manuscript. HR, EJ, RS, AH, ML, CF, FO, MF, KM, ES, MP, KH and F-MS contributed by recruiting patients and collecting data.

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Assessment of Needs in Children Suffering From Refractory Non-neurogenic Urinary and Fecal Incontinence and Their Caregivers' Needs and Attitudes Toward Alternative Therapies (SNM, TENS)

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Background: Non-neurogenic urinary and fecal incontinence (UI, FI) affects approximately 6% of North American children with 1% of cases becoming refractory (nonresponsive to standard therapies). Incontinence has major potential long-term physiological and psychological implications for patients and their families. While Sacral Neuromodulation (SNM) and Transcutaneous Nerve Stimulation (TENS) are alternative therapies available for the treatment of refractory UI/FI, these are not approved for use in children in Canada. The present study assessed participants' perception of current treatments, incontinence burden, and attitudes toward novel therapies in a single pediatric institution.

Methods: Multiple validated questionnaires including Dysfunctional Voiding Scoring System (DVSS), Bristol Stool Chart (BSC), Pediatric Incontinence measurement (PinQ), and Time-Driven Activity Based Costing were used to perform a needs assessment for patients with non-neurogenic refractory incontinence, and to determine patients' and caregivers' attitudes toward alternative therapies.

Results: 75% of patients and 89% of caregivers reported a moderate to severe impact of incontinence on QoL with diminished social interactions among the primary concerns. Caregivers were frustrated with current treatments and were open to trying alternative therapies (SNM and TENS), which, at least in the case of SNM, seems to be less expensive, possibly less burdensome and more effective than current surgical options.

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Conclusion: Pediatric refractory UI/FI has a large impact on patients' and caregivers' QoL and alternative therapies with the potential to improve QoL of patients and caregivers should be further investigated as a substitute for surgery.

Keywords: child health, non-neurogenic refractory incontinence, quality of life, sacral neuromodulation, transcutaneous nerve stimulation

INTRODUCTION

Non-neurogenic urinary and/or fecal incontinence (UI/FI) refers to the involuntary leakage of urine or stool in the absence of central or peripheral neurological causes. Approximately 10% of adults and 6% of children in both Canada and the US (1–3) suffer from these conditions, however the actual prevalence is likely underreported due to the social stigma associated with incontinence. The most common cause of non-neurogenic incontinence in children is Bladder and Bowel Dysfunction (BBD), which can be responsible for up to 40% of pediatric urology clinical visits (4). BBD describes a multitude of lower urinary tract symptoms (LUTS), often accompanied by constipation and/or encopresis. BBD is also commonly associated with vesicoureteral reflux (VUR) and recurrent (febrile) urinary tract infections (UTIs), which can ultimately impact renal function (5). Incontinence also affects children's psychosocial well-being including anxiety, self-esteem, shame, isolation, poor school performance, and other behavioral changes (4).

Standard therapy for both children and adults with non-neurogenic UI/FI in Canada includes behavioral and dietary changes, biofeedback, and pharmacological treatments (6). Medications, in particular antimuscarinics, may elicit adverse effects (dry eyes, dry mouth, constipation, GI upset, heat intolerance) but can provide effective treatment outcomes in most patients. Despite the success of these therapies, 1% of patients will become refractory and will not respond to any treatment (7, 8). Currently, the only options for refractory cases have been surgical. These include botulinum toxin injections for urinary incontinence and transanal irrigation and anterograde continence enema for fecal incontinence. However, these surgical alternatives can be burdensome for the patient, are not always successful and carry their own risks of morbidity (9–11).

Incontinence-afflicted adults who do not respond to first-line therapies are eligible for sacral neuromodulation (SNM) or transcutaneous nerve stimulation (TENS). SNM and TENS are minimally invasive and reversible treatments that rely on delivering electrical stimulation to the S3 foramen or saphenous nerve, respectively. In the US, SNM is considered standard of care for adults suffering from non-neurogenic refractory incontinence while in Canada, SNM is performed on case-by-case basis. SNM and TENS are not approved by the FDA or Health Canada for use in children even though international studies have shown that SNM therapy can positively affect symptoms associated with constipation and urinary dysfunction, decreases reliance on pharmacological interventions and improves QoL in children (12–23). TENS is associated with immediate and short-term improvement in children suffering from nocturnal enuresis, although there is a paucity of data in the contemporary literature (21, 24).

It is essential to investigate these therapies as a potential treatment for non-neurogenic refractory incontinence in children as an alternative to current surgical options. While SNM is considered a minimally invasive procedure, it requires an operating room and an implantation of a neurostimulator that sends electrical signals to the sacral nerves, as such, it is important to understand patient willingness to undergo these procedures. This study aims to determine the impact of non-neurogenic refractory UI/FI on children and their caregivers, assess patient's and caregiver's attitudes toward the potential use of SNM/TENS therapies in Canada and to provide a preliminary cost analysis of SNM therapy vs. standard surgical options within the Canadian context.

METHODS

Study Design, Population, and Recruitment

This was a single-center, cross-sectional study of patients with non-neurogenic refractory UI/FI followed at the urology outpatient clinic of a quaternary care pediatric hospital in Ontario, Canada. Following institutional Research and Ethics Board approval (REB# 1000058568), we prospectively recruited patients who presented with non-neurogenic refractory incontinence and their caregivers from January to August, 2018. All patients were evaluated with videourodynamics (refractory UI only) and spine MRI (refractory UI and/or FI). Patients with abnormal findings on MRI were excluded from this study. We defined refractory incontinence as persistent UI/FI after 6 months of conservative management (bladder retraining and constipation treatment ± biofeedback and pelvic floor physiotherapy), followed by lack of response to adequate medical therapy (maximized antimuscarinics ± beta-3 adrenoceptor agonists; or alpha-blockers for dysfunctional voiding with high post void residuals) for at least 3 months in patients aged 5–17 years. We included patients who regularly attended our institution for treatment of BBD, LUTS, voiding dysfunction, urinary retention, constipation, and UTI; spoke English and; could provide consent. We excluded patients with spinal cord injury, developmental delays, behavioral/psychiatric disorders, immunodeficiency, bleeding disorders, and inflammatory bowel disease; did not speak English; or were unable to provide consent. Families were recruited during scheduled visits. After obtaining consent, patients completed the Dysfunctional Voiding Scoring System (DVSS), Bristol Stool Chart (BSC), the Pediatric Incontinence measurement (PinQ) tool for children (or the parental proxy), and a needs assessment questionnaire with

open-ended questions (**Supplementary Appendices A, B**) (12, 13, 25–27).

Measurement Tools

DVSS is a validated 10-parameter instrument used to measure pediatric non-neurogenic dysfunctional voiding. DVSS scores of ≥6 for females and ≥9 for males are indicative of incontinence (26). The BSC detects the presence of functional defecation disorders and has been validated for use in children (27). The PinQ is a cross-culturally validated test with proven test-retest reliability and good parental proxy, developed for non-neurogenic enuretic children to evaluate social, self-esteem, family function and body image domains of QoL (12, 13, 25). A mild, moderate or severe impact of incontinence on QoL is defined by a PinQ score of <20, 20–50, or >50, respectively (28). Descriptive statistics were used to evaluate demographic data and questionnaire scores. The Mann-Whitney/Wilcox test was used to assess differences between patient and caregiver PinQ scores.

Qualitative Analysis

Patient and caregiver responses to open-ended questions were de-identified and transcribed from written to electronic text. Codes were generated manually by two independent investigators and inconsistencies were discussed and resolved. Responses were analyzed using NVivo software (NVivo 12, QSR International, Cambridge, MA).

Time-Driven Activity-Based Cost Analysis

TDABC calculates costs by combining the capacity cost rates (CCRs) of key direct and indirect resources as well as time estimates from electronic medical records (EMR) for processes involved in patient care (29). Direct resource costs include the cost of providers and material resources. Indirect costs include the cost of using hospital spaces which includes general administrative and overhead support activities. To calculate capacity cost rates, the total costs of staff, supplies, and clinical working spaces were captured using budgets and salary information from the 2018–2019 fiscal year divided by the total available hours of those resources for patient care. Time estimates from EMR data were then applied to generate costs for each step of the procedure. TDABC model includes cost of OR and PACU space, materials (device, drugs, equipment, etc.), and the cost of non-physician staff (OR nurses, etc.). Physician costs are not included in this model. Similarly, follow up costs are also not included. For SNM the costs for one stage and two stage procedures are provided. All costs are provided in Canadian dollars.

RESULTS

Study Participants

Of the 30 patient families approached, 29 agreed to participate in this study (96.7%). The median age of patients was 9.6 (range 5–15) and 79% were female. Of the 21 patients with siblings, 32% of those siblings also had issues with incontinence (**Table 1**). The median DVSS of patients as reported by their caregivers was 12 (range 2–23; **Table 2**) and 61% of children attained a DVSS score >11 indicating at least moderate incontinence. There was no statistically significant difference in DVSS with sex, patient age, parental marital status, or household income.

TABLE 1 | Characteristics of study population.

Patients ($n = 29$)	Gender	Male	6 (21%)
		Female	23 (79%)
	Mean Age	9.6 years (5–15 years)	
Parents ($n = 29$)	Marital Status	Single	0 (0%)
		Married/Common Law	24 (83%)
		Divorced	5 (17%)
	Employment Status	Employed	22 (76%)
		Not employed	7 (24%)
	Parents with >1 child		21 (72%)
	Parents with >1 child with UI		7 (32%)

TABLE 2 | Participants' DVSS and QOL scores.

	DVSS	QOL
Parents	NA	36.50 (5–56)
Patients	12 (2–23)	32 (2–50)

DVSS scores of the children were based on parental responses.

Twenty-eight patients and their caregivers (96.5%) completed the PinQ. Patient PinQ results showed no significant difference in QoL with sex (**Table 2**). Parental PinQ results showed no significant differences with patient age, sex, or parental marital status. Overall, 71% of patients and 89% of parents reported moderate to severe impact of incontinence on QoL (PinQ ≥20). Patient and parental PinQ scores were strongly correlated ($p < 0.0001$, rho = 0.758), but no significant correlations were observed between PinQ score and patient's age or caregiver's marital status.

Needs Assessment

Based on patients' responses, incontinence burden was grouped into five major themes: physical, emotional, social, family, and financial burden (**Figure 1**).

Physical Burden

"Change" was the predominant word in this theme (**Figure 2**). Patients reported the need for frequent change of clothing, restricting fluid intake, going to the washroom before bed and setting alarms. Caregivers reported frequent clothing changes and stressed the need to always be prepared which resulted in feelings of tiredness: "This can be tiring, (and) time consuming to always have to change him and bring clothes and pull-ups everywhere." Caregivers often monitored the frequency of their children's washroom visits and encouraged proper diet and voiding habits, which could be "exhausting," as one caregiver described it. Caregivers also worried about the long-term consequences of incontinence such as infections, impact on future kidney function, medication use, treatments, and the potential need for surgery.

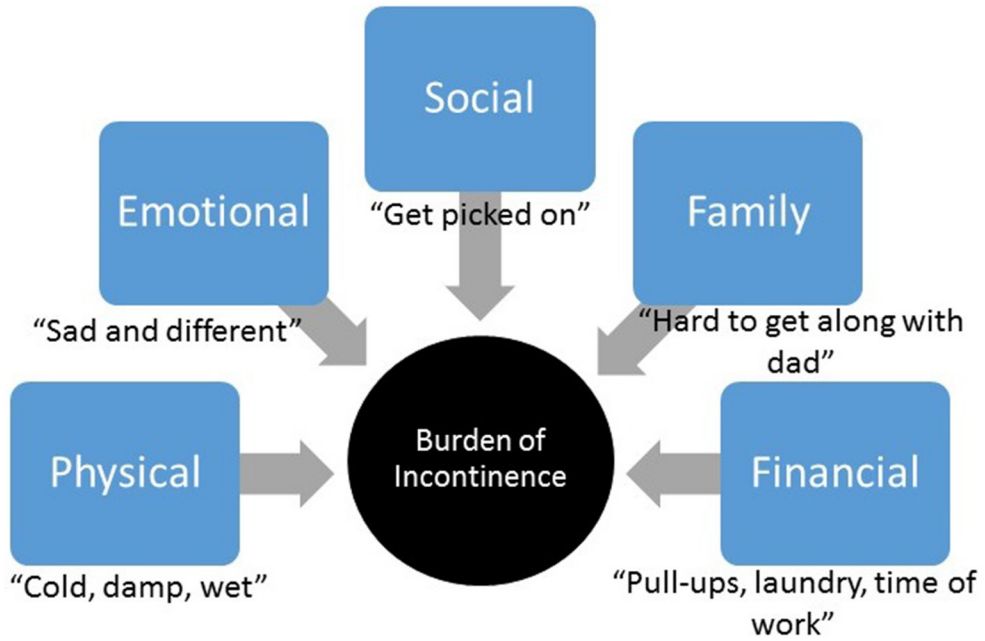

FIGURE 1 | Incontinence burden. Major themes identified within patients' and parents' responses related to incontinence burden.

Emotional Burden

Incontinence took an emotional toll on both patients and caregivers. Caring for a child with refractory UI/FI contributed to lost sense of control, heightened stress and increased frustration. Patients expressed feeling "different," "embarrassed," "sad," and "angry" (**Figure 2**). Patients also reported feeling bad for their caregivers: "I was sad when I peed (the) bed and daddy had to change." Caregivers reported wanting to control situations and being frustrated and anxious when it was impossible. Caregivers also worried about their children's emotional well-being and did not want to see them feeling embarrassed, ashamed or guilty. When discussing their daughter, one parent reported the "stress of feeling like she (the daughter) had done something wrong."

Social Burden

Both patients and caregivers expressed that refractory UI/FI contributed to a loss of social interaction: "It's hard missing out on things with my friends," "I can't go to sleepovers," "(I) Don't have many friends because of (incontinence)," and "(I) get picked on" (**Figure 2**). Social alienation, lowered self-esteem, reduced independence, and future implications were also the major concerns of caregivers. Caregivers also worried about their children's self-esteem, and how "(incontinence) affects (their child's) confidence outside of home," "I am worried he'll get picked on."

Family Burden

The most prevalent word among patients with respect to incontinence management was "mom" (**Figure 2**): "My mommy wakes me," "My mom changes me and cleans up," "talk with mommy," "find solutions with mommy for school and friends," and "tell mommy and she helps." Patients also reported that incontinence is stressful on their caregivers and on family life in general: "mom gets stressed," incontinence "makes it hard to get along with daddy sometimes." Stress was the predominant descriptor used when caregivers talked about their children's incontinence in relation to family life. Caregivers constantly monitored and reminded their children to visit the washroom frequently, ensure soiled clothes are changed, and encourage appropriate exercises: "I feel for my daughter. I don't like to see her embarrassed and don't enjoy nagging (her) to drink more or use (the) washroom." Still, another caregiver summed it up simply as, "you do what has to be done for your child."

Financial Burden

Approximately 61% of parents reported that their child's UI/FI is a significant financial burden, primarily due the cost of incontinence-related items and missed work. Laundry was the most common word in this theme (**Figure 2**). One caregiver expressed choosing buying diapers over groceries when money was tight. Caregivers reported taking time off work for hospital visits or to stay home when necessary. One caregiver reported "(I) have lost weeks worth of pay to be in the hospital to talk with doctors." Another reported "(I) lose sleep and miss work."

Current and Alternative Treatments

The majority (72%) of caregivers reported limited, no improvement or a "cycle of improvement and setbacks" with current treatments. Caregivers were hopeful new treatments may be helpful in reducing their child's "on-going symptoms,"

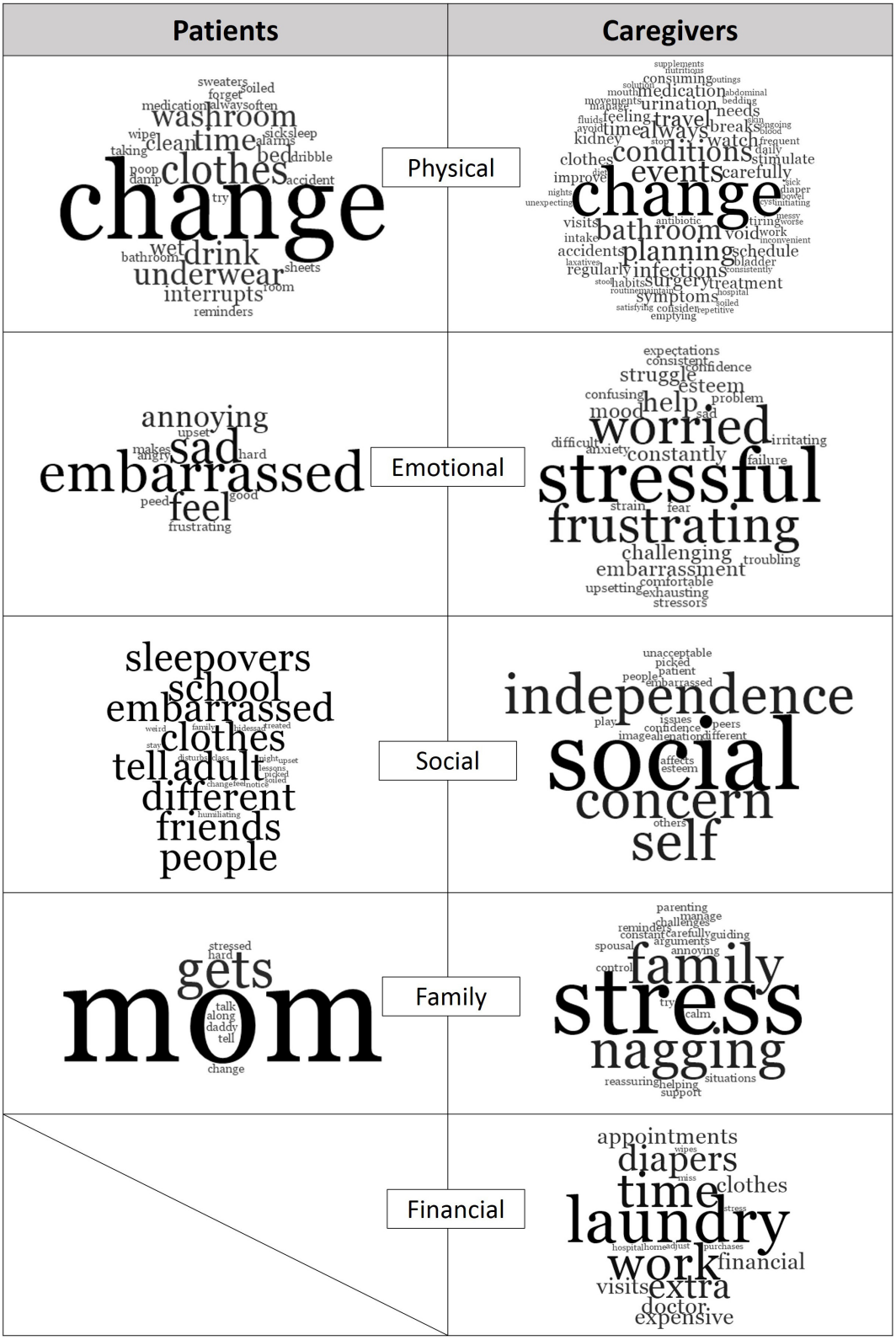

FIGURE 2 | Study themes and most common words mentioned by patients and caregivers. Generated with NVIVO.

TABLE 3 | TDABC of costs for management of refractory urinary and fecal incontinence.

Sacral Neuromodulation (SNM) ($CAD)		Standard Treatment Options ($CAD)		
SNM Lead Test & Implantation One-Stage	$11,930	Urinary Incontinence	Botulinum Toxin Surgery*	$9,030–15,050
SNM Lead Test & Implantation Two-Stage	$13,269	Fecal Incontinence	Transanal Irrigation System+	$14,198–23,664 (32, 33)
			Malone Anterograde Continence Enema[x]	$6,433–8,547 (34–36)

Two injections/year over 3–5 years.

+System use over 3–5 years.

[x]Includes daily flushing over 3–5 years.

or limit their child's antibiotic intake. Others were hopeful new treatments may improve their child's QoL, self-esteem and make them "feel normal." One caregiver stated that a new treatment is "worth trying even if it doesn't cure." Over half (54%) of caregivers were open to trying TENS therapy. Of those, 42% were also open to trying SNM therapy. Approximately 21% of caregivers were unwilling to try either therapy while 25% indicated they were unsure or required additional information.

Cost Analysis

A preliminary cost analysis was performed to compare the surgical costs of SNM therapy vs. common established treatment modalities for children with refractory non-neurogenic urinary and fecal incontinence which include but are not limited to botulinum toxin injections for urinary incontinence, open/laparoscopic caecostomy for antegrade enemas, and retrograde transanal irrigation systems for fecal incontinence. Surgical costs were calculated using the time-driven activity-based cost analysis (TDABC) methodology (29). Costs are given in Canadian dollars, using Canadian public health system, and based on our institutional experience and available literature. The costs do not include physicians time, follow up appointments, ongoing costs into adulthood (30, 31), nor the time required daily for personal care (>1 h/day). The costs indicated here include the initial surgery/set up and supplies for 3–5 years. The preliminary longitudinal cost assessment indicates that SNM therapy is less expensive than current standard surgical options for both UI/FI (**Table 3**). This is mainly due to the continuous need for re-administration, or significant accumulative daily costs for equipment/materials (catheters/flushes) required with the current options.

DISCUSSION

Refractory non-neurogenic incontinence is complex, with short- and long-term consequences that affect patients and their immediate circle of care. Two-thirds of patients indicated that incontinence has a moderate to severe impact on their lives. The remaining one-third expressed minimal impact and consisted of patients aged <12. Younger children are more dependent on their parents and thus the loss of independence, friends, and sleepovers, may play a smaller role. Bedwetting has been shown to lead to behavioral problems and both wetting and bowel accidents carry social stigma that may affect self-esteem and feelings of shame and isolation (2, 4, 28). Bullying, name-calling and having few friends affect not only the child's self-esteem but also his/her social development and academic performance at school. Less is known about the parental burden of a child's refractory UI/FI. Studies that examined the impact of parental well-being in similar populations indicate a general decrease in QoL (5, 6). In this study, pressures on caregivers led to altered family dynamics, marital discourse, and financial burden, consistent with previous publications (28).

As a result, 42 and 54% of caregivers welcome the potential use of novel therapies, SNM and TENS, respectively. Caregivers were more reluctant to try SNM, presumably due to its invasiveness. Several caregivers indicated that they require more information about SNM and TENS, suggesting these therapies are not well described as treatment alternatives. Twenty-one percent of caregivers were unwilling to try either SNM or TENS. This may be due to the length of time children struggled with this condition. While all the children enrolled in the study have previously tried multiple non-invasive therapies, some have suffered for less than a year while others struggled with the condition for much longer. The shorter time since the diagnosis might have led some parents to still hope that the condition will resolve itself and their unwillingness to try invasive therapies. In addition, parents of younger children might have also hoped that the condition will resolve itself without invasive treatments. Due to the small sample size of our study, we were unable to determine correlations between children's age, condition persistency, and willingness to try SNM or TENS. Moreover, while caregivers were asked about their willingness to subject their children to SNM or TENS therapies this question did not relate SNM or TENS therapies as alternatives to surgical options (Botox injection or MACE). It is possible that if provided with a choice, parents will more readily opt for SNM or TENS. Approximately 50% patients were willing to undergo SNM or TENS, indicating that caregivers are willing to try anything to ease suffering for their children.

One potential advantage of the SNM system is that it demonstrates efficacy in treating both fecal and urinary incontinence, as opposed to current standard surgical options which may require a combination of multiple surgical therapies to treat this dual issue. In our institution, botulinum toxin injections are a standard surgical option used to alleviate symptoms of urinary incontinence (associated with refractory

overactive bladder, small bladder capacity or low compliance) while transanal irrigation and MACE are surgical options used to alleviate symptoms of fecal incontinence. SNM has demonstrated higher success rates compared to botulinum toxin surgery in improving symptoms of urinary incontinence but also leads to the decrease and finally elimination of antegrade continence enema use in children with severe constipation (19, 23, 37, 38). This point must be considered in the context of conventional treatment failure which is associated with high costs of health care resource use (ER visits, clinic visits), and community costs including the cost of continence supplies which could be in excess of CAN$2000/year for some families (3, 34, 39, 40). Furthermore, once permanently implanted, SNM does not require further repetitive treatments (as opposed to Botulinum toxin injections) or multiple episodes of general anesthesia, and does not have the same revision risks and side effect profile seen with cutaneous abdominal stomas (41). Morever, botulinum neurotoxin injections can lead to antibody production in patients and subsequent therapy failure (42). This, of course is not a factor in SNM therapy. This preliminary cost description can be used in future cost-effectiveness work that accounts for health outcomes, community costs, and transition of health states, which is needed to definitively establish the cost-effectiveness of the SNM implantation in this population.

This study was conducted in a major Canadian pediatric center where SNM has been demonstrated to have fewer outright costs than current standard surgical options available for patients suffering from refractory urinary and/or fecal incontinence. This was a preliminary cost description, and hence therapy failure, future emergency visits, additional surgical and pharmacological treatments, and treatments for other disorders that might stem from incontinence (mental health), and the economic impacts of therapy failure, such as loss of work time were outside the scope of this study. However, the preliminary cost description presented here, together with the QOL findings reported in this paper, future studies and literature reports can be used for future cost-effectiveness analysis that accounts for health outcomes, community costs, and transition of health states. SNM therapy has already been shown to be highly effective, improves QOL of children and carries minimal complications (18, 23). In addition, it is possibly less burdensome for patients and caregivers than botulinum toxin injections, antegrade stomal irrigation, or retrograde transanal irrigation systems, although, direct comparisons in term of quality of life or costs between these various options are not available in the literature. On the other hand, it is important to consider and discuss potential limitations when presenting SNM to families as an alternative treatment, such as risk of lead displacement with growth, jumping, high contact sports and falls, and possible need for surgical revisions.

In cases where all other options have been exhausted, the potential use of orphan therapies, such as SNM, that may take a long time to receive regulatory approval should be considered. This was a single center study with a small convenience sample of children with refractory incontinence and as such might not be generalizable to other populations. The use of questionnaires instead of interviews limits our ability to provide in depth assessments of the impact of refractory non-neurogenic UI/FI on QoL. Despite these limitations, our study highlights the challenges associated with non-neurogenic refractory incontinence on children and their families, as well as the value of patient and caregiver feedback when searching for alternative treatment options. Our findings support further investigation of the use and effectiveness of therapies, such as SNM and TENS that have not yet received regulatory approval in Canada, as alternatives to surgery in children suffering from non-neurogenic UI/FI. These novel therapies may be key to reducing the burden of this complex condition on families and improving patient and caregiver QoL.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospital for Sick Children Research Ethics Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JD, EM, and RV conceived and executed the project and wrote the preliminary draft. MP administered the survey to the participants. EM and MP performed the qualitative analysis. JD, EM, and MP wrote the paper. AA performed quantitative analysis. LR and FO'K performed cost-effectiveness analysis. DE, RF, AL, and MK contributed to the project conception and execution with MK supervising the project as well. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00558/full#supplementary-material

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Risk Factors for Testicular Atrophy in Children With Testicular Torsion Following Emergent Orchiopexy

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Objective: To analyze the risk factors for testicular atrophy (TA) in children with testicular torsion (TT) following emergent orchiopexy.

Methods: Clinical data of patients with TT undergoing orchiopexy were retrospectively reviewed, including age at surgery, affected side, delayed surgery (12–24 h and more than 24 h), echogenicity of testicular parenchyma on ultrasonography (ETPU), testicular blood flow on Color Doppler ultrasonography (CDUS), surgical findings (intraoperative blood supply, the degree of torsion, and surgical approaches), and follow-up. The primary outcome was the rate of TA after orchiopexy. The secondary outcome was the testicular volume loss (TVL) between the affected testis and the contralateral.

Results: A total of 113 patients were enrolled in this study with a median age of 11 years. The median follow-up was 21 months. Patients had a median TVL of 51.02% and 44 (38.94%) of them developed severe TA during follow-up. TA was significantly associated with age at surgery ($P < 0.0001$), delayed surgery ($P = 0.0003$), ETPU ($P = 0.0001$), and intraoperative blood supply ($P = 0.0005$). Multivariate logistic regression analysis showed that school-age children (OR = 0.069, $P < 0.001$) and puberty (OR = 0.177, $P = 0.007$) had a decreased risk of TA compared with preschool children, and that heterogeneous ETPU (OR = 14.489, $P = 0.0279$) and delayed surgery >24 h (OR = 3.921, $P = 0.040$) increased the risk of TA. Multivariate analysis demonstrated that ETPU ($F = 16.349$, $P < 0.001$) and delayed surgery ($F = 6.016$, $P = 0.003$) were independent risk factors for TVL.

Conclusions: Age at surgery, delayed surgery, and ETPU may play a crucial role in predicting the TA in children with TT following emergent orchiopexy. Moreover, blood flow measured by CDUS could not predict the outcome properly.

Keywords: pediatrics, testicular torsion (TT), atrophy, risk factors, urogenital system, urgent care

INTRODUCTION

Testicular torsion (TT) has been considered as a serious surgical emergency that requires prompt diagnosis and surgical intervention. It is not only the most common cause of testicle loss in children but also may impair fertility and endocrine function of the testis in the future (1). The annual incidence of TT has been estimated to be around 0.004% among children <18 years (2). TT may occur at any age, but the vast majority of cases occur after age 10 years with a peak at 12 to 16 years (3). This disease features a dramatic reduction of testicular blood supply due to the torsion of the spermatic cord, with left-sided predominance, and rare bilaterality. The testicular torsion may be present intravaginally and extravaginally, and extravaginal torsion occurs more often in infants. Therefore, correct diagnosis and immediate treatment are crucial for the salvage when clinical assessments support or raise suspicion for spermatic cord torsion. Children and adolescents presenting acute scrotal pain for 12 h or more should still undergo surgical exploration because the viability of the testis is difficult to predict. Relevant research showed that testicular atrophy (TA) developed postoperatively in 12–68% of such cases, even though orchiopexy had been successfully performed (4–6). Most previous studies have focused on the predictors of intraoperative blood supply in the affected testis, mainly the degree and duration of torsion. However, no study has explored their combined influence on the prognosis of TT along with other factors. As such, the present study aimed to evaluate the occurrence of TA in children with TT following emergent orchiopexy and to identify risk factors involved in the development of testicular loss.

MATERIALS AND METHODS

Patient Population

The protocol was approved by the local institutional review board. We identified all children with TT treated in the Children's Hospital of Chongqing Medical University between January 2009 and January 2019.

Clinical data were retrieved on age at surgery, affected side of testes, delayed surgery, echogenicity of testicular parenchyma on ultrasonography (ETPU), testicular blood flow on Color Doppler ultrasonography (CDUS), surgical findings, and follow-up. Inclusion criteria: TT confirmed by surgical exploration, successful orchidopexy, provision of informed consent by a parent or legal guardian. Exclusion criteria: age at surgery <2 years, follow-up <6 months or loss to follow-up, bilateral or congenital TT, cryptorchidism, testicular tumor, combined with systemic disease, or postoperative complications.

Outcomes and Variables

The primary outcome was the rate of TA after orchiopexy. The secondary outcome was the testicular volume loss (TVL) between the affected testis and the contralateral. Patients were divided into 3 groups according to their age at surgery: preschool children (2–6 years), school-age children (7–12 years), and puberty (13–17 years). The delayed surgery was defined as the surgery more than 12 h after onset of pain. In addition, delays were further separated into two periods: 12–24 h and more than 24 h. CDUS findings included testicular size (L, length; W, width; H, height), ETPU, and testicular blood flow. The conditions of testes were evaluated based on the ETPU (homogeneous or heterogeneous), in which the testicular blood flow was either decreased or absent. Moreover, surgical findings consisted of intraoperative blood supply, the degree of torsion (<360° or more than 360°), and surgical approaches (orchidopexy or orchiectomy). Intraoperative rich blood supply was defined as the color of the affected testis changed to bright red; in contrast, poor blood supply meant that the color was maintained dark red.

Management and Follow-Up

All operations were performed in a standard fashion. First, the affected testis was reset without tension. A moderate amount of 1% lignocaine was infiltrated within the spermatic cord. Second, the tunica vaginalis was opened to notice the color of the testis, the number of rotations, and the anatomy of the tunica vaginalis. The tunica albuginea was incised to lower intraparenchymal pressures. The exposed surface was covered with a piece of gauze moistened in warm saline and the blood supply was observed for 30 min. The contralateral testis was fixed with non-absorbable suture to reduce the risk of metachronous torsion. The affected testis was re-examined for potential viability, and the decision for orchidopexy or orchiectomy was made. Testicular infarct was characterized by coagulative necrosis of the testicular parenchyma, resulting in a diffuse reddening or blackening of the tissue. In this setting, surgical resection was taken into practice.

Patients had a routine follow up, with CDUS performed to evaluate the TVL between the affected testis and the contralateral. Testicular volumes were calculated using the modified ellipsoid volume formula, $V = L^*W^*H^*0.523$. Currently, there is no standard definition of TA. We used a similar definition as suggested by relevant references (7, 8); TA was defined as the following: TVL ≥80% or no sustained blood supply in the follow-up.

Statistical Analysis

Continuous variables and categorical variables were demonstrated as median value (range: min–max) and a percentage, respectively. Comparisons of variables between patients with and without TA were done with the chi-square test or Fisher's exact test for categorical data. Differences between groups were determined by non-parametric Mann–Whitney test (two groups) or Newman–Keuls test (multiple groups). Multivariate logistic regression analysis was used to examine the association of variables that were statistically significant in univariate analysis with TA, presented as odds ratios (OR) with 95% confidence intervals (95% CI). Multivariate analysis of variance was performed to evaluate the association between TVL and predictors. $P < 0.05$ were considered statistically significant. All analyses were performed using SPSS®, version 26.0 (IBM Corp., Armonk, NY, United States).

Abbreviations: TT, Testicular torsion; TA, Testicular atrophy; TVL, Testicular volume loss; CDUS, Color Doppler ultrasonography; ETPU, Echogenicity of testicular parenchyma on ultrasonography.

TABLE 1 | Univariate analysis of testicular atrophy and testicular volume loss.

	Normal testis ($n = 69$)	Testicular atrophy ($n = 44$)	*P*-value	Testicular volume loss Median (IQR)	*P*-value
Age at surgery (years)			<0.0001*		0.0372*
2–6	8 (7.08%)	26 (23.01%)	Ref	69.31 (48.98–90.51)	Ref
7–12	33 (29.20%)	8 (7.08%)	<0.0001*	26.80 (0.67–73.35)	0.0178*
13–17	28 (24.78%)	10 (8.85%)	<0.0001*	28.64 (9.70–90.28)	0.0495*
Side			0.5847		0.6080
Left	57 (50.44%)	40 (35.40%)		51.02 (10.76–87.65)	
Right	12 (10.62%)	4 (3.54%)		44.73 (1.48–85.92)	
Delayed surgery (hours)			0.0003*		<0.0001*
0–12	33 (29.20%)	8 (7.08%)	Ref	11.57 (3.61–42.24)	Ref
12–24	20 (17.70%)	10 (8.85%)	0.1860	62.29 (20.02–87.18)	0.0037*
>24	16 (14.16%)	26 (23.01%)	<0.0001*	78.40 (54.97–95.67)	<0.0001*
Degree of torsion			0.7201		0.7212
0–360°	40 (35.40%)	24 (21.24%)		54.21 (2.46–89.66)	
>360°	29 (25.66%)	20 (17.70%)		51.02 (12.04–76.14)	
ETPU†			0.0001*		<0.0001*
Homogeneous	27 (23.89%)	2 (1.77%)		12.03 (0.45–28.64)	
Heterogeneous	42 (37.17%)	42 (37.17%)		69.03 (13.68–91.18)	
Blood flow on ultrasonography			0.1146		0.5033
Decreased	18 (15.93%)	6 (5.31%)		54.58 (22.36–90.23)	
Absent	51 (45.13%)	38 (33.63%)		51.02 (6.30–87.65)	
Intraoperative blood supply			0.0005*		<0.0001*
Rich blood supply	48 (42.48%)	16 (14.16%)		17.82 (1.48–72.32)	
Poor blood supply	21 (18.58%)	28 (25.78%)		68.75 (51.02–92.87)	

Data are expressed as median (IQR) or n (%) as appropriate.
† ETPU, Echogenicity of testicular parenchymal on ultrasonography.
**P < 0.05.*

RESULTS

A total of 313 patients with TT were evaluated and 174 patients (55.59%) underwent orchiopexy. Of these, 128 patients had a complete follow-up. One child suffered continuous testicular enlargement at 5 months postoperatively, which was later diagnosed as a yolk-sac tumor by re-exploration, without any other complications such as infection or recurrence. Taking the effect of cryptorchidism on testicular development into account (9), we excluded 11 patients who had a history of cryptorchidism. Finally, a total of 113 patients were included after we identified some patients with other exclusion criteria. The median age at surgery was 11 years (range: 2–16) and the most frequently affected side of the testes was left-sided (85.84%). After a median follow-up of 21 months (range: 6–63), the patients in this cohort had a median TVL of 51.02% (range: 0–100), and 44 patients (38.94%) developed severe TA at their last visit.

As reported in **Table 1**, univariate analysis revealed that the following variables were significantly associated with TA: age at surgery ($P < 0.0001$), delayed surgery ($P = 0.0003$), ETPU ($P = 0.0001$), and intraoperative blood supply ($P = 0.0005$). Multivariate logistic regression analysis (**Table 2**) showed that school-age children (OR = 0.069, 95% CI: 0.018–0.256, $P < 0.001$) and puberty (OR = 0.177, 95% CI: 0.050–0.624, $P = 0.007$) had a decreased risk of TA compared with preschool children, and that heterogeneous ETPU (OR = 13.69, 95% CI: 2.424–77.31, $P = 0.003$) and delayed surgery >24 h (OR = 3.921, 95% CI: 1.061–14.486, $P = 0.040$) increased the risk of TA.

TABLE 2 | Multivariate logistic analysis of testicular atrophy.

Variables	OR	95% CI	*P*-value
Echogenicity of testicular parenchymal on ultrasonography	13.69	2.424–77.31	0.003*
Intraoperative blood supply	0.844	0.264–2.692	0.774
Age at surgery (control group: pre-school age)			
School age	0.069	0.018–0.256	<0.001*
Puberty	0.177	0.050–0.624	0.007*
Delayed surgery (control group: 0–12 h)			
12–24 h	1.329	0.354–4.992	0.674
>24 h	3.921	1.061–14.486	0.040*

**P < 0.05.*

Table 1 showed that the following variables were significantly associated with TVL: age at surgery ($P = 0.0372$), delayed surgery ($P < 0.0001$), ETPU ($P < 0.0001$), and intraoperative blood supply ($P < 0.0001$). The results in **Table 3** indicated

TABLE 3 | Multivariate analysis of variance of testicular volume loss.

Variables	df	*F*	*P*-value
Echogenicity of testicular parenchymal on ultrasonography	1	16.349	<0.001*
Delayed surgery	2	6.016	0.003*
Age at surgery	2	1.780	0.174
Intraoperative blood supply	1	1.805	0.185

$R^2 = 0.403$.
*$P < 0.05$.

that 40.3% ($R^2 = 0.403$) of the total variation in TVL could be explained by the regression model in multivariate analysis, in which ETPU ($F = 16.349$, $P < 0.001$) and delayed surgery ($F = 6.016$, $P = 0.003$) were independent risk factors for TVL.

DISCUSSION

TT is one of the most common causes of "acute scrotum" in children. Persistent ischemia can result in acute testicular damage, requiring immediate surgical intervention. However, testicular torsion-detorsion is an ischemia-reperfusion process of the testis. The injury induced by ischemia-reperfusion is notably more severe than the damage resulted from ischemia alone (10, 11). Besides, multiple theories were implicated to support the cross-injury theory and adverse effects on fertility, such as autoimmunization against the spermatogonia, decrease in testicular blood flow caused by a reflex sympathetic response, autoimmune reactions due to the disruption of the blood-testis barrier, and the generation of reactive oxygen species after detorsion (12). As with any medical treatment, the surgeon must weigh different complications associated with retention of testes (infection, malignant transformation, atrophy, and adverse effects on fertility) and removal of testes (trauma and psychosocial problems during puberty). While these concerns were acceptable preoperatively, the actual rate of orchiectomy after surgical detorsion was fairly high in general, and so was TA following emergent orchiopexy. Therefore, it is important to identify the preoperative or intraoperative factors related to prognosis, allowing emergency practitioners to figure out the correct surgical planning and the appropriate patient education. In this study, several potential risk factors for TA have been retrospectively evaluated, showing that age at surgery, delayed surgery, ETPU, and intraoperative blood supply were significantly correlated to the prognosis.

Delayed surgery and the degree of torsion were known prognostic factors for testicular viability during operation (13, 14). In a retrospective study, Lian et al. found that a higher incidence of postoperative TA was related to a delay of more than 24 h (6). Through a more detailed division of delay, the present study revealed that delayed surgery for more than 12 h increased significantly the TVL compared with the operation within 12 h and that delayed surgery for more than 24 h was significantly associated with an increased risk of TA. In the meantime, delayed surgery for more than 24 h was an independent risk factor for TA in the multivariate analysis after the effects of other variables were excluded. To the best of our knowledge, no other studies have demonstrated the association of the degree of torsion with TA. Compared with the incomplete twist of the spermatic cord, the complete twist (more than 360°) did not increase the risk of TA and demonstrated no significant reduction of testicular volume in our study, indicating the critical importance of timely surgical detorsion without preoperative clarifications about the degree of rotation. The intraoperative blood supply was another significant risk factor related to the prognosis but not an independent predictor.

CDUS offered a rapid, available, and safe modality in the diagnosis of TT to assess testicular architecture, intraparenchymal blood flow, and other anatomic details (hydrocele, scrotal thickening), with the advantages of non-invasive and repeatable examinations. But this study showed no significant correlation between blood supply on CDUS and outcomes. Nonetheless, CDUS showed accuracy in the imaging of affected testis with a high degree of sensitivity and specificity. We may pay attention to the heterogeneous ETPU in CDUS reports, which are signs of parenchymal edema or necrosis due to severe and persistent ischemia or hypoxia. This study found that TA and TVL could be predicted by heterogeneous ETPU, which was further confirmed by multivariate analyses. Another study also found that ETPU could predict testicular salvage after torsion (15). Based on these findings, emergency testis-sparing surgery could be performed for testis with homogeneous ETPU as soon as possible, to some extent, if no other risk factors were identified.

A previous study has reported a correlation between age at surgery and the rate of orchiectomy (16). However, the patients were not stratified based on age at surgery and no relevant investigations have found its influence on testicular prognosis. In our study, we realized that age at surgery was a predictor for the development of TA. Meanwhile, further analyses showed that the risk of TA reached a peak in the preschool period, which may be helpful in clinical decision-making and postoperative family counseling.

It has been reported that the outer membrane of affected testis was usually under high tension, due to obstruction of venous return and lymphatic circulation. This presentation resembles that of the testicular "compartment syndrome," which might increase the possibility of testicular ischemia and necrosis. Previous studies concluded that a tunica albuginea fasciotomy to relieve compartment pressure followed by a tunica vaginalis flap may enhance salvage (17, 18). However, a major disadvantage of this technique is the higher rate of postoperative TA. The incision of tunica albuginea was adopted for decompression in our surgical procedures, yet this may impact spermatogenesis. On the other hand, the reliability of these techniques lacks large-scale validation at the moment. Of note, our intraoperative findings revealed that the rate of orchiectomy in our cohort was 44.41%. But if the proportion of patients with TA were included, more

than 65% of all patients diagnosed with TT at our institution would ultimately develop severe testicular loss. Overall, surgical exploration without any delay remains the optimum strategy for improved outcomes.

Some principal limitations are noteworthy in our study. First, this was a retrospective, single-centered study, and so selection bias may exist. Secondly, CDUS is a subjective modality on the basis of a physician's experience in ultrasonic imaging, which may affect accuracy due to less consistency. Additionally, the follow-up was relatively short-term, and the absence of the examination of semen quality means that more atrophic testes and infertility are expected to occur as years come. It is possible that the torsed testicle of pre-pubertal boys, despite no TVL at present, will not develop during puberty and a significant volume loss may be appreciated later.

In summary, age at surgery, delayed surgery, and ETPU may play a crucial role in predicting the development of TA in children with TT following emergent orchiopexy. Blood flow on CDUS could not predict the outcome properly and intraoperative blood supply should not be the only foundation for the judgment of TA. Comprehensive assessments of these preoperative factors are necessary, which can be helpful for the surgeon to implement the correct surgical plan. If these indicators combined with intraoperative evaluation indicate a potential TA, the testis with a high risk of atrophy could be removed to avoid long-term complications. Otherwise, emergent surgery must be performed without any delay to save the testis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board, Children's Hospital of Chongqing Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

PL, XL, X-LL, D-WH, TL, and G-HW contributed to conception and design. XL, D-WH, TL, and G-HW contributed to administrative support. X-MT, Q-LS, and SW contributed to collection and assembly of data. X-MT, X-HT, and Q-LS contributed to data analysis and interpretation. X-MT and X-HT contributed to manuscript writing. All authors contributed to manuscript revision, read, and approved the submitted version.

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The BAL-Score Almost Perfectly Predicts Testicular Torsion in Children: A Two-Center Cohort Study

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Introduction: Testicular torsion (TT) is a common emergency that warrants immediate exploration to prevent infertility or testicular loss. To improve diagnostic reliability, various scoring systems have been published. The aim of this study was to evaluate and validate different testicular torsion scores in a large cohort of children with acute scrotum.

Methods: Retrospective analysis of all male children that were admitted for acute scrotum at the Pediatric Surgery Department of the Altonaer Kinderkrankenhaus and University medical Center Hamburg-Eppendorf from 01/2013 to 03/2019. Two testicular torsion scores (Boettcher Alert Score, Testicular Workup for Ischemia and Suspected Torsion Score) were applied to all data sets. Furthermore, an artificial intelligence (AI)-based score was developed and compared to the two current scores.

Results: In total, 460 boys were included in the study. Of those, 48 (10.4%) had TT. Children with TT suffered most often from short duration of pain, nausea and vomiting, high riding testicle and absent cremasteric reflex. The BALS and the AI-based score had excellent predictive values and all patients with TT would have been detected.

Conclusion: The BAL and the AI score show excellent predictive capabilities and may be used to identify all cases of TT in a pediatric population. The scores are easy to apply. As the BALS was slightly better, we advocate to use this score but to validate our findings in prospective multicenter studies.

Keywords: testicular torsion, acute scrotum, prediction score, epidydimitis, hydatide torsion, scrotal edema, clinical

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INTRODUCTION

Testicular torsion is an immediate surgical emergency, without prompt intervention it results in an ischemia-reperfusion (IR) injury with subsequent infertility or testicular loss (1, 2). The incidence of testicular torsion in children has been estimated at 3.8% (3, 4). A recent study in 558 patients showed that early intervention (within 6 h) results in testicular salvage rate of 90–100%, whereas this rate decreases after 6 till 12 h to 20–50% and after 12 till 24 h to 0–10% (5). However, testicular torsion accounts for only a small fraction of acute scrotum cases in children (10–15%) and

exploration in all children would result in unnecessary surgery in most cases (3, 6–8). Therefore, fast and definite diagnosis is essential (9).

In the past diagnosis relied heavily on clinical factors such as short symptom duration, nausea or vomiting, absence or abnormality of ipsilateral cremaster reflex, high testicle position or hard testicle (7, 10, 11). However, conclusive diagnosis is hampered by clinical overlap between the common reasons of acute scrotum (12). Hence, Doppler ultrasonography in addition to clinical examination appears to be very useful. It provides excellent sensitivity of 85–100% and specificity of 75–100% (13–15). But it is very operator dependent with a relevant risk of false-negative results (16).

To improve diagnostic reliability, by combining clinical with imaging factors, several scores have been established. Boettcher et al. defined a prediction score (Boettcher Alert Score, BALS) based on a retrospective and prospective analysis of 242 children in a center in which all children with acute scrotum were surgically explored. They identified four factors that predict testicular torsion: (1) pain duration less than 24 h, (2) nausea or vomiting, (3) high position of testis, (4) abnormal cremasteric reflex. A score of $\geq$ 2 was considered as high probability of testicular torsion (7, 8). Moreover, the Testicular Workup for Ischemia and Suspected Torsion (TWIST) score was described by Barbosa et al. It is based on five variables which are weighted differently: (1) swelling, (2) hard testicle, (3) nausea or vomiting, (4) high position of testis, (5) abnormal cremasteric reflex. A score of $\geq$3 was considered as high probability of testicular torsion and a score of $\geq$5 warrants immediate surgery without imaging (10, 17). Until, today only the TWIST has been validated (in a small cohort of 128 children) but not the BAL score (17). Thus, the aim of the current study was to validate and compare the two current testicular torsion scores in a very large cohort of children with acute scrotum.

TABLE 1 | Display of the different scoring systems for diagnosing testicular torsion, including the different weighing factors for each score.

	BALS	TWIST	AIS
Duration of pain <24 h	1		1
Nausea / vomiting	1	1	1
High riding testicle	1	1	2
Abnormal cremastic reflex	1	1	
Testicular swelling		2	
Hard testicle		2	
Abdominal pain			1
	2/4	3/7	2/5

METHODS

Study Design

Retrospective cohort study of all male children (age 1–17) that were admitted for acute scrotum at the Department of Pediatric Surgery at the Altonaer Children's Hospital Hamburg (AKK) and the University Medical Centre Hamburg-Eppendorf (UKE) between January 2013 till March 2019.

Methods

The patients were selected from the hospital database using the ICD10 codes for acute scrotum including testicular torsion, testicular appendage torsion, epididymitis and idiopathic scrotal edema. Patients suffering a testicular trauma were excluded. Additionally, patients with reduced perception of symptoms of testicular torsion due to chronic illness as well as patients with peripartal testicular torsion were excluded. Medical files, including patient charts, operating theater records and office notes, were reviewed and routinely obtained characteristics were recorded. Following items were assessed: age, duration, nausea or vomiting, position of the testicle, presence of erythema or swelling, testicle side, abdominal pain, fever, dysuria, presence of cremasteric reflex (an absent or reduced cremasteric reflex was considered an abnormal findings), tenderness of testicle/epididymis and blue dot sign as well as findings of ultrasound, serum infectious parameters, urine analysis using urine dipstick and intraoperative and microbiological findings. Physical examinations were performed by a resident and/or a senior physician of pediatric surgery and ultrasound examinations were performed by a resident of Pediatric Surgery or Pediatric Radiology.

Two different testicular torsion scores including the BALS and the TWIST score were assessed in all children (7, 8, 10, 17). In addition, an artificial intelligence (AI) based score was developed and compared to current scores (**Table 1**).

Nationwide Database Analysis

A controlled remote analysis of the nationwide DRG database from 2016 to 2017 was undertaken to evaluate inpatient data provided by the Research Data Centers of the Federal Statistical Office and the Statistical Offices of the Länder. The German adaptation of the International Classification of Diseases (ICD) Tenth Revision and the procedure coding system [Operationen- und Prozedurenschlüssel, OPS] were used to identify diagnoses and procedures. Every inpatient case with a procedure code for testicular torsion was included. Used ICD codes were N44.0, and the procedure codes were 5–622 and 5–624.

Data Analysis

Statistics were performed using SPSS Statistics 26 (IBM, NY, USA) and R 4.0 (Foundation for Statistical Computing, Vienna, Austria). The AI-based approach was calculated using the R package randomForest (18). Random forest is a method of regression which can capture non-linear relationships by averaging the prediction of multiple decision trees (19). All features assessed in this study were utilized for the creation of the score. In terms of validation, the model was trained by randomly splitting the entire data into two parts, where 70% of the data were used for training and 30% of the data for testing. This was performed 20 times with new random distribution of the data, to eliminate outliers, and the average of the results were taken. In order to prevent over-fitting of the model to the data of the current study which could limit generalization in future real-world use, the random forest method comprises the use

TABLE 2 | Clinical data for children TT compared to non-TT.

	TT	Non-TT	*P*
Age (years)	11.85 (4.29)	9.09 (4.01)	$p < 0.001$
Short duration of pain	43/48	206/412	$p < 0.001$
High riding testicle	40/48	29/412	$p < 0.001$
Nausea and vomiting	28/48	12/412	$p < 0.001$
Hard testicle	12/48	41/412	$p = 0.006$
Erythema	10/48	210/412	$p < 0.001$
Swollen testicle	33/48	234/412	$p > 0.05$
Dysuria	0/48	8/412	$p > 0.05$
Fever	0/48	4/412	$p > 0.05$

of different trees of which each tree is trained on a different bootstrapped dataset. In our case 200 trees were used.

The level of significance was set to 0.05.

RESULTS

In total 460 boys were included in the current study and 161 patients were excluded due to exclusion criteria. Due local policy all patients with elevated probability of testicular torsion underwent surgical exploration, which was nearly half of all patients presented with acute scrotum (218/460, 47.4%). In 48/460 (10.4%) patients' testicular torsion was confirmed and no testicular torsion patients was missed. The most common diagnosis was testicular appendix torsion (115/460, 25.0%), followed by epididymitis (72/460, 15.7%) and idiopathic scrotal edema (13/460, 2.8%). In 18 children no defining clinical or surgical feature was found. These cases were considered as intermitted testicular torsion (18/460, 3.9%).

Mean patient age was 9.38 (4.12) years. As shown in **Table 2**, children with TT were significantly older, had more often a short duration of pain (<24 h), nausea and vomiting, a high riding testicle, and an absent or reduced cremasteric reflex as well as a hard testicle than patients without TT. Erythema was significantly less common in TT than non-TT (**Table 2**). Swelling of the testicle, dysuria and fever was not significantly more frequent in TT or non-TT (**Table 2**).

As shown in **Table 3**, BALS and AIS had excellent sensitivity. The BAL-Score detected all 48 cases of testicular torsion. All patients with testicular torsion showed a BAL-Score ≥ 2 (BALS ≥ 2: 48/48, BALS ≥3: 24/48, BALS 4: 8/48). Only 28 of patients without testicular torsion had a BAL-Score ≥ 2 (BALS ≥2: 28/412, BALS ≥3: 2/412, BALS 4: 0/412). Also, AI Score detected all cases of testicular torsion. An AI Score of ≥2 was reached by all 48 patients with testicular torsion (AIS ≥2: 48/48, AIS ≥3 44/48). Forty two non-TT patients also reached an AI Score of ≥2 (AIS ≥2: 42/412, AIS ≥3: 15/412). TWIST Score did not identify all cases of testicular torsion, 9 patients would have been missed (TWIST ≥3: 39/48, TWIST ≥5: 16/48). Moreover, 56 without testicular torsion had a TWIST Score ≥3 (TWIST ≥3: 56/412, TWIST ≥5: 7/412).

TABLE 3 | Predictive capabilities of the current and the new score in predicting testicular torsion in the entire cohort of children with acute scrotum.

	Sensitivity (CI 95%)	Specificity (CI 95%)	PPV (CI 95%)	NPV (CI 95%)
BALS ≥ 2	100% (91.5–100%)	92.7% (91.7–92.7%)	61.5% (56.3–61.5%)	100% (98.9–100%)
BALS ≥ 3	66.7% (57.3–70.1%)	99.5% (98.4–99.9%)	94.1% (80.9–99.0%)	96.2% (95.2–96.6%)
TWIST ≥ 3	81.3% (68.2–90.3%)	86.4% (84.9–87.5%)	41.1% (34.4–45.6%)	97.5% (95.5–98.8%)
TWIST ≥ 5	66.7% (55.7–74.1%)	98.3% (97.0–99.2%)	82.1% (68.5–91.2%)	96.2% (94.9–97.1%)
AIS ≥ 2	100% (91.4–100%)	89.9% (88.8–89.8%)	53.3% (48.7–53.3%)	100% (98.9–100%)
AIS ≥ 3	91.7% (81.2–97.2%)	96.4% (95.1–97.0%)	74.6% (66.1–79.0%)	99% (97.8–99.7%)

BALS and the AI score have perfect sensitivity with very good specificity for testicular torsion in children.

Nationwide Database Analysis

The nationwide database analysis revealed that in 2 years 3,374 procedures for confirmed testicular torsion were performed in Germany. Of these 443/3,374 consisted or orchiectomy without -pexie of the opposite site, 2,644/3,374 consisted orchidopexy only and finally 287/3,374 surgeries included simultaneous orchiectomy of the affected side and orchidopexy of the opposite site. Thus, in a total of 730 cases orchidectomy was necessary. Mean age was 11.20 (6.11) years and mean hospital stay was 2.19 (1.42) days. In the 2,644 children in which the testicle could be rescued (orchidopexy only) mean age was 13.25 (3.89) years and mean hospital stay was 1.56 (1.00) days. Thus, these boys in which the testis was rescued were significantly older in comparison to the boys who underwent orchidectomy ($p < 0.001$) and hospital stay was significantly shorter ($p < 0.001$). The above-mentioned 3,374 procedures were performed in a total of 447 centers (mean caseload per center: 7.55). Of these, 1,056 procedures were performed in low-volume institutions (1–8 procedures/2 years), and 2,318 in high-volume centers (≥9 procedures/2 years).

Complications were rare: T81 (Complications of procedures) arose in 68/3,374 cases and T88 (Other complications of surgical and medical care) occurred in 6/3,374 cases.

DISCUSSION

As shown by the nationwide database analysis yielding around 1,687 cases annually, testicular torsion is a very common reason for consultation. In order to prevent testicular atrophy and infertility, TT warrants immediate surgical intervention (20). Unfortunately, diagnosis may be challenging as most reasons of acute scrotum show a high clinical overlap and imaging may not be readily available (12, 21). In this current study, 460 male pediatric patients with acute scrotum that presented within 5 years were analyzed and only 10% suffered from testicular torsion. Fortunately, the BALS and AIS showed

excellent predictive capabilities and none of the patients with testicular torsion would have been missed. Using the BALS, only 28 of all children in this current would have been operated upon without having TT (negative exploration rate of 6.1%), whereas the AIS would have resulted in 42 operated children without TT (negative exploration rate of 9.1%).

Even though, the BALS and AIS are similarly effective, we recommend using the BAL-Score, in particular due to its enhanced specificity in comparison to the AIS. The BALS is very easy and quick to access. It includes only four features, which are directly to access via medical history and physical examination, so no time delay is expected. Even for inexperienced residents BAL-Score is well applicable. Almost all patients with TT had (1) a duration of pain < 24 h (2) a high riding testicle (3) nausea and/or vomiting and (4) absent cremasteric reflex.

It is remarkable, that while the AIS and the BALS depend more or less on the same anamnestic features plus the clinical feature "high riding testicle," the TWIST comprises amongst others the factors "hard testicle" and "testicular swelling," even weighing them double. In our experience evaluation of both factors can be rather complex, especially for the less experienced residents. Hence, the TWIST underperforms the AIS and BALS both in terms of sensitivity and specificity.

The combination of a clinical prediction score, e.g., BAL-Score, with ultrasonography (especially doppler US) enabled even safer diagnosis of the different reasons of acute scrotal pain, within an estimated sensitivity for identifying testicular torsion ranging between 85 and 100% (4, 22, 23). Ultrasonography become a standard imaging tool for acute scrotum, especially due to its prompt availability, fast duration of application and low costs (13). A limitation of ultrasound as diagnostic tool for acute scrotum is the high investigator dependence. Most residents are rather less experienced and a false negative US is not uncommon (16). Therefore, the application of a clinical scoring tool in combination with ultrasonography, if appropriate, will be the safest approach for a conclusive diagnosis with respect to abbreviate ischemia time of testicle.

Most limitations of the current study are inherent in a retrospective study. As in most studies that rely on clinical features, another limitation is the inter-observer variability, as experience may significantly affect the examiner's interpretation of the clinical findings (16). However, the BALS should be very robust in this regard, as the four items are easy to assess aside from the cremasteric reflex. Cremasteric reflex in adolescent patients sometimes is difficult to assess and inconclusive due to the flat response by a heavier testicle.

In conclusion, different clinical tools are available and helpful to predict testicular torsion. In our population, BAL-Score showed excellent capabilities to predict testicular torsion and reduce negative explorations to 6.1%. Due to its easy and quick application, BAL-Score is convenient for unexperienced residents as well and reduces time to conclusive diagnosis, resulting in less ischemia time.

Since, the modifications of the BAL-Score refer to the results in our cohort, prospective validation should be performed in future studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MK conceptualized and designed the study, acquired and analyzed clinical and experimental data, performed statistics, drafted the initial manuscript, and approved the final manuscript as submitted. JE, CS, and JW acquired and analyzed clinical and experimental data, drafted the initial manuscript, and approved the final manuscript as submitted. TG and KR acquired clinical data, drafted the initial manuscript, and approved the final manuscript as submitted. MB conceptualized and designed the study, analyzed clinical and experimental data, drafted the initial manuscript, and approved the final manuscript as submitted. All authors contributed to the article and approved the submitted version.

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Laparoscopic Ureteroureterostomy vs. Common Sheath Ureteral Reimplantation in Children With Duplex Kidney Anomalies

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Purpose: Laparoscopic ureteroureterostomy (LUU) has been proposed as an alternative to common sheath ureteral reimplantation (CSUR) in children with symptomatic duplex kidneys. However, data is limited for LUU in the pediatric population. The aim of this study was to analyze our experience with LUU and to compare the results with those after CSUR to assess whether a less invasive surgical approach could be a valid alternative.

Patients and methods: The data of all children with duplex kidneys who underwent either LUU or CSUR at our center from 2006 to 2018 were reviewed retrospectively. After parental counseling, the option of LUU was provided as an alternative to CSUR for unilateral procedures and in the absence of vesicoureteral reflux to the receiving ureter. Baseline characteristics, indication for surgery, hospitalization and operative times, and intraoperative, post-operative, and late complications were analyzed. Preoperative and 1-year post-operative sonographies were reviewed by a pediatric radiologist. Increasing renal pelvic diameter ($\Delta > 5$ mm) was regarded as a sign of ureteral obstruction.

Results: Forty children were included in this study, with 16 children receiving LUU and 24 children receiving CSUR. The children had a mean age of 2.7 years (7 months−9.8 years) and were followed up in our outpatient clinic for an average of 3.9 years (3 months−10.6 years) after surgery. The median hospital stay was 2 days shorter after LUU. Initially, a considerably longer time was needed for LUU, but after more experience was gained, similar operative times were observed for both procedures. Complications were encountered in both groups. After LUU, two patients developed anastomotic leakage: one was managed conservatively, and one required temporary nephrostomy. In the CSUR group, one patient developed vesicoureteral obstruction during follow-up and required reoperation with LUU. The occurrence of post-operative urinary tract infections was similar in both groups. No complications related to the ureteral stump after LUU arose.

Conclusion: LUU is a safe and efficacious treatment option for children with duplex kidney anomalies and can be used as an alternative to CSUR. All children receiving LUU showed a non-obstructive, patent anastomosis and no signs for stenotic compromise of the receiving ureter.

Keywords: ureteral reimplantation, ureteroureterostomy, laparoscopy, duplex kidney, pediatric

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INTRODUCTION

Duplex kidney is the most common renal abnormality in children (1–3). Although its prevalence is high (up to 1% of the population) (4–6), medical problems requiring treatment are rarely encountered (7). However, associated pathologies require attention. Ectopic insertion of the upper pole ureter, ureteroceles, and vesicoureteral reflux (VUR) may cause renal damage due to obstruction and/or urinary tract infection (UTI) (8). Furthermore, incontinence in ectopic ureters and obstruction of the bladder neck by large ureteroceles can occur.

The aims of the surgical treatment are preservation of renal function by relieving obstruction and preventing UTIs or attainment of urinary continence in ectopic inserting ureters. Surgical strategies encompass procedures for acute decompression (nephrostomy, cutaneous ureterostomy, and transurethral ureterocele incision) and delayed reconstruction procedures (ureteroureterostomy or common sheath ureteral reimplantation). Lastly, in cases with non-functioning moieties, heminephrectomy may be considered (9, 10).

Although ureteroureterostomy, whether open or laparoscopic, has been described as a valid alternative to reimplantation, it is still not used routinely in many centers, and provided literature is limited by small case numbers (11–16). At our center, laparoscopic ureteroureterostomy (LUU) was introduced in 2006 after experience had been gained in other laparoscopic techniques in infants.

The aim of this study was to analyze our experience with LUU as a treatment option for children with duplex kidney anomalies and to compare it with the most widely used surgical treatment, the common sheath ureteral reimplantation (CSUR). Focus was put on efficacy, post-operative outcome, and surgical learning curve.

MATERIALS AND METHODS

For this retrospective study, we included all patients with duplex kidneys who underwent LUU at our center between 2006 and 2018. Patients with duplex kidneys undergoing CSUR during the same period were used as controls. CSUR represents the established and most performed procedure for patients with symptomatic duplex kidneys. The use of CSUR as control group is not based on surgical technicalities but, rather, on clinical application and practice, as both LUU and CSUR fix the same problem. During the pre-operative outpatient visit, LUU was presented to the child's parents as an alternative to CSUR for unilateral procedures and in the absence of VUR to the receiving ureter. After providing detailed information on both operative approaches, we left the decision on the choice of method to the parents. To avoid selection bias, all patients with CSUR were included despite the resulting unbalanced group size. Surgery-related codes and a full-text search in our electronic data management system were used to identify patients. Relevant data were collected from the patients' files. This included age at time of surgery, sex, indication for surgery, laterality of surgical site in case of LUU ipsilateral or translateral anastomosis, intraoperative and post-operative complications, time of hospitalization, and operative time. Pre-operative and 1-year post-operative sonographies with measurement of the renal pelvic diameter were retrospectively reassessed and re-evaluated by a specialist pediatric radiologist (RG). A post-operative increasing renal pelvic diameter ($\Delta > 5$ mm) was regarded as a sign of ureteral obstruction. The study was approved by the Ethical Committee of the Canton of Zurich (2019-00305).

Operation Techniques

LUU was performed with a 5-mm camera port and two 3-mm working ports. Before laparoscopy began, a ureteral stent was inserted cystoscopically into the receiving ureter. The end-to-side anastomosis was performed as described by González et al. (11) at the level of the ureteral crossing of the iliac vessels. Resection of the ureteral stump was carried out as low as possible. Care was taken not to compromise the vascular supply of the receiving ureter. The anastomosis was performed with a running 5–0 monofilament absorbable suture. Before completion of the anastomosis, the proximal end of the ureteral stent was slightly retracted and then pushed over the anastomosis into the donor ureter and corresponding moiety. All patients received perioperative intravenous antibiotic prophylaxis with cefuroxime for 48 h and oral antibiotic prophylaxis with trimethoprim/sulfamethoxazole until cystoscopic ureteral stent removal 6–8 weeks post-operatively. Standardized sonographic follow-up occurred post-operatively at 3, 12, and 24 months and every 4 years thereafter.

CSUR was performed as described by Cohen or by Politano-Leadbetter. Ureteral stents were left in place in 16 patients but omitted in eight patients in whom a distinct urine jet was observed intraoperatively, and therefore vesicoureteral obstruction due to swelling was unlikely. All patients received perioperative intravenous antibiotic prophylaxis with cefuroxime for 48 h and oral antibiotic prophylaxis with trimethoprim/sulfamethoxazole for 3 months. Standardized sonographic follow-up occurred post-operatively at 3, 12, and 24 months and every 4 years thereafter.

No routine post-operative voiding cystourethrograms (VCUGs) were planned. At our center, VCUGs are only scheduled after recurrent UTIs, not for single events.

RESULTS

Sixteen patients had a LUU (2 male, 14 female), and the mean age at surgery was 2.2 years (7 months—9.8 years). The mean post-operative follow-up was 3.8 years (3 months—9.3 years). One patient was lost to follow-up (relocation to another country). Hospitalization time was 2–26 days, with a mean of 6.8 days and a median of 5 days. The operative time including cystoscopy was 112–495 min, with a mean of 216 min and a median of 188 min (**Figure 1**). Indications for LUU were ectopic ureter insertion in 10 patients (62.5%), causing obstruction in eight and incontinence in two patients. Four patients presented with

Abbreviations: CSUR, common sheath ureteral reimplantation; LUU, laparoscopic ureteroureterostomy; UTI, urinary tract infections; VCUG, voiding cystourethrogram; VUR, vesicoureteral reflux.

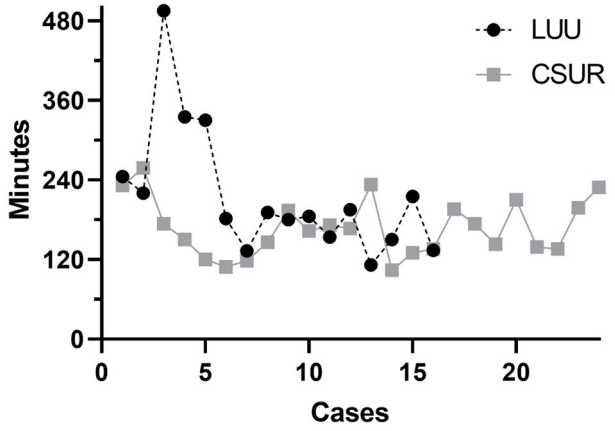

FIGURE 1 | The operative times for both procedures gradually converge. Cases 3 and 4 in the laparoscopic ureteroureterostomy group were salvage procedures after failed common sheath ureteral reimplantations with anastomosis to the contralateral ureter and a bilateral procedure, respectively. Operative time was therefore significantly longer than in standard cases.

recurrent febrile UTI due to VUR. Three patients had primary VUR to the lower moiety (18.8%), and one patient developed iatrogenic VUR after ureterocele incision (6.2%). In two patients (12.5%), LUUs were performed as salvage operation after failed CSURs due to post-operative vesicoureteral obstruction. Nine were left-sided procedures, six were right-sided, and one was bilateral. Anastomosis was performed 15 times to the ipsilateral ureter and once to the contralateral ureter (**Table 1**). There were no intraoperative complications. No patient required conversion to open surgery. Two patients (12.5%) suffered anastomotic leaks: one of these patients required a percutaneous nephrostomy, and the other was treated with bowel rest and intravenous antibiotics for paralytic ileus, urinoma, and UTI. Four (25%) post-operative febrile UTIs occurred during follow-up; all were single events, so we omitted invasive diagnostics by using VCUGs. The 1-year post-operative ultrasound was available in all but two patients. It showed a stable (Δ <5 mm) or decreasing renal pelvic diameter, thus showing no signs of post-operative obstruction. In two patients, only 3-month and 2-year post-operative ultrasounds were available. Those showed no signs of obstruction.

Twenty-four patients received a CSUR (4 male, 20 female), and the mean age at surgery was 3.1 years (10 months−9.3 years). The mean post-operative follow-up was 4.4 years (11 months−10.6 years). One patient was lost to follow-up (relocation to another country). The hospitalization time was 3–10 days, with a mean of 6.6 days and a median of 7 days. The operative time was 104–258 min, with a mean of 169 min and a median of 167 min (**Figure 1**). Indications for CSUR were recurrent febrile UTI in 14 patients (58.3%) with primary VUR to the lower moiety (one combined lower/upper moiety), iatrogenic VUR after ureterocele incision in seven patients (29.2%), and ureteral obstruction (two ureteroceles and one vesicoureteral obstruction) in three patients (12.5%). CSUR was performed in 12 cases unilaterally (seven right, five left) and in 12 cases bilaterally (**Table 1**). There were no intraoperative complications. Five patients (20.8%) suffered febrile UTI during follow-up; all were single events, so we omitted invasive diagnostics with VCUGs. One patient (4%) showed increasing renal pelvic diameter and required reoperation with LUU due to post-operative vesicoureteral obstruction. Apart from that, the 1-year post-operative renal pelvic diameter was stable (Δ <5 mm) or decreased in all patients; there were no further signs for post-operative vesicoureteral obstruction.

Due to poor image quality, post-operative renal diameter assessment was not possible in two patients with LUUs and four with CSURs.

DISCUSSION

Our data demonstrate that LUU is a safe and efficacious treatment option for selected patients with duplex kidney anomalies and thus can be used as a minimally invasive alternative to CSUR. All patients receiving LUUs showed a non-obstructive, patent anastomosis and, most importantly, no signs of stenotic compromise of the receiving ureter. Furthermore, none of the patients suffered complications related to the ureteral stump. However, complications after ureteroureterostomy such as anastomotic leaks, recurring febrile UTIs, ureteral strictures, worsening of hydronephrosis, reoperation on the distal ureter stump due to infection, and new-onset reflux have been described (17, 18). Lashley et al. (19) reported on 100 open ureteroureterostomies in children and described failure because of obstruction in three cases (3%), reflux in two (2%), and a non-draining ureteral stump in one (1%). Lee et al. (20) identified infections in the remnant ureteral stump requiring re-operation in 12% in their series of 74 adult patients. Michaud et al. (18) reviewed the available literature regarding complications in LUU and robot-assisted LUU in pediatric patients. A total of 51 cases had an overall complication rate of 7.8%, all with febrile UTIs and one reoperation for ureteral stent exchange. There was no reported case of post-operative obstruction, ureteral stricture, new-onset reflux, or ureteral stump excision (18). It is quite plausible that ureteral stump complications are less frequent when ureteroureterostomy is performed laparoscopically because the ureteral stump is likely to be left shorter when dissected laparoscopically.

Our LUU cohort included two patients (12.5%) with anastomotic leakage and consecutively prolonged hospitalization. Both patients presented with febrile UTI and paralytic ileus. Both patients were treated with antibiotics and needed parenteral nutrition. One case was managed conservatively, and the other required percutaneous nephrostomy. This particular case was a salvage procedure due to vesicoureteral obstruction after failed CSUR and the only patient in our series without a ureteral stent to protect the anastomosis. Similarly, Lashley et al. (19) reported a prolonged output from perianastomotic drains in 13% of patients from an average of 15 days (7–31 days). A shunt across the Y-junction, causing stasis, was described for extravesical bifid ureter (21). We observed no problems with yo-yo reflux in any of our patients. This is consistent with the studies referenced above, none of which reported any complication attributed to yo-yo

TABLE 1 | Patient characteristics and summary of results [operative time: the statistical outliers causing the difference between mean and median in the laparoscopic ureteroureterostomy (LUU) group were one case of bilateral anastomosis (operative time, 335 min) and one case of contralateral LUU (operative time, 495 min); hospitalization: the statistical outliers causing the difference between mean and median in the LUU group were two cases with anastomotic leak (hospitalization time, 13 and 26 days)].

Parameters	Laparoscopic ureteroureterostomy ($n = 16$)	Common sheath ureteral reimplantation ($n = 24$)
Demographics		
Sex (male; female)	2; 14	4; 20
Mean age (range)	2.2 years (7 months–9.8 years)	3.1 years (10 months–9.3 years)
Mean follow-up (range)	3.8 years (3 months–9.3 years)	4.4 years (11 months–10.6 years)
Indication		
Febrile urinary tract infection (UTI) and vesicoureteral reflux (VUR)		
Primary VUR	3 (18.8%)	14 (58.3%)
Secondary VUR	1 (6.2%)	7 (29.2%)
Ectopic insertion		
Obstruction	8 (50%)	3 (12.5%)
Incontinence	2 (12.5%)	-
Salvage procedure	2 (12.5%)	-
Side of procedure		
Right	6	7
Left	9	5
Bilateral	1	12
Anastomosis		
Ipsilateral	15	
Contralateral	1	
Operative time (min)		
Range	112–495	104–258
Mean	216	169
Median	188	167
Hospitalization (day)		
Range	2–26	3–10
Mean	6.8	6.6
Median	5	7
Complications		
Intraoperative	-	-
Postoperative	2 (12.5%) anastomotic leaks with UTI	-
Follow-up	4 (25%) UTI	5 (20.8%) UTI, 1 (4%) obstruction

reflux. Furthermore, Steyaert et al. (13), Storm et al., and Chandrasekharam et al. (22) did not observe perioperative or post-operative complications at 6, 8, or 19 months of mean follow-up.

The rate of post-operative complications was similar following both LUU and CSUR. In both of our groups, post-operative febrile UTIs occurred. However, these were only single events, and post-operative VCUG was therefore not performed. Owing to two complicated courses with anastomotic leaks and consecutive prolonged hospitalization (13 and 26 days), the mean hospitalization time after LUU was quite high (6.8 days), even higher than after CSUR (6.6 days). The effect of these outliers is eliminated when the median time of hospitalization is considered: this was shorter after LUU (5 days) than after CSUR (7 days). The mean hospital stay after LUU reported in the comparable literature is 3 days, only one prolonged hospitalization (7 days) due to pyelonephritis has been reported (11–14).

Operative time was longer in our first five cases using LUU but became similar to the operative time for CSUR thereafter. Our most recent 10 LUUs exhibit a clear learning curve, with a mean duration of 166 min including cystoscopy and repositioning of the patient from lithotomy to supine position. This operative time is similar to the 169 min for CSUR. González et al. (11) reported a mean operative time of 256 min, and Storm et al. (14) took 187 min mean, both including cystoscopy. The teams around Chandrasekharam et al. (22) and Steyaert et al. (13), who did not clearly include or exclude time for cystoscopy, both reported requiring 120 min per procedure in case series of eight and two patients, respectively. In summary, LUU offers the advantages of a minimally invasive procedure, resulting in smaller scars and shorter hospitalization times, without

concerns about more complications or longer operation times than CSUR. Owing to the retrospective design of this study, certain points have to be commented. The goal of this study was to assess, whether in children with symptomatic duplex kidney anomalies, who would typically get scheduled for CSUR, a less invasive surgical approach could be a valid alternative. For this reason, we wanted to compare LUU to CSUR and not open ureteroureterostomy to CSUR.

For the same reason, we included patients with varying indications for surgery, even exceptional cases with excessive operative times such as a bilateral LUU (05:35 h) or a salvage procedure LUU with anastomosis to the contralateral ureter (08:15 h). Such a heterogeneous population with a different surgical risk profile is problematic in a retrospective study. Nevertheless, our study shows that LUU can be used as an alternative to CSUR irrespective of the indication for surgery.

Although LUU is not a novel method, we believe that the comparison with CSUR, the large case number with the extended follow-up, and the diversity of our population provides valuable information for pediatric urologists, especially considering the fact that there is little comparable literature.

CONCLUSION

LUU is a safe and efficacious treatment option for children with duplex kidney anomalies and can be used as an alternative to CSUR.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of the Canton Zurich (2019-00305). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TG and LM contributed to structure, content, and writing of the manuscript. DW and RGn contributed to structure, content, and reviewed the manuscript. RGn evaluated the ultrasounds and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Pediatrics

ORIGINAL RESEARCH
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Evaluation of a New Tubular Finger Oxygen-Enriched Oil Inside-Coated Dressing Device in Pediatric Patients Undergoing Distal Hypospadias Repair: A Prospective Randomized Clinical Trial Part II

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Background: This study was the second part of a prospective randomized clinical trial and aimed to evaluate the use of a tubular finger oxygen-enriched oil inside-coated dressing device and its effect on the post-operative outcome of children undergoing distal hypospadias repair.

Methods: A prospective single-blinded randomized clinical trial was carried out between September 2019 and September 2020. We included all patients with distal hypospadias, who received Snodgrass urethroplasty and preputioplasty. The patients were randomized in two groups according to the type of dressing: tubular finger oxygen-enriched oil inside-coated device (G1) and elastic net bandage with application of oxygen-enriched oil-based gel (G2). The patients were evaluated at 7, 14, 21, 30, and 60 post-operative day (POD).

Results: Sixty-four patients (median age 14 months) were included in the study and randomized in two groups, each of 32 patients. Post-operative preputial edema rate was significantly lower in G1 (3/32, 9.3%) compared with G2 (10/32, 31.2%) ($p = 0.001$). The median duration of preputial edema was significantly shorter in G1 compared with G2 (6 vs. 10.5 days) ($p = 0.001$). Penile diameter measurements at 4th, 7th, 14th POD proved that entity and duration of post-operative swelling were objectively decreased using the new dressing. The wound healing was significantly faster in G1 compared with G2 (14.2 vs. 18.5 days) ($p = 0.001$). The post-operative complications rate was significantly lower in G1 (0%) compared with G2 (3/32, 9.3%) ($p = 0.001$). Foreskin dehiscence occurred in two G2 patients (6.2%) whereas, breakdown of urethroplasty and preputioplasty occurred in one G2 patient (3.1%) due to scratching injuries. The dressing management was subjectively assessed by nurses to be easier in G1 patients compared with G2 ones (median score 1.2 vs. 3.5) ($p = 0.001$). The median treatment costs were significantly lower in G1 compared with G2 (55 vs. 87 eur) ($p = 0.001$). No adverse skin reactions occurred.

Conclusions: Post-operative dressing using tubular finger oxygen-enriched oil inside-coated device was highly effective, easy to manage, cheaper and associated with a lower rate of foreskin and urethral complications compared with the standard dressing method in pediatric patients undergoing distal hypospadias repair. It was also clinically safe without allergy or intolerance to the product.

Keywords: hypospadias, dressing, oxygen-enriched oily gel device, wound, complications, children

INTRODUCTION

Probably one of the most controversial aspects of hypospadias surgery is the post-operative dressing (1). Analyzing the international literature, no consensus emerged about this argument (2) and an enormous number of surgical techniques as well as type of dressings have been described for operative and post-operative management of pediatric patients affected by hypospadias (3–10).

The ideal dressing should be soft and mobile, adaptable to the penis during the child's movements but at the same time rigid to stabilize the penis during the dynamic changes occurring in physiological erections (11). It should also keep the penis clean, preventing the fecal and urinary contamination, that occurs constantly in infants under 2–3 years of age, who wear the diaper. In addition, the dressing should protect the penis from external injuries, secondary to child's scratching, occurring very frequently in the post-operative period. Regarding the dressing type, it is not only important the structure of the dressing but also its substrate (3–7). In children wearing the diaper, the penis is always wet. For this reason, it is important to cover the inner part of the dressing, that is in direct contact with the wound, with a product that improves healing and avoids infections. Finally, the dressing should be easy to manage by nurses and its change or removal should be comfortable for the child (12–14).

We recently published a prospective randomized clinical trial reporting the efficacy of oxygen-enriched oil-based gel associated with an elastic band dressing on the post-operative wound healing outcome of children undergoing distal hypospadias repair (15).

More recently, a special medical device in the form of a single-use tubular finger or toe stall, coated on the inside with oxygen-enriched oily gel, has been marketed for treatment of specific finger or toe wounds, including surgical wounds, superficial burns, traumatic wounds, ulcers. The shape of this device was specifically designed to fit the anatomical form of the fingers or toes. We decided to apply this device to surgical wound following hypospadias surgery, since its shape seemed to perfectly fit the anatomical form of the penis.

This article represented the second part of the cited prospective randomized clinical trial and aimed to evaluate the use of a new tubular finger oxygen-enriched oil inside-coated dressing device and its effect on the post-operative outcome of children undergoing distal hypospadias repair.

MATERIALS AND METHODS

The second part of this prospective single-blinded randomized clinical trial was carried out between September 2019 and September 2020 and included all patients with distal hypospadias, who underwent Snodgrass urethroplasty and preputioplasty. This study received the appropriate approval by the Institute Review Board (IRB) and the Ethics Committee. Written informed consent was obtained from all patients before surgery.

Patients' Selection

The study included all patients aged <3 years with distal hypospadias, who received Snodgrass urethroplasty and preputioplasty in our surgical unit. Patients with proximal hypospadias or with distal hypospadias aged >3 years or receiving circumcision or toilet-trained at time of surgery were excluded from the study.

Sample Size and Sampling Method

It was calculated that the minimum sample size to obtain, at 80% power and at type 1 error of 5%, an absolute difference in rates of at least 14%, should be 24 in each treatment arm, with a total number of 48. Adding an expected attrition rate of 30% (to account for eventual loss to follow-up), the calculated sample size came to 64, randomized to 32 participants in each arm.

Randomization and patient allocation were performed using simple random sampling method, which entailed an equal number of ballot papers pre-labeled with either tubular finger oxygen-enriched oil inside-coated device (G1) and elastic net bandage with application of oxygen-enriched oil-based gel (G2), sealed in similarly opaque envelopes and picked before the surgical procedure.

Operative Technique

All the patients received Snodgrass urethroplasty and preputioplasty. All the surgical procedures were performed by two experienced surgeons. The same sutures and post-operative urinary diversion were adopted in all patients, as already described in the first part of the study. All the procedures were performed under general anesthesia. After degloving the penis and removing the chordees, the urethral plate was incised and tubularized over an 8 Fr Foley catheter. The reconstruction of the urethra was performed using a running suture of 6/0 monofilament polyglyconate suture followed by a second layer of interrupted stitches using the same suture. The urethra was then covered with a well vascularized subcutaneous dartos flap, that was fixed to the new urethra using interrupted mattress stitches.

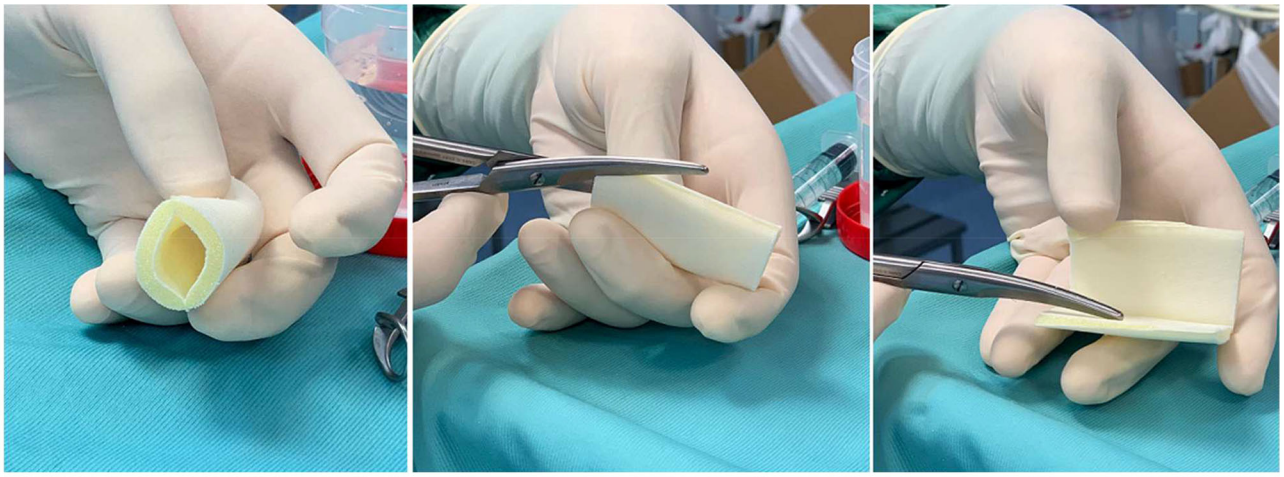

FIGURE 1 | The device is reduced in length according to the penis' size and opened longitudinally.

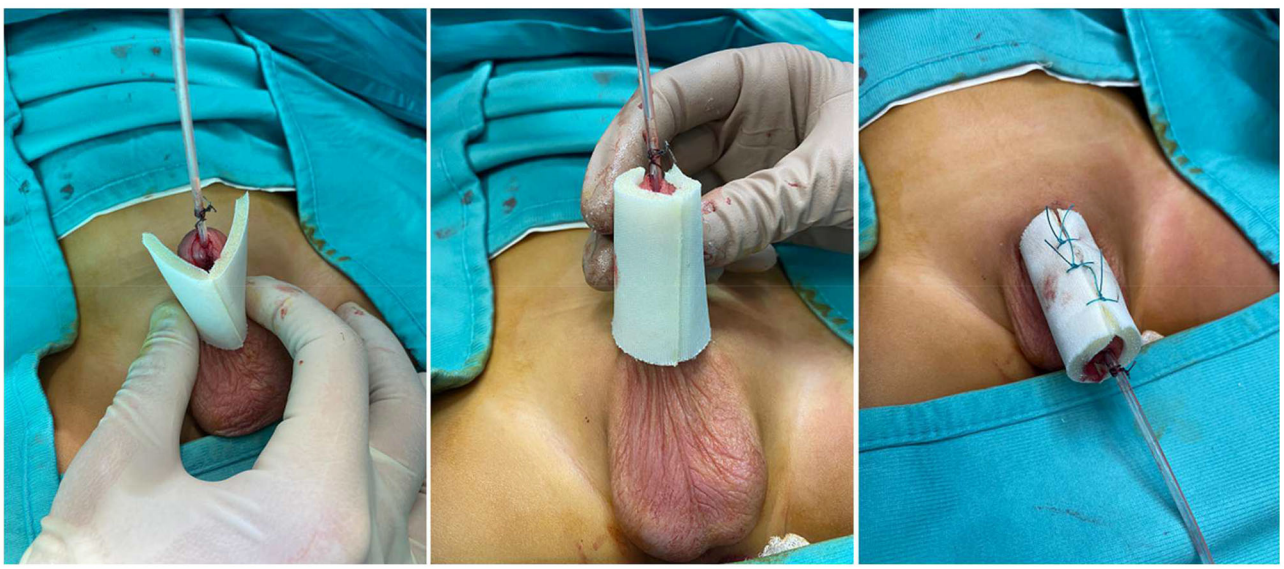

FIGURE 2 | The device is applied onto the penis and stabilized on the dorsal aspect using 3 stitches.

Glanuloplasty was then performed using 5/0 polyglyconate interrupted sutures. Preputioplasty was performed with a three-layer closure. All the patients had an 8 Fr Foley silicon catheter inside the urethra, that was managed using a double diaper layer.

Post-operative Wound Management

After completing the surgical correction of the hypospadias, the area was washed with saline and dried with gauze. In G2 patients, as already described in the previous study (15), a layer of oxygen-enriched oil-based gel was directly applied on a wet gauze composed by hyaluronic acid. This impregnated gauze was wrapped around the penis and subsequently covered by an elastic net bandage to obtain hemorrhage compressive effect. In G1 patients, a new medical device in the form of a tubular finger with a closed tip was adopted. The substrate of this device was polyurethane and polyester whereas, the inner part was coated with oxygen-enriched olive oil and lavender essential oil. The device was available in two different sizes, small (S) measuring 11 cm in length × 3 cm in diameter and large (L) measuring 11 cm × 4 cm. The S size was adopted in all the patients of our study. Before applying the device, it was cut to reduce its length in accordance with the penis size to ensure that the whole penis was covered. The closed tip of the device was cut off and the device was opened longitudinally to easily apply it and wrap the entire length of the penis (**Figure 1**). Finally, the device was stabilized onto the penis by closing the two edges using three interrupted stitches on the dorsal side of the penis taking care not to deform or crumple it excessively and ensuring that it sticked firmly to

FIGURE 3 | The final aspect of dressing using tubular finger device.

the entire wound area (**Figure 2**) and was fixed to the skin using adhesive tape (**Figure 3**).

The dressing was changed in all the patients of both groups on the 4th post-operative day (POD), then repositioned and finally removed on the 7th POD at the time of bladder catheter removal. After hospital discharge, all parents were asked to apply topically 2% eosin solution and oxygen-enriched oil-based gel without any bands or gauzes during the diaper change twice a day until completion of wound healing.

Assessment of Outcome Measures

The follow-up evaluations were performed by one independent pediatric surgeon and one pediatric nurse, not involved in the operation and blinded to the patient group. The follow-up schedule included a clinical control at 7, 14, 21, 30, and 60 days postoperatively. At each control, the wound was observed and photographs of the penis were obtained to document the wound healing and the cosmetic result.

The primary endpoint of the study was to compare the two groups about the wound healing time defined as the time to return to normal structure and appearance of the penis following surgery. The wound healing was scored using the Southampton Wound Assessment Scale (SWAS) (15), that evaluated normal healing (grade 0), normal healing with mild bruising or erythema (grade 1), presence of erythema plus other signs of inflammation (grade 2), clear or hemo-serous discharge (grade 3), or major complications such as pus (grade 4) and deep or severe wound infection with or without tissue breakdown or hematoma requiring aspiration (grade 5) (**Table 1**).

Furthermore, we evaluated the presence and duration of post-operative preputial edema. We measured the penile diameter before surgery and at fourth, seventh and fourteenth post-operative day (POD) to achieve an objective evaluation of swelling and its evolution at these timelines. The penile diameter was always measured 1-cm below the apex of reconstructed foreskin to obtain a homogeneous measurement in all patients.

Secondary outcome parameters included adverse reactions to the product, foreskin retractability, post-operative complications, level of nurses' difficulty to manage the dressing, and dressing costs in each group. Adverse skin reactions such as hypersensitivity or allergic reactions to the product were categorized by the researcher as absent, limited to the foreskin, or extended to other areas.

The retractability of the foreskin was also evaluated at > 30days postoperatively. At this time, the first retraction of the reconstructed prepuce was performed by the evaluating

TABLE 1 | Southampton wound assessment scale (SWAS).

Grade	Appearance
0	Normal healing
1	Normal healing with:
a	Some bruising
b	Considerable bruising
c	Mild erythema
2	Erythema plus other signs of inflammation:
a	At one point
b	Around sutures
c	Along wound
d	Around wound
3	Clear or hemoserous discharge:
a	At one point only (<2 cm)
b	Along wound (>2 cm)
c	Large volume
d	Prolonged (>3 days)
Major complication	
4	Pus
a	At one point only (<2 cm)
b	Along wound (>2 cm)
5	Deep or severe wound infection with or without tissue breakdown; hematoma requiring aspiration

surgeon and continued by parents at home during daily hygienic care.

Post-operative complications including infections, foreskin dehiscence, meatal stenosis and urethrocutaneous fistula were also assessed and graded according to Clavien-Dindo classification (16).

The level of nurses' difficulty to manage the dressing was subjectively assessed by nurses on a 1–5 Likert-type scale, with 1 = very easy; 2 = easy; 3 = average; 4 = hard; 5 = very hard.

We performed a comparative analysis of all secondary outcome parameters between the two groups.

Statistical Analysis

Statistical analysis was carried out using the Statistical Pack- age for Social Sciences (SPSS Inc., Chicago, Illinois, USA), version 13.0. Continuous variables were summarized and presented as median and interquartile range. The categorical variables were presented as absolute numbers and percentages.

The associations between qualitative variables were measured by the chi-square test and quantitative variables were measured with the parametric Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Sixty-four patients, with median age of 14 months (interquartile range, IQR 12–28), were included in the study. All the children wear the nappy at the time of surgery. The patients were randomized in two groups, each of 32 patients, according to

TABLE 2 | Comparative analysis of patients' demographics and outcomes between G1 and G2.

	G1 Tubular oxygen -enriched oil inside-coated device *n = 32*	G2 Elastic band + Oxygen-enriched oil-based gel *n = 32*	*P*-value
Median patients age, years (IQR)	15 (13–28)	13 (12–26)	0.44
Balanic hypospadias, n (%)	12/32 (37.5%)	10/32 (31.2%)	0.33
Coronal hypospadias, n (%)	15/32 (46.9%)	17/32 (53.1%)	0.33
Subcoronal hypospadias, n (%)	5/32 (15.6%)	5/32 (15.6%)	0.33
Median length of stay, days (IQR)	8 (7–9)	8 (7–9)	0.55
Median follow-up, months (IQR)	11 (2–13)	10 (2–14)	0.37
Median length of surgery, minutes (IQR)	82 (70–105)	88 (75–110)	0.44
Median wound healing time, days (IQR)	14.2 (11–16)	18.5 (16–21)	0.001
Post-operative preputial edema, n (%)	3/32 (9.3%)	10/32 (31.2%)	0.001
Pre-operative mean ± SD penile diameter, cm	1.08 ± 0.17	1.07 ± 0.14	0.33
Post-operative mean ± SD penile diameter POD 4th, cm	1.14 ± 0.12	1.28 ± 0.15	0.001
Post-operative mean ± SD penile diameter POD 7th, cm	1.11 ± 0.11	1.22 ± 0.13	0.001
Post-operative mean ± SD penile diameter POD 14th, cm	1.07 ± 0.16	1.12 ± 0.11	0.03
Median duration of preputial edema, days (IQR)	6 (2–7)	10.5 (4–12)	0.001
Adverse skin reaction to the product, n (%)	0	0	n/a
Post-operative complications, n (%):	0	3/32 (9.3%)	0.001
- Foreskin dehiscence, n (%)	0	2/32 (6.2%)	0.001
- Urethroplasty and preputioplasty breakdown, n (%)	0	1/32 (3.1%)	0.001
Foreskin retractability at >30 days follow-up:			
- Retractile, n (%):	31/32 (96.9%)	31/32 (96.9%)	0.55
- Phimosis, n (%):	1/32 (3.1%)	1/32 (3.1%)	0.87
Nurses' scoring of dressing (l–5 Likert type scale: 1-very easy; 2-easy; 3-average; 4-hard; 5-very hard), n (IQR)	1.2 (1–3)	3.5 (2–4)	0.001
Median costs, eur (IQR)	55 (33–60)	87 (38–116)	0.001

IQR, interquartile range; n/a, not applicable; SD, standard deviation; POD, post-operative day.

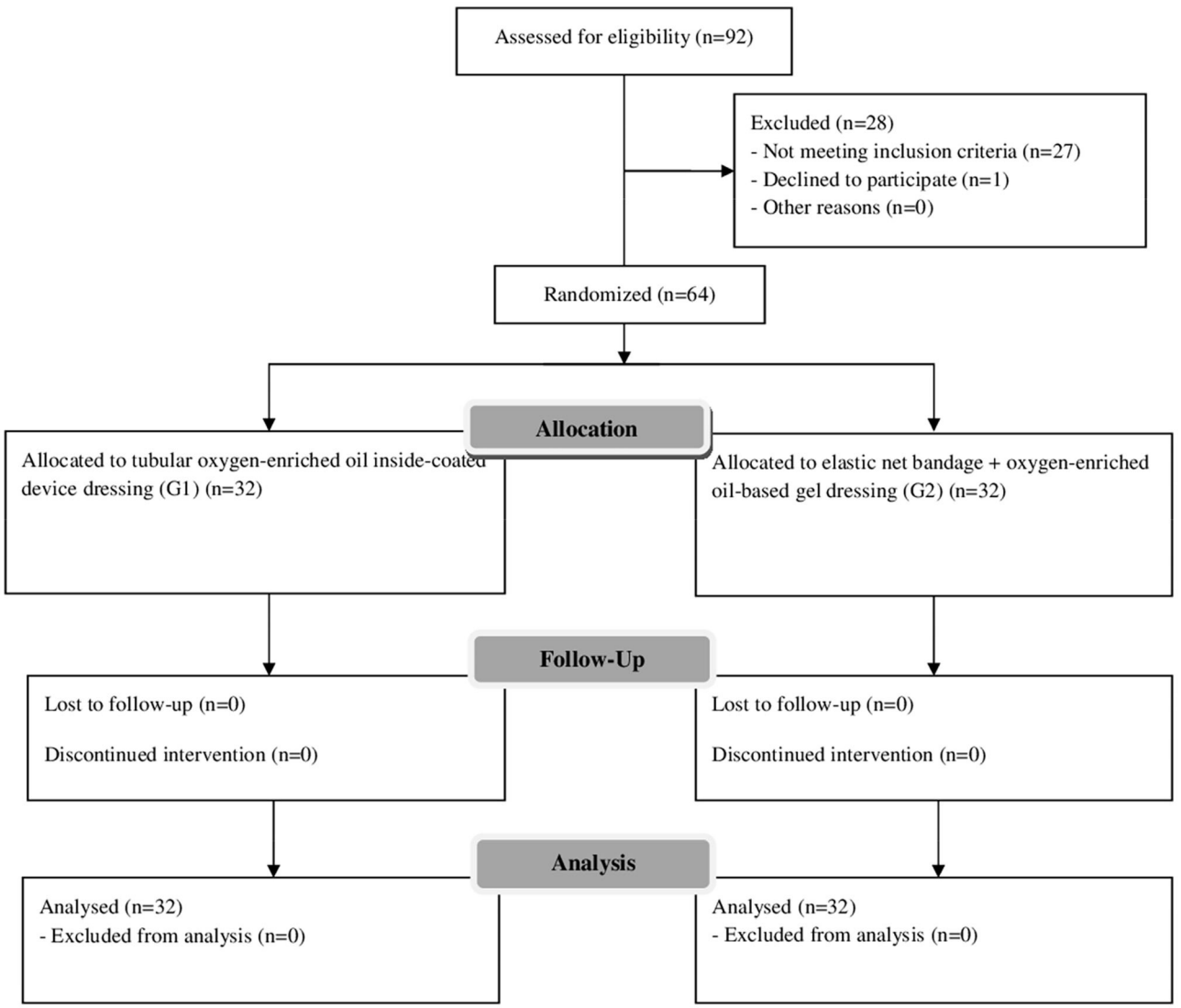

FIGURE 4 | Patients' allocation.

the type of post-operative dressing: the treatment group (G1) included patients in whom the tubular finger oxygen-enriched oil inside-coated device, NOVOX TOUCH ® (MOSS SpA, Lesa, Novara, Italy), was adopted whereas, the control group (G2) included patients receiving the standard dressing consisting of elastic net bandage with application of oxygen-enriched oil-based gel NOVOX ® (MOSS SpA, Lesa, Novara, Italy).

There was no significant difference in the age at presentation ($p = 0.44$), the hypospadias degree ($p = 0.33$), the length of stay ($p = 0.55$) and the follow-up ($p = 0.37$) between the two groups.

The patients' demographics are summarized in **Table 2**.

The length of surgery ranged between 70 and 110 min (median 85). All the patients received analgesic therapy through an elastomeric infusion pump (tramadol 2 mg/kg) for the first 24 h post-operatively, followed by oral therapy (paracetamol 15 mg/kg/8h and tramadol 2 mg/kg/8h). The bladder catheter was removed on the 7th POD in all patients, who were discharged on the following day.

No patients were lost to follow-up (**Figure 4**). At clinical examination, the wound healing time was significantly shorter in G1 compared with G2 [14.2 vs. 18.5 days] ($p = 0.001$) (**Figures 5**, **6**). Wound healing evaluation using SWAS reported a significantly higher rate of normal healing (≤ 1) in G1 compared with G2 at 7 days (84.4 vs. 59.4%), 14 days (100 vs. 68.8%), 21 days (100 vs. 84.4%) and 30 days (100 vs. 90.6%) follow-up ($p = 0.001$). No significant difference in SWAS scores was found between G1 and G2 ($p = 0.33$) at 60 days follow-up (**Table 3**).

Post-operative preputial edema incidence was significantly lower in G1 (3/32, 9.3%) compared with G2 (10/32, 31.2%). Post-operative mean ± standard deviation (SD) penile diameter (cm) was significantly lower in the treatment group (G1) compared with the control group (G2) at 4th POD (1.14 ± 0.12 vs. 1.28

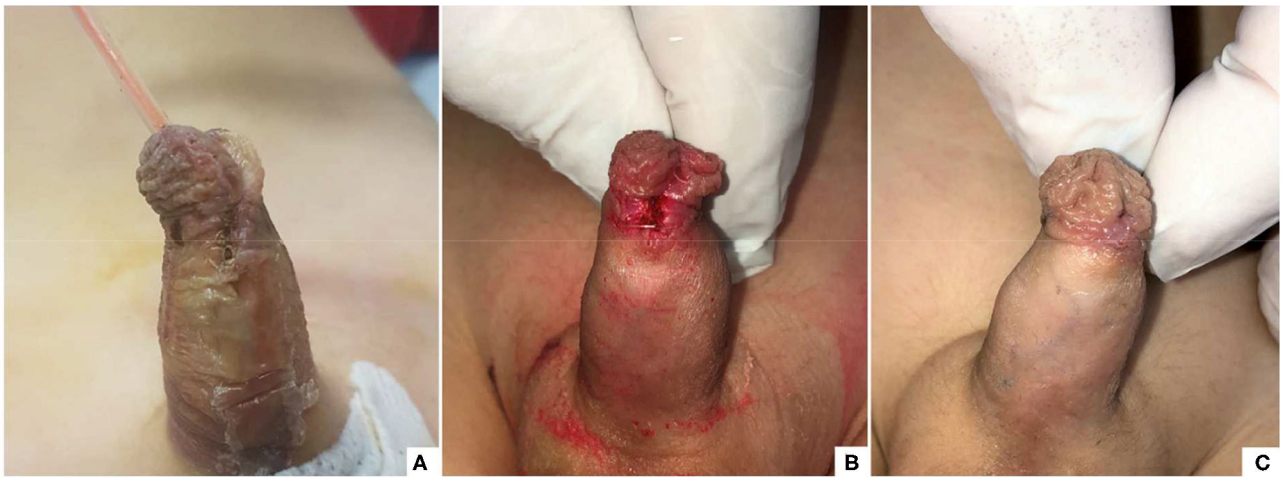

FIGURE 5 | Wound healing in G1 at 7 days **(A)**, 14 days **(B)** and 30 **(C)** days post-operatively.

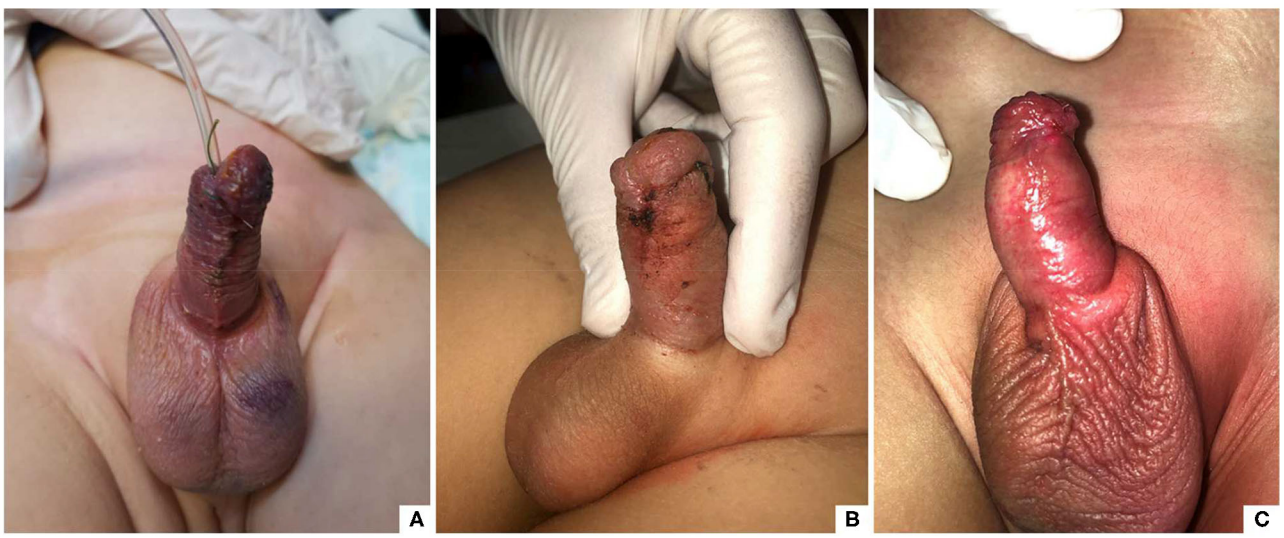

FIGURE 6 | Wound healing in G2 at 7 days **(A)**, 14 days **(B)** and 30 **(C)** days post-operatively.

TABLE 3 | Southampton wound assessment scale (SWAS) scores in G1 and G2.

	G1 Tubular oxygen-enriched oil inside-coated device *n = 32*		**G2 Elastic band + Oxygen-enriched oil-based gel *n = 32***		***P*-value**
	≤1	**>1**	**≤1**	**>1**	
7 days follow-up	84.4% (27/32)	15.6% (5/32)	59.4% (19/32)	40.6% (13/32)	0.001
14 days follow-up	100% (32/32)	0	68.8% (22/32)	31.2% (10/32)	0.001
21 days follow-up	100% (32/32)	0	84.4% (27/32)	15.6% (5/32)	0.001
30 days follow-up	100% (32/32)	0	90.6% (29/32)	9.4% (3/32)	0.001
60 days follow-up	100% (32/32)	0	100% (32/32)	0	0.33

± 0.15) (p = 0.001), 7th POD (1.11 ± 0.11 vs. 1.22 ± 0.13) ($p = 0.001$) and 14th POD (1.07 ± 0.16 vs. 1.12 ± 0.11) ($p = 0.03$). The median duration of preputial edema was significantly shorter in G1 compared with G2 (6 vs. 10.5 days) ($p = 0.001$).

No adverse skin reactions or allergies to the product occurred in both groups. The evaluation of foreskin retractability showed no significant difference about incidence of post-operative phimosis between the two groups ($p = 0.87$). The post-operative complications rate was significantly lower in G1 (0%) compared with G2 [(3/32 Clavien IIIb) 9.3%] ($p = 0.001$). In two G2 patients (6.2%), foreskin dehiscence occurred whereas, in one G2 patient (3.1%) breakdown of urethroplasty and preputioplasty occurred due to scratching injuries caused by the child on the 5th POD.

The dressing management was subjectively evaluated by nurses to be easier in G1 patients (median score 1.2) compared with G2 ones (median score 3.5) ($p = 0.001$).

The median treatment costs were significantly lower in G1 (55 eur) compared with G2 (87 eur) ($p = 0.001$).

The comparative analysis of outcomes between G1 and G2 is summarized in **Table 2**.

DISCUSSION

Hypospadias repair is one of the most common surgical procedures performed by pediatric surgeons (17). Limited evidence is available regarding several aspects of surgical management of hypospadias, including details of surgical technique, type of suture, indications for foreskin reconstruction, type and length of urinary diversion, and post-operative dressing (18–20).

One of the most controversial aspects of hypospadias surgery is the choice of an appropriate wound dressing (1). Multiple dressings have been previously described (3–10); however, there is no evidence in the current literature about the best method for post-operative dressing following hypospadias repair (2). The choice of an adequate dressing is crucial because the success of the surgical procedure may be influenced by post-operative management of the penis. The dressing is particularly important in those patients undergoing preputioplasty and urethroplasty, who may develop a considering preputial edema in the post-operative period. This may cause a high tension on the suture line and increase the risk of foreskin and urethra dehiscence.

The ideal hypospadias dressing should be rigid but at the same time flexible, elastic, resistant, it should keep the penis straight and provide an effective pressure on the wound (11). In addition, hypospadias dressing should present minimal adverse reactions when in contact with tissues, protect the penis against the contamination by pathogens and also against the traumatic injuries, frequently caused by the child's scratching on the wound, that may occur in the early post-operative period. Finally, the ideal dressing should be easy and painless to change and to remove (12–14).

We discovered incidentally this new device, that was originally created to treat fingers or toes wounds, ulcers, injuries and burns, and its shape and size caught our attention. In fact, it was cylindric, with a closed tip and was long 11 cm and large 3 cm in the size S. This new device seemed to fit all the characteristics of an ideal hypospadias dressing, because it was stable and kept the penis straight with a lower incidence and duration of post-operative swelling compared with the standard dressing, as reported in our study. It was an effective mechanical barrier through a double mechanism: first, the non-impregnated outside of the device was fairly water-repellent and protected the penis against urinary and fecal contamination but also against traumatic injuries, caused by the child's scratching on the wound area, as happened in our experience. Second, the inner part of the device, coated with oxygen-enriched oily gel, created a micro-environment, that was unfavorable to the proliferation of the pathogens commonly found on skin lesions, allowing activation of the microcirculation and thanks also to the protective, barrier and soothing action of oxygen-enriched olive oil (21–26). In fact, seepage of small amounts of oxygen-enriched oil from the edges of the dressing allowed the oil to be in direct contact with the wound and the pungent smell was an intrinsic characteristic, indicating the release of reactive oxygen species.

The oxygen-enriched oil-based gel we adopted in the study comes in the form of an oily gel, derived from extra virgin olive ozonated oil, hyperoxidized and standardized in peroxides. Regarding the mechanism of action, the application of the gel promotes the physiological repair of the wound, having a film-forming protective action and improves re-epithelialization, promoting the proliferation of fibroblasts. The beneficial effects of ozone on wound healing are related to the reduction of microbial infection, debridement effect, modulation of the inflammatory phase, stimulation of angiogenesis as well as biological and enzymatic reactions that favor oxygen metabolism improving wound healing (23). It acts as insulator, producing heat that results in local peripheral vasodilation, increased blood flow, oxygenation and cellular metabolism, accelerating the healing process. The effect on the skin is due to its reaction with the polyunsaturated fatty acids and traces of water present in the upper layer of the dermis, generating reactive oxygen species (ROS) and lipo-oligopeptides, among which is H_2O_2. ROS are the most effective natural agents against antibiotic-resistant pathogens and favor the degradation of organic material which could disturb the healing process. In addition, it improves metabolism and immune functions (23). Ozone therapy activates the production of some nuclear factors to induce cytokines transcription, such as IL-2, TNFα, IL-6, IFNγ and IL-8, participating in the immune response of our body. Furthermore, ozone enhances a higher expression of growth factors TGF-β and vascular endothelial growth factor (VEGF), which play important roles in the wound repair process. In this way, remodeling of the extracellular matrix (ECM) begins and collagen fibers proliferate and reorganize into a stronger network (27). In these situations, ozone promotes the release of nitric oxide (NO), endothelium-independent vasodilator, which increases blood circulation for tissue remodeling (27).

For all these reasons, this new device was very effective in our series in promoting a faster healing of penis wound compared with the standard dressing method. The beneficial effects on the wound healing were also associated with a better post-operative outcome in our study. In fact, post-operative

complications rate was statistically significant lower in G1 patients compared with G2 ones ($p = 0.001$). Furthermore, penile diameter measurements at different timelines proved that entity and duration of post-operative swelling were objectively decreased using the new dressing method.

The clinical use of the device was easier compared with the standard dressing method for different reasons, as subjectively assessed by nurses. First, the tubular device was fairly water-repellent and protected the penis against urinary and fecal contamination whereas, the elastic bandage was not water-repellent and was more frequently exposed to contamination with urines or stools, especially of fluid-consistency, and needed more frequently to be changed. Second, the dressing change was also easier using the tubular device compared with the standard method; in fact, it only needed to remove the posterior anchoring stitches and the device was detached off the penis. A new tubular device was then re-applied and closed on the dorsal side of the penis using 2–3 pieces of adhesive tape without any discomfort for the child. The device never sticked to the penis thanks to the constant release of oil from the inner coated side. Conversely, the elastic bandage frequently sticked to the penis because the layer of oxygen-enriched oil-based gel directly applied on the wound was quickly absorbed by tissues.

The use of the device was clinically safe because it was a preparation for topical use and had no systemic effects. Additionally, the topical use in our series did not report any hypersensitivity or allergic or other serious skin reactions to the dressing.

Finally, the cost analysis showed that the new tubular device for hypospadias dressing was also cost-effective. The lower costs of wound treatment in G1 patients were related to the faster healing process in such patients, who required a shorter treatment following hospital discharge, compared with the G2 patients.

One the main limitations of the study is the short follow-up time which could influence the overall surgical complications rate as previous papers (28, 29) have shown urethroplasty complications to develop well-passed the 60 days follow-up of our paper. We must also consider that in other countries such as the United States (US) most distal hypospadias are done as outpatient. As reported by Snodgrass et al. (30), a simple clear plastic wrap (Tegaderm) was used as dressing for a few days postoperatively and their complications rate tended to be around 8%. The lack of a control group using a similar type of dressing in our study could be considered a further limitation.

Additionally, the way this dressing is being described would possibly suggest a limit for its utilization in the outpatient setting. We preferred that the patients remained in the hospital after the operation with the aim to assess different aspects of this dressing during the hospital stay such as the tolerability by patients and the ease of management by nurses and parents. However, this does not mean that it cannot be adopted in the outpatient setting. Probably, it is better than the previous dressing methods because it does not require any manipulation by parents. It could be conceived that patients are operated as outpatient and discharged following the operation and then come back to the hospital for dressing change and thereafter for catheter removal.

Another limitation is represented by the restrictive inclusion criteria, limited to distal hypospadias repairs. The next step will be to test this new dressing method in a larger number of patients with a longer follow-up and include also proximal hypospadias to validate these preliminary results.

In conclusion, based upon the preliminary results of this study, post-operative dressing using tubular finger oxygen-enriched oil inside-coated device was highly effective, easy to manage, cheaper and associated with a lower rate of foreskin and urethral complications compared with the standard dressing method in pediatric patients undergoing distal hypospadias repair. It was also cost-effective and clinically safe without allergy or intolerance to the product.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CE contributed conception and design of the study and wrote the first draft of the manuscript. VC, FD, MCe, GE, FC, MCa, AC, and ME organized the database and wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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REVIEW
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Management of Vesicoureteral Reflux: What Have We Learned Over the Last 20 Years?

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Vesicoureteral reflux (VUR) is associated with increased risks of urinary tract infection, renal scarring and reflux nephropathy. We review advancements over the last two decades in our understanding and management of VUR. Over time, the condition may resolve spontaneously but it can persist for many years and bladder/bowel dysfunction is often involved. Some factors that increase the likelihood of persistence (e.g., high grade) also increase the risk of renal scarring. Voiding cystourethrography (VCUG) is generally considered the definitive method for diagnosing VUR, and helpful in determining the need for treatment. However, this procedure causes distress and radiation exposure. Therefore, strategies to reduce clinicians' reliance upon VCUG (e.g., after a VUR treatment procedure) have been developed. There are several options for managing patients with VUR. Observation is suitable only for patients at low risk of renal injury. Antibiotic prophylaxis can reduce the incidence of UTIs, but drawbacks such as antibiotic resistance and incomplete adherence mean that this option is not viable for long-term use. Long-term studies of endoscopic injection have helped us understand factors influencing use and the effectiveness of this procedure. Ureteral reimplantation is still performed commonly, and robot-assisted laparoscopic methods are gaining popularity. Over the last 20 years, there has been a shift toward more conservative management of VUR with an individualized, risk-based approach. For continued treatment improvement, better identification of children at risk of renal scarring, robust evidence regarding the available interventions, and an improved VUR grading system are needed.

Keywords: antibiotic, bladder/bowel dysfunction, endoscopic injection, NASHA/Dx, vesicoureteral reflux, ureteral reimplantation, urinary tract infection, voiding cystourethrogram

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INTRODUCTION

Vesicoureteral reflux (VUR) is associated with increased risks of urinary tract infection and renal scarring or reflux nephropathy (1). Reflux nephropathy in children with VUR may be attributable to scars from upper urinary tract infection (UTI) as well as congenital renal dysplasia (1). The severity of VUR is described by a grading system according to the findings of a voiding cystourethrogram (VCUG), with grades ranging from I (mild) to V (severe). In most cases, VUR does not directly cause any symptoms; it is diagnosed either antenatally in children with hydronephrosis, or later following the occurrence of symptomatic UTIs (2, 3). Diagnosing the condition can be challenging

due to the lack of direct symptoms and, in neonates and young infants, this is compounded by the non-specific manner with which UTIs present. Estimated prevalence rates for VUR range between 0.4 and 1.8% (4, 5).

Numerous studies have examined the links between VUR, UTIs, pyelonephritis, renal scarring and impaired renal function. In a study of 115 infants with grade III–V reflux, single-kidney glomerular filtration rate (GFR) was below 40% of the individual's total expected value in 71% of the patients, and a deterioration in renal status was observed in 18% of the patients (6). Recurrent febrile UTIs (fUTIs), bilateral renal abnormalities and reduced total GFR were identified as risk factors for renal deterioration. Swerkersson et al. evaluated VUR and renal scarring in children aged <2 years presenting with UTI (7). VUR and renal scarring were each present in 26% of the study participants, and the rate of renal abnormality increased significantly with increasing grade of VUR. A later study by the same group assessed changes over time in children aged <2 years presenting with UTI who were found to have renal scarring (8). Over a follow-up time of at least 2 years, 19% of the children exhibited renal deterioration. Grade III–V VUR and recurrent UTI were identified as risk factors for deterioration. Hidas et al. developed an instrument for predicting the risk of breakthrough UTI in children with VUR (9). VUR grade, gender, circumcision status, presence of bladder/bowel dysfunction (BBD) and cause of presentation of VUR enabled stratification of children into different risk groups. When the instrument was applied to a validation cohort, the predicted 2-year incidence of breakthrough UTI was 19.5%, compared with an actual rate of 21% (9). Arlen et al. similarly developed a tool for calculating the risk of a breakthrough fUTI in children with VUR based on risk factors for UTIs [including age, gender, VUR grade, reflux at low bladder volume, bladder/bowel dysfunction (BBD) and UTI history] (10). In a cohort of 255 children, the calculator was shown to have 76% accuracy. A study by Keren et al. investigated risk factors for recurrent UTI and renal scarring in children aged 2–71 months who had experienced one or two febrile or symptomatic UTIs (11). VUR, BBD and renal scarring were all associated with increased likelihood of recurrent UTIs. In males, circumcisional status may also be an important risk factor for UTI. One review reported that circumcision is associated with an 87% reduction in the incidence of UTI among boys with high-grade VUR (12).

In a Turkish study of 156 children aged 0–16 years with UTIs, increasing grade of VUR was associated with increasing rates of renal scarring (13). A longitudinal study with median follow-up of 5.6 years was conducted to investigate the association between renal scarring and adverse renal outcomes in children with a diagnosis of UTI or VUR (14). Patients with, vs. without, renal scarring showed significantly increased risk of developing proteinuria (5.1 vs. 1.6%, $p = 0.005$) and kidney disease (2.0 vs. 0.0%, $p = 0.005$). The available data support intervention in patients with VUR to reduce the risks of pyelonephritis and renal scarring, which can have permanent consequences.

VUR has long been known to resolve spontaneously over time. However, a decision to wait for this to occur rather than treating or curing the condition should only be taken in the absence of repeat fUTIs that could cause renal scarring. In 1998, Wennerström et al. reported that grade III–V reflux resolved spontaneously (to grade 0–I) in 73% of cases over a follow-up period of 10 years (15). Early investigations also showed that older age, high-grade VUR and female gender were associated with a lower likelihood of spontaneous VUR resolution (15, 16). Later studies identified high-grade VUR, renal abnormalities, prenatal hydronephrosis, bladder dysfunction, low bladder filling volume at reflux onset, breakthrough UTI and older age upon diagnosis of VUR as independent predictors of a lower likelihood of spontaneous resolution (17–21). Evidence suggests that effective treatment of BBD can increase the chance of spontaneous resolution of VUR (22). Kirsch et al. performed multivariate analysis on outcomes from 229 patients diagnosed with VUR before the age of 2 years, and reported that patients with the following had significantly longer time to spontaneous resolution: grade IV–V VUR, duplicated ureters or periureteral diverticula, occurrence of reflux during bladder filling, and female gender (23). The occurrence of reflux early during bladder filling has been associated with low spontaneous resolution rates and increased risk of fUTI, independent of the grade of VUR (18, 23, 24).

VUR and BBD are closely related and around half of patients with VUR also have BBD (25). Among patients with VUR, additional presence of BBD approximately doubles the risk of UTIs (11, 25, 26). As mentioned above, co-existent BBD may also reduce the likelihood of spontaneous resolution of VUR, and BBD has been associated with reduced success in patients undergoing endoscopic injection for VUR (17, 27). On the other hand, intervention for VUR can lead to the improvement or cure of BBD, indicating a degree of interdependence between the two conditions (28–30). Treatment of BBD as well as VUR in patients with both conditions appears to be advisable (22, 27).

In females, VUR is associated with increased risk of pregnancy-related complications such as pre-eclampsia and UTI (31, 32). This is mainly attributable to the presence of renal scarring, supporting the notion that preventing renal damage should be a key goal of VUR management. However, UTI prevention may also be important since the risk of fetal complications is elevated among women with frequent UTIs (32).

There are four main options for managing patients with VUR: observation, antibiotic prophylaxis, endoscopic injection and ureteral reimplantation (33–35).

DIAGNOSIS AND ASSESSMENT

The key aims of assessment are to determine how and when the patient should undergo treatment. VCUG has been described as the only definitive method of diagnosing VUR and defining its severity (36–38). However, inter-rater variability is common with this assessment. Some studies have reported favorable intraclass correlation coefficient (ICC) values between 0.8 and 0.9, but lower levels of inter-rater agreement (50–60%) have also been reported (39–42). To improve the reliability of results, a standardized protocol should be adhered to when performing this assessment (37, 43). Specifications such as choice of contrast, method for infusing contrast, timing and quality of spot images,

and documentation of bladder volume at onset of VUR ensure consistency (37). Also, more than one cycle of filling and voiding may be needed to avoid the possibility of underdiagnosing VUR (44). Optimal VCUG methodology is not always followed, and this represents an opportunity to improve routine clinical practice (43).

Perceived importance of the available assessments of VUR patients has changed significantly over the last 20 years. Historically, the grade of VUR was often the only determinant of treatment decisions. The VCUG procedure causes considerable distress and exposes patients to radiation. Strategies to reduce clinicians' reliance upon VCUG have therefore been developed (45). As well as the grade of VUR, treatment decisions are routinely based on age, gender, UTI occurrence and the presence of renal scarring. Determination if the patient has BBD is particularly important. Reproducible, validated methods for diagnosing BBD are limited and an 18-item questionnaire developed in 2019 was shown to enable reliable diagnosis and subcategory classification (46). VCUG assessment is considered necessary in patients with recurrent fUTIs (38, 47, 48). For children aged 2–24 months presenting with their first fUTI, routine VCUG is supported by the American Academy of Pediatrics only if there is an abnormality on a renal and bladder ultrasound scan (36, 49). Assessments other than VCUG that can help determine appropriate therapies include the frequency of UTIs or fUTIs, renal function tests [e.g., dimercaptosuccinic acid (DMSA) or mercaptoacetyltriglycine (MAG-3) scanning], ultrasound scanning, and assessment of bowel and bladder function (49–54).

A future shift toward assessing the severity of VUR in patients with methods that are more objective and easily measurable than the current grading system is recommended by the authors. The distal ureteral diameter ratio (UDR; diameter of distal ureter normalized to the L1–L3 vertebral body distance; measurable using VCUG images) has been shown to be predictive of spontaneous resolution of VUR and risk of breakthrough fUTI (55–58). Each unit increase of UDR of 0.1 is associated with a significant increase in the probability of VUR persistence (55, 56, 59). A significant decrease in inter-grader variability has been reported with the UDR assessment compared with VUR grading, with ICC values of 0.95 and 0.87, respectively (59). Knowledge of the factors involved in spontaneous resolution of VUR prompted the development of computational prediction methods (60). Subsequently, a VUR index was proposed, where a patient's clinical characteristics (e.g., gender, grade of VUR, timing of VUR) are used to predict the likelihood of spontaneous resolution (23). Reliability of this index was initially shown in a cohort of VUR patients aged <2 years ($n = 229$), and then validated in a second cohort of patients aged <2 years ($n = 369$) as well as in 271 patients aged >2 years (23, 61, 62). The VUR index score has also been shown to correlate with the risk of developing UTIs (**Figure 1**) (23, 62, 63). Importantly, both UDR and VUR index appear superior to international VUR grading in predicting either spontaneous resolution of VUR or risk of a breakthrough UTI in children aged <2 years at diagnosis (55, 61, 63). Contrast-enhanced voiding urosonography (ceVUS) could potentially be used as a replacement for VCUG. This method enables determination of the presence and grade of VUR in a similar manner to VCUG, without exposing the patient to ionizing radiation. Available evidence suggests that ceVUS may provide acceptable diagnostic accuracy (64–66). In one study using VCUG results as the reference point, ceVUS was shown to provide sensitivity of 92% and specificity of 98% (65). The concordance rate between the two methods in determining the grade of VUR was 82%. Further data are needed to establish the suitability of ceVUS for use in routine clinical practice.

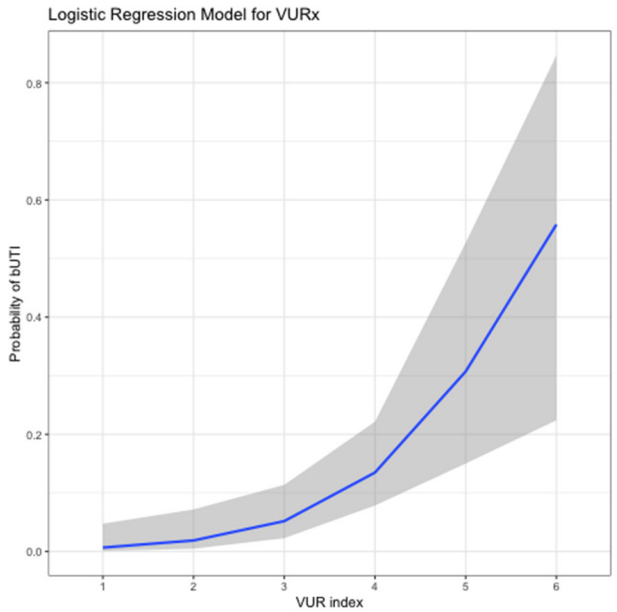

FIGURE 1 | Relationship between vesicoureteral reflux index (VURx) score and subsequent occurrence of breakthrough urinary tract infections (bUTIs) (63). The graph is based on data from a cohort of 139 patients, mean age at VUR diagnosis 5.45 years (standard deviation, 4.7 years), followed for a mean of 32.1 months (standard deviation, 24.5 months) after diagnosis. Reproduced with permission purchased from the Copyright Clearance Center.

TREATMENT APPROACHES

The principal aim of VUR management is to reduce kidney infections and renal scarring. In addition, clinicians should aim to prevent UTIs and minimize long-term assessment and treatment procedures. Management may be non-surgical (e.g., urotherapy, antibiotic therapy), minimally invasive (endoscopic injection) or surgical (ureteral reimplantation), and these approaches are detailed below.

Observation

The selection of "observation" may be perceived as favorable due to the avoidance of medical intervention. However, regular follow-up visits to the clinic are required to enable adequate monitoring of the patient's status. In addition, parents must always be vigilant to ensure that all UTIs are reported and managed. Antibiotic therapy should be administered promptly to treat fUTIs, while frequent occurrence of UTIs is an indication for a different management strategy (1, 22, 67). Observation is

only considered suitable for patients with a relatively low risk of renal injury (i.e., males with low-grade VUR) (68–70).

Antibiotic Prophylaxis

Antibiotic prophylaxis has been reported to be effective in preventing UTIs. In the randomized intervention for children with vesicoureteral reflux (RIVUR) trial, the risk of recurrent infection was reduced by 50% vs. placebo among VUR patients with one or two prior UTIs (71). Similarly, in the randomized Swedish Reflux study which compared antibiotic prophylaxis with endoscopic injection and observation in children with VUR, the incidence of recurrent fUTIs was significantly lower in girls receiving antibiotic prophylaxis vs. observation (19 vs. 57% over a median period of 2 years; $p = 0.0002$) (72). In boys, the numbers of recurrent fUTIs were low in both study groups, with no significant difference. The RIVUR trial and others have demonstrated that delayed treatment of UTIs increases the risk of renal scarring (71, 73, 74). Despite the data showing possible benefits of antibiotic treatment, it is important to consider that VUR often persists for years, meaning that antibiotic prophylaxis is often needed for a prolonged duration. In contradiction to the studies above, a 2019 Cochrane review reported that long-term antibiotic prophylaxis "makes little or no difference to the risk of repeat UTI causing a person to be unwell" (34). Other studies also suggest that prophylactic antibiotic therapy can often be discontinued without incurring significantly increased UTI rates (75). The extent to which patients adhere to their prescribed treatment may explain some of the variability between studies, with real-world compliance rates tending to be considerably lower than those in clinical trials. In 2007, Hensle et al. reported a compliance rate of only 17%, suggesting widespread exposure to the same risk of UTIs as children under observation only (76).

The risk of antibiotic resistance in children receiving prophylactic antibiotics is an important consideration when choosing between management options (34, 71, 77, 78). Another possible drawback of antibiotic prophylaxis is deleterious effects on the microbiome of the gut, which can have a significant impact on patients' overall health (77, 79–81). These aspects are now recognized to a much greater extent than they were 20 years ago.

A cost-effectiveness analysis of antibiotic therapy was performed using results from the RIVUR trial (82). This study showed that antibiotic prophylaxis has marginally higher costs than placebo, while significantly reducing the incidence of infection. A second cost-utility analysis reported that antibiotic prophylaxis is only cost-effective if administered to patients with grade IV VUR; costs per quality-adjusted life-year gained in patients with grade I–III VUR were deemed prohibitively high (83).

In the future, it may become possible to better select specific patients who would benefit from antibiotic prophylaxis (84). However, the viability of long-term antibiotic prophylaxis as a treatment option for all patients with VUR remains questionable.

Endoscopic Injection

Clinical data from numerous studies have confirmed long-term safety and efficacy of endoscopic injection. In a meta-analysis published in 2016, the overall resolution rate ranged between 71 and 83%, depending on the injection technique. Studies with long-term follow-up [3–22 years, mostly performed with NASHA/Dx (Deflux)] have similarly reported resolution rates ranging between 69 and 100% (28, 85–88). In addition to results in "uncomplicated" VUR, numerous studies have provided evidence that endoscopic injection is also effective in specific patient populations that may be deemed more difficult to treat (historically not considered for endoscopic therapy). These include high-grade VUR, duplicated systems, adult women and kidney transplant patients (85, 86, 89–104). Resolution rates may be reduced in these groups of patients: for example, Läckgren et al. reported a positive response rate of 63% in patients with duplicated ureters, compared with 68% in the broader population of VUR patients (104, 105). However, the success rates are high enough for endoscopic injection to remain viable in these groups of patients.

A range of factors have been shown to influence the resolution rate with endoscopic injection. Statistically significant effects on outcomes have been reported with VUR grade, injection technique, physician experience, patient age, and the extent of renal scarring at time of treatment (35, 106–108). In addition, high UDR values have been associated with reduced likelihood of VUR resolution following endoscopic injection (109). Baydilli et al. recently studied associations between a range of clinical parameters and the outcome of endoscopic therapy with NASHA/Dx (110). The factors associated with greatest increase in the likelihood of failure of NASHA/Dx to resolve VUR were: onset of reflux during the early filling phase of the voiding cycle, UDR value above 0.24, and a delay in upper urinary tract drainage after voiding. Presence of renal scarring, presence of BBD, history of fUTI and high-grade VUR were also associated with significantly increased risk of treatment failure.

There is little evidence of major differences in VUR resolution rates between injectable agents in current use (34). This appears contingent upon formation of a long-lasting bolus following injection; experience with bovine collagen indicated lower efficacy than with other injectable agents (111–113). This was attributable to degradation of collagen post-injection, and collagen is not currently used for endoscopic treatment of VUR. The choice of injectable agent may have a more significant impact on the safety of endoscopic injection. Early investigations of endoscopic injection were performed using polytetrafluoroethylene (PTFE) and polydimethylsiloxane (silicone). Safety concerns with these products include granuloma formation (a foreign-body reaction), migration from the injection site, and, because of their lack of biodegradability, permanent accumulation within the body (113–115). These considerations led to PTFE and silicone falling out of common use in patients with VUR.

Recently developed injectable agents include polyacrylate-polyalcohol copolymer, polyacrylamide hydrogel, and small-size (80–120 μm) dextranomer/hyaluronic acid copolymer (116). Like PTFE and silicone, polyacrylate-polyalcohol copolymer and polyacrylamide hydrogel are non-biodegradable, meaning they can remain within the body permanently. They both have a favorable histopathologic profile, but foreign-body reactions are possible (117–120). Polyacrylate-polyalcohol

copolymer has been associated with risks of periureteral fibrosis (potentially complicating subsequent ureteral reimplantation) and obstruction of the vesicoureteral junction (116, 121). Comparative studies suggest that polyacrylate-polyalcohol copolymer, polyacrylamide hydrogel and small-size dextranomer/hyaluronic acid copolymer are at least as effective as NASHA/Dx in resolving VUR (106, 121–124). Only one of these studies was a prospective, randomized trial; the results showed comparable efficacy with NASHA/Dx and polyacrylate-polyalcohol copolymer (121). The only other prospective study (non-randomized) also reported similarity between the two agents being compared (NASHA/Dx and polyacrylamide hydrogel) (123). The remaining comparisons of recently developed materials vs. NASHA/Dx were retrospective, limiting the robustness of the results. Small-size dextranomer/hyaluronic acid copolymer (brand names Urodex, Vurdex and Dexell) differs from NASHA/Dx (brand name Deflux) not only in the size of the dextranomer microspheres, but also in the characteristics of the hyaluronic acid, potentially affecting the safety profile, physical properties and ease/controllability of the injection procedure. Importantly, these differences mean that clinical results obtained with NASHA/Dx are not directly applicable to small-size dextranomer/hyaluronic acid copolymer. Long-term efficacy and safety data (>5 years) are yet to be published with any of the recently developed agents. We advocate NASHA/Dx because of its long-term safety (documented follow-up to 25 years), robust published evidence of efficacy and international regulatory approval (in the USA, it is the only FDA-approved material for endoscopic treatment of VUR).

A limited number of studies have assessed the pharmacoeconomics of endoscopic treatment of VUR. Early data published by Kobelt et al. in 2003 showed that, in the USA, endoscopic treatment with NASHA/Dx could reduce the cost of VUR management without reducing the clinical success rate (125). Another US study, published 3 years later, similarly reported that NASHA/Dx could be more cost-effective than ureteral reimplantation in patients with unilateral grade III VUR, although not in patients with bilateral grade III VUR or grade IV–V VUR, in whom larger volumes of NASHA/Dx are needed (126). In 2008, total reimbursement costs in the USA were found to be lower with outpatient ureteral reimplantation than with endoscopic injection for VUR (127). However, the cost difference was only ∼10%, and total reimbursement for ureteral reimplantation was increased if a proportion of these patients require hospital admission. In 2016, results from patients treated in two European centers were analyzed to compare endoscopic treatment of VUR using NASHA/Dx with two methods of ureteral reimplantation (open Cohen and laparoscopic Lich-Gregoir) (128). Intra-operative costs were highest with endoscopic injection, but the total cost (intra-operative plus post-operative hospitalization costs) was highest with the Cohen procedure (€8201), and similar with endoscopic treatment and laparoscopic reimplantation (€3283 and €3211, respectively). Observations regarding lower product costs with polyacrylamide hydrogel and small-size dextranomer/hyaluronic acid copolymer vs. NASHA/Dx have been made in some publications (88, 123, 129). Simple comparisons of product costs do not provide a complete pharmacoeconomic picture: formal studies that include the total long-term costs of patient management (influenced by long-term safety and efficacy of the treatments concerned) are needed for true pharmacoeconomic comparisons.

Ureteral Reimplantation

Ureteral reimplantation is associated with high resolution rates (>90%) in grade ≤IV VUR. It is considered an invasive procedure that requires hospital admission and time for recovery (130–134). There is a small risk of post-operative complications; these occur in ∼5–9% of children undergoing open surgery (134, 135).

Laparoscopic and robotic methods have the potential to reduce the invasiveness of ureteral reimplantation, and these methods are gaining popularity (136). A multicenter, retrospective analysis of laparoscopic ureteral reimplantation conducted in patients with grade II–IV VUR reported a success rate of 96% (137). A 2016 review of laparoscopic ureteral reimplantation also reported a median success rate of 96%, with a complication rate of 7% (138). Success rates similar to those with open surgery (>90%) have been reported with robot-assisted laparoscopic ureteral reimplantation, although there is evidence that the success rate with this method may be lower (around 80%) when the procedure is performed bilaterally (139–141). Urinary retention has been reported as a complication among patients undergoing robot-assisted ureteral reimplantation, and the overall complication rate appears higher in patients undergoing bilateral procedures (140, 142). The cost-effectiveness of robot-assisted ureteral reimplantation has been questioned due to higher costs and higher complication rates compared with open surgery (135). The learning curve for robotic surgery can be substantial, and is best done at centers with high patient numbers (143–145). In addition, the costs associated with procuring robotic equipment may limit the availability of this approach. Treatment outcomes are likely to improve as techniques are developed further, but current data indicate that open surgery may still be preferable.

Cochrane Review

A recently published Cochrane review evaluated benefits and harms of all the available interventions for VUR. Thirty-four randomized studies met the inclusion criteria (34). Antibiotic prophylaxis was reported to have little effect on the risk of UTI and to increase the likelihood of antibiotic resistance. The benefits with endoscopic injection or ureteral reimplantation vs. antibiotic treatment were deemed unclear due to insufficiencies in study design.

MANAGEMENT RECOMMENDATIONS

The main aim of VUR management recommendations is to ensure that each patient receives the most appropriate intervention for their individual needs. There are variations between countries in the approach to VUR management and in the specialty of the healthcare provider who first sees the patient. International variability is also encountered in the licensed

indications for devices including the injectable agents used in endoscopic treatment.

Developments Over the Last 20 Years

The 1997 AUA guidelines recommended antibiotic prophylaxis as first-line treatment, with surgery (ureteral reimplantation) as second-line treatment for persistent cases or as first-line intervention in severe VUR (particularly in older children) (146). Endoscopic injection was not recommended for routine use at that time, and concerns we now have regarding antibiotic prophylaxis were less well-understood. In 2002, positive results obtained with endoscopic injection of NASHA/Dx led to the proposal of an updated treatment algorithm (147). For most patients, 1 year of antibiotic prophylaxis was recommended in the first instance. For those in whom VUR persisted to the end of the year, endoscopic injection was proposed. Ureteral reimplantation was considered appropriate for patients not responding to endoscopic treatment, and it was also recommended as first-line intervention in high-risk groups (children aged >1 year with grade V reflux, and those aged >5 years with grade bilateral III–IV reflux) (147).

Over the last 20 years, there has been a shift toward more conservative management of VUR. More emphasis is now placed on an individualized, risk-based approach, with less reliance on long-term antibiotic prophylaxis, reduced use of VCUG and a decline in surgical intervention (45). Also, patients with concurrent VUR and BBD are understood to have an increased risk of UTI vs. patients with VUR only, meaning that treatments for both conditions may be needed (22, 25). It remains unclear whether BBD should always be treated before VUR.

Current Guidelines

The European Association of Urology (EAU) guidelines on the management of VUR are 8 years old (69). They state prominently that "there is no consensus on the optimal management of VUR or on its diagnostic procedures, treatment options, or most effective timing of treatment." VUR guidelines from the American Urological Association (AUA) were updated more recently, in 2017, but these too include a comment that "the data were not sufficient to permit development of strict 'standards of care' in many instances" (68). This has undoubtedly contributed to the current variability in VUR management.

The EAU and AUA guidelines recommend VCUG in infants with prenatally diagnosed hydronephrosis, and siblings and offspring of VUR patients (68, 69). EAU guidelines also recommend VCUG examination in children with an fUTI or lower urinary tract dysfunction (69). For infants diagnosed in the first year of life, antibiotic prophylaxis is recommended as first-line treatment, with ureteral reimplantation or endoscopic treatment for those with breakthrough infections (68, 69). Antibiotic prophylaxis is also recommended in the EAU guidelines as initial treatment for children aged 1–5 years with grade III–V VUR, although ureteral reimplantation should be considered as an alternative in those with high-grade VUR (69). For children with lower urinary tract dysfunction (LUTS) as well as VUR, the EAU recommend that initial management should be focused on LUTS. Endoscopic treatment is recommended principally as an option for children with low grades of VUR (up to grade III) and, for high-risk patients with renal impairment, an "aggressive, multidisciplinary approach" is recommended (69). In the AUA guidelines, for patients aged >1 year and no BBD, antibiotic therapy is suggested as an option, while endoscopic injection or ureteral reimplantation are recommended for patients with recurrent UTIs or new renal abnormalities. For patients aged >1 year with concurrent VUR and BBD, the AUA guidelines recommend antibiotic therapy with BBD treatment (68). The AUA guidelines describe lower success rates with endoscopic injection vs. ureteral reimplantation, but definite recommendations on how to choose between these options are lacking (68).

Guidelines on managing patients with UTIs also include recommendations relating to VUR. The American Academy of Pediatrics (AAP) guidelines of 2011 were influential in reducing the use of VCUG. Before 2011, patients between 2 and 24 months of age with an fUTI routinely underwent VCUG assessment. In contrast, the 2011 guidelines recommended renal and bladder ultrasound assessment for patients with their first fUTI, and no VCUG among those without ultrasound-detectable abnormalities (148). This approach was reaffirmed by the AAP in 2016 (149). The UK National Institute for Health and Care Excellence (NICE) similarly recommend VCUG only in selected children with UTIs: those aged <6 months with atypical or recurrent UTIs (150). These UK guidelines recommend surgical treatment of VUR (either endoscopic injection or ureteral reimplantation) only for VUR patients with "symptomatic breakthrough

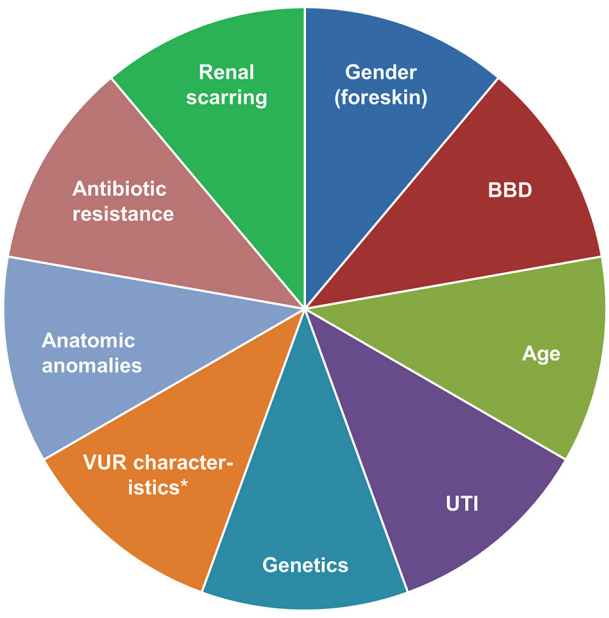

FIGURE 2 | Factors to consider in a risk-based approach to the management of VUR. *Grade, bladder volume at onset of reflux, ureteral diameter ratio. BBD, bladder/bowel dysfunction; UTI, urinary tract infection; VUR, vesicoureteral reflux.

UTIs despite medical management and/or increased renal parenchymal defects."

Current Opinion of the Authors

In all patients with VUR, there is a need to balance risks, benefits and costs of treatment vs. risks (particularly to the kidneys) of not treating the condition (**Figure 2**) (14, 33, 35, 151). We believe that first-line endoscopic injection is preferable for many VUR patients requiring intervention. Ureteral reimplantation is usually performed in patients not responding to endoscopic injection, those with primary obstructive refluxing megaureter, and those with grade V VUR and concomitant narrowing of the vesicoureteral junction. For patients with VUR and bladder or bowel dysfunction (BBD), we recommend treating BBD as early as possible (before VUR intervention). However, in cases with recurrent breakthrough UTIs, endoscopic treatment or ureteral reimplantation should not be delayed and BBD therapy can still be undertaken as needed. We no longer support the routine use of long-term antibiotic prophylaxis for VUR. Long-term monitoring of patients with VCUG assessments after endoscopic treatment or ureteral reimplantation appears unnecessary, due to the high cure rates with both treatment options. Follow-up VCUGs are mainly triggered by the occurrence of symptomatic UTIs. Every decision needs to be taken with due consideration of the individual patient's history and current health status, risk of recurrent UTI, as well as the wishes of the patient and/or their parents.

CONCLUSIONS

Over the last 20 years, our understanding of VUR has increased considerably. A proportion of children with renal scarring after UTI (particularly those with grade III–V VUR and recurrent fUTI) are at risk of renal deterioration. Improved knowledge of how to identify such patients has led to an individualized, risk-based approach to the management of VUR and an overall shift to more conservative management of VUR. Surgical methods of ureteral reimplantation have progressed but our opinion is that endoscopic injection is frequently preferable, based on evidence from the last two decades confirming the long-term tolerability and durability of this procedure. Although a number of materials have been explored as injectable agents during the last 20 years, NASHA/Dx is widely considered the preferred choice with the strongest long-term efficacy and safety data. Key knowledge gaps include the need for better identification of children at risk of recurrent UTIs and future renal scarring, robust evidence from randomized controlled trials, further evaluation of the side effects of chronic antibiotic exposure, and an improved VUR grading system. These gaps will need to be addressed in the coming years to ensure that individual patients' needs are fulfilled to the greatest possible extent.

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All authors reviewed the literature and collaborated in writing and editing the manuscript.

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SYSTEMATIC REVIEW
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Acquired Rectourethral and Rectovaginal Fistulas in Children: A Systematic Review

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Background: Acquired rectourethral (RUF) or rectovaginal fistulas (RVF) in children are rare conditions in pediatric surgery. Prior literature are retrospective studies and based on a small number of patients. The managements and outcomes vary widely across different studies. No standard or recommended management has been universally adopted. The goal was to systematically summarize different causes, provide an overlook of current clinical trend and to derive recommendation from the literature regarding the etiology, managements, and outcomes of pediatric acquired RUF and RVF.

Methods: PubMed, Embase, Cochrane databases were searched using terms: rectourethral fistula, recto-urethral fistula, urethrorectal fistula, urethro-rectal fistula, rectovaginal fistula. All studies were retrospective, in English, and included patients under the age of 18 years. Any series with congenital cases, adult (>18 years), <2 fistula cases less and obstetric related causes were excluded. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline was followed.

Results: Of the 531 records identified, 26 articles with 163 patients (63 RUF and 100RVF) were fully analyzed. Most RUF resulted from trauma, most RVF were from infection of HIV. About 92 patients underwent 1 of 3 categories of definitive repair, including transanal (4.3%), trans-sphincteric (48.9%), and transperineal (30.4%). Tissue interposition flaps were used in 37.6% patients, while temporary fecal diversions were used in 63.9% patients. Fistula was successfully closed in 50.3% patients (98.4% RUF and 20% RVF). 89.1 and 79.7 % of surgical repair patients had optimal fecal and urinary functions, respectively. In the inflammatory bowel disease and HIV infection related RVF patient group, the closure rate was prohibitive poor.

Conclusions: Most RVF are a sign of systematic diseases like HIV-infection or IBD and are associated with poor general conditions. While conservative treatment is recommended, stable patients can benefit from surgery. Further investigation is recommended if RVF are encountered without trauma or surgical history. RUF are likely to result from trauma or surgery, and transperineal or trans-sphincter approach can lead to closure and optimal function results. Fecal diversion and/or urinary diversion are helpful in some cases, while interposition technique may not be necessary. An objective scoring system for long-term follow-up and reporting consensus is needed to address treatment inconsistence.

Keywords: rectourethral fistula, rectovaginal fistula, acquired fistula, pediatric surgery, systematic review

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BACKGROUND

Acquired rectourethral (RUF) or rectovaginal fistulas (RVF) in children are rare conditions but a Gordian knot for pediatric surgeons (1). They may occur as a manifestation of HIV infection (2), in the setting of inflammatory bowel disease (IBD) (3), or resulting from trauma (4), iatrogenic fistulas because of colorectal surgery for congenital anorectal malformation (ARM) (5), or Hirschsprung's disease (6). Despite various reasons leading to acquired RUF and RVF, reported incidence is low and mishandled cases are frequent.

Conservative treatments are attempted by using draining seton, contemporary fecal diversion (FD) and/or urinary diversion (UD). However, irrespective of etiology, most children with RUF and RVF do not respond well to FD alone and require further treatments (7, 8). Various repair procedures: transperineal, trans-sphincteric (i.e., York-Mason, PSARP), transanal, transabdominal, or a combination of the above, have been described. Rarely, fibrin glue therapy or collagen plug are used, but outcomes are prohibitively poor (9).

Definitive repairs for children are considered more difficult than adults. The underdeveloped structure between primitive gut and urogenital sinus leads to two systems intimately attached to each other, leaving a narrow space to repair. The transperineal approach is favored by urologists as it provides a direct view and access to the posterior urethra (10), and allows for muscle flap interposition and concomitant urethroplasty. The trans-sphincteric approach (i.e., York-Mason, PSARP) (1, 4) involves midline sagittal division of posterior and anterior anorectal walls along with the corresponding sphincteric musculature. Fecal and urinary functions can be largely preserved. Flap interposition was once considered impossible or difficult through trans-sphincter incision (11), but recent research provides a new thought (1). The transanal approach (i.e., Latzko) has limited use because of narrow exposure (12). Obstructions of urinary and/or alimentary tract are commonly seen with acquired fistulas and may inhibit wound healing (13). Simultaneous end-to-end urethral anastomosis (14) and pull-through (PT) (15) are used to address these pathologic defects.

In summary, acquired RUF and RVF in children are heterogeneous; managements, and surgical approaches have been proposed. There is no established convention at present. Narrative review reflects the uncertainty and confusion, and fails to provide evidence-based knowledge. In this article, we aimed to systematically review literatures on pediatric acquired RUF and RVF to provide an overlook of current clinical trends and, when possible, outline suggestions for evaluation and treatment.

METHODS

Protocol

This article is in accordance to the preferred reporting items for systematic reviews and meta-analyses (PRIMSA) guideline (16) and registered on INPLASY (INPLASY202110078).

Search Strategy and Sources

Two reviewers (Huang and Tan) screened PubMed and Embase from inception to Jan 2021 and searched records by title and abstract and reviewed eligible articles. A MEDLINE and Embase search was performed using three key terms with no date limits: (1) "rectourethral fistula" OR "recto-urethral fistula," (2) "urethrorectal fistula" OR "urethro-rectal fistula," (3) "rectovaginal fistula." These three key terms were then narrowed: (1) English, (2) Human, (3) Child (<18 years). Ovid and the Cochrane library databases were searched using two key terms: (1) "rectourethral fistula" and (2) "rectovaginal fistula." Pertinent references were searched manually. Five articles (9, 12, 17–19) were also reviewed for backgrounds but not used for qualitative analysis.

Enrolled Criteria

The studies were included using the following criteria: (1) Child < 18y; (2) related to rectourethral or rectovaginal fistulas treatments.

The studies were excluded using the following criteria: (1) studies focusing on radiographic diagnosis of RUF/RVF; (2) case series/studies involving only congenital conditions; (3) reported cases focused on fistula repair with <2 patients (regardless of age); (4) case report; (5) studies focusing on obstetric related conditions; (6) review articles not reporting repairs and outcomes at the author's institution; (7) editorial letters/comments; (8) inadequate documentation of techniques or outcomes; (9) presentation abstracts; (10) others (adult patients, article not found, irrelevant).

Data Collection Process

Two reviewers (Huang and Tan) independently read all full articles and complied pertinent data as follows: (1) author and publication year, (2) number of RUF and/or RVF patients, (3) mean or median age, (4) etiology, (5) number of RUF and/or RVF patients who underwent surgical interventions, (6) number of patients who had temporary FD and/or UD, (7) type of surgical repair, (8) tissue flap usage, (9) treatment outcomes, (10) follow-up period, (11) prognosis. Types of surgical repair were organized into four categories: (1) trans-sphincteric, (2) transperineal, (3) transanal, and (4) others. Combined techniques such as laparotomy or endorectal pull-through were categorized as others. The trans-sphincteric category included York-Mason, PSARP as well as other sphincter-dividing operations. Temporary FD via colostomy or ileostomy alone was categorized as surgical interventions. Temporary UD defined as invasive drainage techniques was categorized as surgical interventions, i.e., suprapubic cystostomy. Use of transurethral Foley catheter was excluded.

Primary treatment outcomes were classified as follows: (1) successful fistula closure, (2) fistula recurrence or persistence; (3) others, i.e., patient refused treatment or died. Functional outcomes regarding fecal and urinary continence were defined as function after surgical repair closure within the study follow-up period and was based on description per respective authors. Successful fistula closure was defined as absence of clinical symptoms and radiographic confirmation of closure within

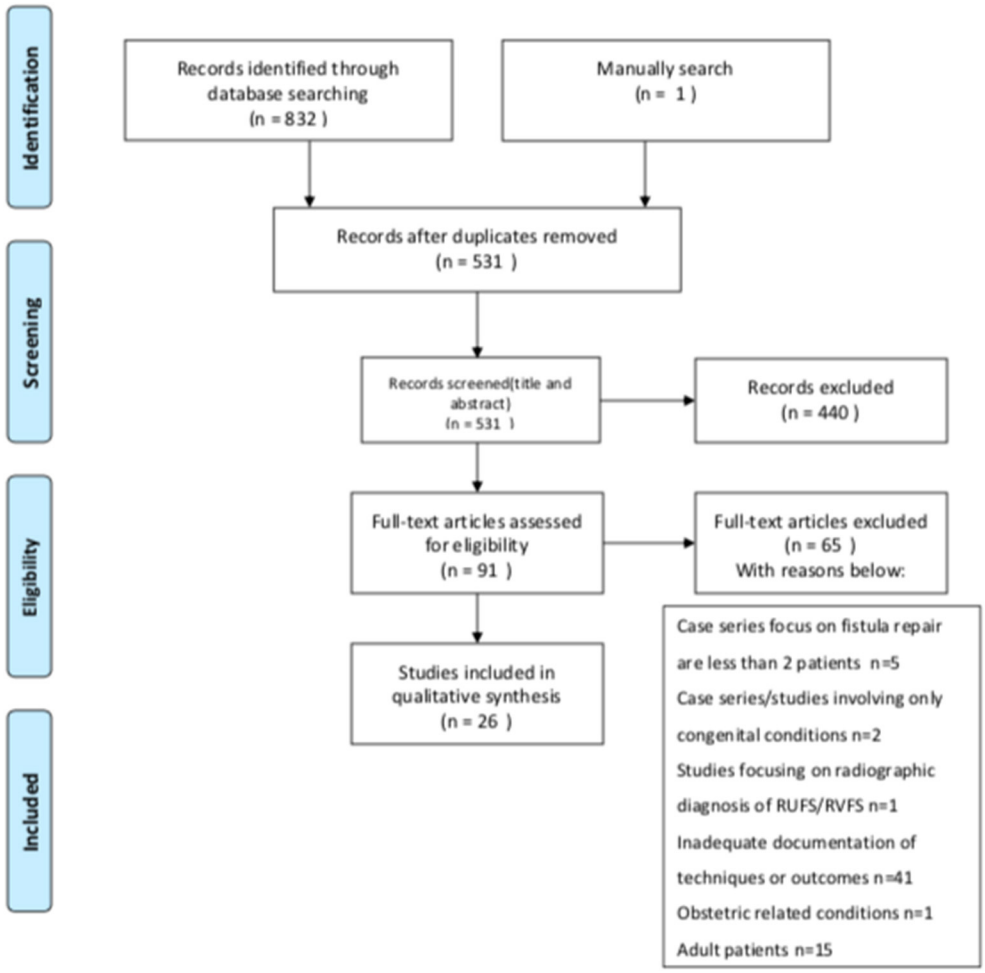

FIGURE 1 | PRISMA search flow diagram.

the study follow-up period. Fistula recurrence was defined as a new fistula emerged after an attempt repair and a period absence of clinical symptoms, fistula persistence was defined as failed closure after surgical or conservative treatment within the study follow-up period. The follow-up period was defined as the mean or median time when patients were followed within the study. Prognosis included postoperative complications (i.e., anatomic pathologic features or impaired functional continence) and patient living status within the study follow-up period.

Bias Among Individual Studies

Given the rarity of pediatric acquired RUF and RVF, all studies were retrospective case series from single center with a small number of patients, and all were classified as level 4 evidence. Within some series, the surgical approach details were vague and heterogeneous, and the descriptions of outcomes were varied, which makes it difficult to compare within and between institutions. As with many retrospective studies, there is no control group, medical records are not consistent and accurate, and there is personal bias regarding surgical approaches.

RESULTS

Study Selection

As shown in the PRISMA flow diagram for study selection process (**Figure 1**), 1,561 titles were generated with three key terms (451 "rectourethral fistula" OR "recto-urethral fistula," 106 "urethrorectal fistula" OR "urethro-rectal fistula," 1,004 "rectovaginal fistula.). The Ovid database search under the two key terms generated 2,544 titles (531 "rectourethral fistula", 2,013 "rectovaginal fistula"). A total of 832 results were identified by using previously described limits. One article was added from the manual reference research. Three hundred and two duplicates were removed, leaving 531 citations for screening. After citations were screened, 440 were excluded, leaving 91 articles for full review. After 91 full-articles were reviewed, 65 were removed, leaving 26 articles for qualitative synthesis. No record was identified in the Cochrane Library.

Study Characteristics

Overall patient-specific data are reported in **Table 1**. Surgical repairs, interposition flap usage, outcomes, follow-up, and

TABLE 1 | Summary statistics for overall patient-specific data across all 26 selected studies.

References	Total RUF patients, *n*	Total RVF patients, *n*	Mean or median age, y	Etiology, *n* (%)				Nonsurgical patients, *n* (%)	Surgical patients, *n* (%)	Temporary FD, *n* (%)	Temporary UD, *n* (%)
				Trauma	Iatrogenic	Inflammatory	Infection				
Nakayama et al. (20)	1	0	Age: 1.7	0	1 (100)	0	0	0	1 (100)	1 (100)	1 (100)
Mukerji et al. (21)	1	0	Age: 12	1 (100)	0	0	0	0	1 (100)	0	1 (100)
Wiseman and Decter (22)	1	0	Age: 5.5	0	1 (100)	0	0	0	1 (100)	1 (100)	1 (100)
Bukowski et al. (11)	1	0	Age: 14	1 (100)	0	0	0	0	1 (100)	1 (100)	1 (100)
Borgstein and Broadhead (23)	0	9	Range: 2–8 months	0	0	0	9 (100)	9 (100)	0	0	0
Hyde and Sarbah (24)	0	22	Range: 0–24 months	0	0	0	22 (100)	22 (100)	0	0	0
Sarioglu et al. (25)	1	2	Range: 1–17	0	3 (100)	0	0	0	3 (100)	2 (66.7)	1 (33.3)
Youssef et al. (26)	3	0	Range: 7–12	3 (100)	0	0	0	0	3 (100)	3 (100)	3 (100)
Rius et al. (27)	0	1	Age: 17	0	0	1 (100)	0	0	1 (100)	1 (100)	0
Zhou et al. (28)	3	0	Mean: 7.1	3 (100)	0	0	0	0	3 (100)	3 (100)	3 (100)
Wiersma (2)	2	37	Mean: 1.35	0	0	0	39 (100)	27 (69.2)	12 (30.8)	12 (30.8)	0
Culp and Calhoon (29)	1	0	Age: 11	1 (100)	0	0	0	0	1 (100)	0	1 (100)
Kubota et al. (30)	1	0	Age: 1	0	1 (100)	0	0	0	1 (100)	0	0
de Ridder et al. (31)	0	4	Range: 14.8–17.5	0	0	4 (100)	0	3 (75)	1 (25)	1 (25)	0
Tang et al. (15)	1	0	Age: 3.7	0	1 (100)	0	0	0	1 (100)	0	0
Liu et al. (4)	15	4	Mean: 6.2	10 (52.6)	9 (47.4)	0	0	0	19 (100)	0	0
Razi et al. (32)	1	0	Age: 7	0	1 (100)	0	0	0	1 (100)	1 (100)	0
Nerli et al. (13)	17	0	Mean: 7	2 (11.8)	15 (88.2)	0	0	0	17 (100)	17 (100)	2 (11.7)
Abdalla (33)	4	0	Range: 5–16	1 (25)	3 (75)	0	0	0	4 (100)	1 (25)	0
Helmy et al. (14)	4	0	Mean: 6.8[a]	0	4 (100)	0	0	0	4 (100)	1 (25)	4 (100)
Osifo and Egwaikhide (7)	0	3	Range: 6–11 months	0	0	0	3 (100)	0	3 (100)	3 (100)	0
Sheng et al. (34)	0	1	N/A	0	1 (100)	0	0	0	1 (100)	1 (100)	0
Levitt et al. (1)	3	6	Range: 2–10	N/A	N/A	N/A	N/A	0	9 (100)	6 (66.7)	0
Sun et al. (6)	0	2[b]	Median: 4.7	0	2 (100)	0	0	0	2 (100)	2 (100)	0
Ye et al. (3)	0	9	Median: 7days	0	0	9 (100)	0	5 (55.5)	4 (44.5)	3 (33.3)	0
Nikolaev (35)	3	0	Median: 13	0	3(100)	0	0	0	3 (100)	3 (100)	3
Total: *n* (%)	63	100	N/A	22/154[c] (14.3)	45/154 (29.2)	14/154 (9.1)	73/154 (47.4)	66/163 (40.5)	97[d]/163(59.5)	63/163(38.7)	21/163(12.9)

FD, fecal diversion; UD, urinary diversion (i.e., suprapubic cystostomy); N/A, not applicable (i.e., not reported or not clearly identified throughout the study). Studies listed with only 1 RUV/RVF pediatric patient also included similar adult surgical patients, as stated in results section Study Characteristics.

[a] *one patient had a congenital malformation but could not be removed from the overall age analysis.*

[b] *ten patients had neither RUF nor RVF but could not be removed from the overall age analysis.*

[c] *one study (1) (nine total patients) did not specific etiology type, and these patients were removed from the denominator.*

[d] *62 RUFs and 35 RVFs underwent surgical repairs.*

prognosis are reported in **Table 2**. We identified 163 patients in 26 studies selected for qualitative analysis, including 63 RUF patients, and 100 RVF patients. Many case series written by urologists, describing both children and adult cases together. This was especially so for articles describing surgical techniques. This portion of surgeons and pediatric urologists would have accumulated substantial experience in treatment of fistulas. We tried to include these relatively more reliable articles in our analysis. Some of these case series only included one pediatric RUF/RVF case, but this nonetheless fulfilled our enrolment criteria. The mean or median age per study ranged from 7 days to 17.5 years. No studies met criteria for quantitative analysis.

Etiology

Of 163 patients, 73 (47.4%) were caused by HIV infection in African since 1990s. Fistulas reported in those literature (2, 7, 23, 24) were predominantly RVF; HIV infection is the major cause of acquired RVF in the pediatric population. Second to retroviral infection, 45 (29.2%) iatrogenic fistulas were a complication of invasive operations, with rates as follows: 13.6% rectal surgery, 9.7% Hirschsprung's disease, 4.5% anorectal malformation, and 1.3% urethroplasty. Trauma-related fistulas were the third leading cause and the major cause of RUF, with over 80% of RUF (18 cases). The main types of trauma-related fistulas arose from pelvic fracture (14 patients), traffic accidents (six patients) and blunt/penetrating injuries (two patients). Although, they can present at any age, children beyond school-age are most affected (**Table 1**). There remained a small subset (14 patients, 9.1%) of RVF patients who were associated with inflammatory bowel disease (IBD). These patients are not many, but there is a trend of growing and early onset of symptoms.

Interventions

In total, 97 patients (59.5%) in 24 studies underwent surgical interventions, comprising of 98.4% (62/63) RUF and 35% (35/100) RVF patients. Among them, 92 patients had definitive surgical repairs, four RVF patient had FD alone with one healed spontaneously, one RUF patient healed after prolonged recovery followed removal of bladder calculus and UD. The remaining 66 patients (65 RVF and one RUF) were all exclusively in the IBD and HIV-infection group. Among them, nine patients died, 54 patients' fistulas persisted, three patients were lost to follow-up. Non-surgical treatments for these patients were anti-retroviral drugs, infliximab, broad spectrum antibiotics, supportive treatments, umbilical cord blood stem cell transplantation (three patients), or declined medical care (three patients).

Urinary and Fecal Diversions

Sixty two patients (63.9%) had temporary FD and 20 patients (20.6%) had temporary UD before or during definitive repair. Most studies (19/26) performed FD, among them 11 studies suggested that the variable time interval between effective FD and fistula repair ranged from 3 months to 8 years (data not shown). The indication of FD varied considerably across different etiology group. Patients in infection and inflammation groups exhibited (1) severe and advanced perianal infection or sepsis (2, 24, 27); (2) perforation (3). Patients in trauma and iatrogenic groups (1) were hopeful for spontaneous closure (4, 11); (2) had to prevent infection and maximize chance of closure (4, 13, 20, 22, 35); (3) classified as recurrent fistulas (11, 34). In general, temporary FD was closed 3 to 6 months after confirmation of closure, and temporary UD was removed 2–4 weeks postoperatively if there was no sign of leakage or infection.

Definitive Repairs

The number of definitive repairs (total 93) in 92 patients [one study (1) reported 10 surgical approaches for nine patients] for each surgical category is as follows: (1) four transanal (4.3%), (2) 45 trans-sphincteric (48.9%), (3) 28 transperineal (30.4 %), (4) three redo-Swenson procedure (3.2%), (5) one transabdominal (1.1%), (6) 12 non-specific (13.0%). One patient (14) required additional laparotomy to harvest the omentum for interposition and to create a FD at the same time. Another author (15) claimed that laparotomy was required because of severe pelvic fibrous adhesion. Some (30) advocated combined endorectal pull-through (PT) technique to prevent refistulization.

Tissue Interposition Flaps

Tissue interposition flaps were used in a little over one-third repairs (32/85, 37.6%) (**Table 2**). Vascularized tunica vaginalis flap were the most common (11/85, 12.9%) but were used in only one study (13), followed by ischiorectal fat (10/85, 11.8%), gracilis muscle (4/85, 4.7%), dartos (3/85, 3.5%), omentum (2/85, 2.4%), gluteus muscle (1/85, 1.2%), and Colle's fascia (1/85, 1.2%). Only seven literature performed tissue interposition, predominantly (28/31, 90.3%) *via* transperineal approach. Levitt et al. (1) and Abdalla et al. (33) used flap *via* trans-sphincteric or transanal approach. However, it is notable that across all definitive surgery repairs, there was no obvious advantage in flap usage regarding closure rate (all were 100%).

Successful Closure

In total, 82 (50.3%) patients had successful closure, 67 patients had persistent fistulas, 11 patients died because of underlying diseases and three patients missed follow-up. Successful closure rate in RUF was 98.4% (62/63), and 20% (20/100) in RVF. In surgical group, 82 patients had successful closure, 13 fistulas persisted and two died. The ultimate successful closure rate was optimal across all surgical repair cases (82/97, 84.5%) (**Table 2**), among them delayed closure was observed in four patients (4, 20, 21) (4/97, 4.1%) after adequate drainage of postoperative infection. At least seven studies (1, 15, 22, 27–29, 35) reported 18 patients who had 35 failed attempts of repair before ultimate closure, mainly *via* transperineal approach. Fistulas persisted in 67 patients (67/163, 41.1%), who were almost exclusively RVF patients (66/67, 98.5%)

TABLE 2 | Summary statistics for types of surgical repair, tissue flap usage, primary outcomes, follow-up period, and prognosis.

References	Total RUF patients, *n*	Total RVF patients, *n*	Surgical patients, *n*	Trans-sphincteric repairs, *n*	Transperineal repairs, *n*	Transanal repairs, *n*	Other techniques, type, *n*	Tissue flaps usage, type, *n*	Successful closure rate, *n* (%)	Follow-up time, months	Prognosis
Nakayama et al. (20)	1	0	1	1	0	0	0	0	1 (100)	Time: 10	/
Mukerji et al. (21)	1	0	1	0	0	0	Bladder calculus removal, 1	0	1 (100)	Time: 3	/
Wiseman and Decter (22)	1	0	1	0	0	0	Kraske, 1	0	1 (100)	Time: 12	Occasional urinary incontinence, 1
Bukowski et al. (11)	1	0	1	1	0	0	0	0	1 (100)	Time: 12	/
Borgstein and Broadhead (23)	0	9	0	/	/	/	/	/	0	N/A	Died 2; persistent fistulas 4; missing 3
Hyde and Sarbah (24)	0	22	0	/	/	/	/	/	0	N/A	Died 6; persistent fistulas 16
Sarioglu et al. (25)	1	2	3	0	0	0	Redo-Swenson, 3	0	3 (100)	Time: 120[b]	/
Youssef et al. (26)	3	0	3	0	3	0	0	Subcutaneous dartos pedicled, 3	3 (100)	Mean: 42	/
Rius et al. (27)	0	1	1	0	0	1	0	Gracilis muscle and perineal skin, 1	1 (100)	Time: 6	/
Zhou et al. (28)	3	0	3	0	3	0	0	0	3 (100)	Mean: 10.1	Stress incontinence and impotence [a]
Wiersma (2)	2	37	12	1	0	0	Not specified, 11	0	1 (2.56)	N/A	persistent fistulas 38
Culp and Calhoon (29)	1	0	1	0	1	0	0	0	1 (100)	N/A	persistent urethral stricture 1

(Continued)

TABLE 2 | Continued

References	Total RUF patients, *n*	Total RVF patients, *n*	Surgical patients, *n*	Trans-sphincteric repairs, *n*	Transperineal repairs, *n*	Transanal repairs, *n*	Other techniques, type, *n*	Tissue flaps usage, type, *n*	Successful closure rate, *n* (%)	Follow-up time, months	Prognosis
Kubota et al. (30)	1	0	1	1	0	0	Combined endorectal pull-through, 1	0	1 (100)	N/A	/
de Ridder et al. (31)	0	4	1	0	0	0	FD alone, 1	0	1 (25)	Mean: 24.8	persistent fistulas 3
Tang et al. (15)	1	0	1	1	0	0	Combined laparotomy, 1	0	1 (100)	Time: 6	/
Liu et al. (4)	15	4	19	19	0	0	0	0	19 (100)	N/A	/
Razi et al. (32)	1	0	1	0	0	1	0	0	1 (100)	Time: 44	/
Nerli et al. (13)	17	0	17	6	11	0	0	Vascularized tunica Vaginalis flap, 11	17 (100)	Mean: 18	urethral stricture 1; fecal incontinence 1; urinary incontinence 1
Abdalla (33)	4	0	4	4	0	0	0	Gluteus muscle, 1	4 (100)	Mean: 21	/
Helmy et al. (14)	4	0	4	0	4	0	Combined laparotomy, 1	• Omental, 2 • Ischiorectal fat, 1 • Colle's fascia, 1	4 (100)	Mean: 22	urethral stricture 1
Osifo and Egwaikhide (7)	0	3	3	0	3	0	0	0	3 (100)	Mean: 34	/
Sheng et al. (34)	0	1	1	0	0	0	Transabdominal, 1	0	1 (100)	Time: 36	/
Levitt et al. (1)	3	6	9[b]	8	0	2	0	Ischiorectal fat, 9	9 (100)	Mean: 6	/
Sun et al. (6)	0	2	2	2	0	0	0	0	2 (100)	N/A	/
Ye et al. (3)	0	9	4	N/A	N/A	N/A	FD alone, 3; Not specified, 1	N/A	0	N/A	died 3; persistent fistulas 6
Nikolaev (35)	3	0	3	0	3	0	0	Gracilis muscle, 3	3 (100)	Time: 48~60	urethral stricture 1
Total: n	63	100	97[c]	44	28	4	N/A	32	82	N/A	N/A

N/A, not applicable (i.e., Not reported or not clearly identified throughout the study). Studies listed with only one RUV/RVF pediatric patient also included similar adult surgical patients, as stated in results section Study Characteristics.

[a] Two patients who did not have ruf had complications but could not be removed from overall analysis.

[b] Ten surgical approaches were performed for nine patients in this study.

[c] 62 RUFs and 35 RVFs underwent surgical repair.

(**Table 2**). The definition of successful fistula closure varied in 23 studies as follows: (1) absence of clinical symptoms alone (14, 22, 28, 32); (2) radiographic confirmation of closure alone (6, 7, 13, 20, 26, 29, 30, 33, 35) (i.e., retrograde urethrogram or contrast enema); (3) evidence of radiographic closure and absence of symptoms (21); or (4) not specified (1, 2, 4, 11, 15, 25, 27, 31, 34).

Follow-Up

In 15 studies with a total of 64 definitive surgical repair, follow-up for postoperative function demonstrated that good fecal continence was preserved in 57 patients (57/64, 89.1%) and urinary continence was preserved in 51 patients (51/64, 79.7%). Ten studies (7, 11, 13, 14, 22, 26, 32–35) claimed to have mean or median follow-up period over one-year. Major postoperative anatomic and/or incontinence complications were rare, and only observed in six patients as follows: urethral stricture in four, urinary incontinence in two, fecal incontinence in one, urinary incontinence in one, and impotence in one (**Table 2**). Most functional results were based on description per authors. High mortality rate was observed in RVF (**Table 2**), all were secondary to underlying pathologic infection disease.

DISCUSSION

Main Findings

Most RVF manifest as signs of systematic diseases including HIV-infection and IBD, which are associated with poor general conditions. While conservative treatment is recommended, stable patients can benefit from surgery. Further, investigation is recommended if RVF is encountered without trauma or surgical history. RUF are likely to result from trauma or surgery, and transperineal or trans-sphincter approach can lead to closure and optimal function results. Fecal diversion and/or urinary diversion is helpful in some cases, while interposition technique may not be necessary. An objective scoring system for long-term follow-up and reporting consensus is needed to address treatment inconsistence.

Interpretation

Acquired RUF and RVF are more common in adults and are rarely encountered in pediatric population. In children, infection with HIV is becoming an important and rapidly increased cause of acquired RUF. It is estimated that about 3 per 2.8 million women of reproductive age had babies with HIV-related RVF in Africa (7). The underlying pathophysiology, although, not yet fully-understood, shares a consistent clinical pattern (17): there is a significantly higher female prevalence (23, 24), but the few male patients (2) showed more complex fistulas. Most affected children were diagnosed within 1 year of age due to observation of abnormal feces discharge following a period of diarrhea. Presumably caused by anal gland abscess, fistula tended to arise from the dentate line, but biopsy showed non-specific inflammatory features (2).

Second to HIV-related fistulas, iatrogenic fistulas are also prevalent causes of RUF. Re-do Hirschsprung's disease (36) or re-do ARM repair (5) are two major sources of acquired fistulas in pediatric surgical practice. Fistulas in re-do Hirschsprung's disease are considered to be totally preventable and unacceptable (36), regardless of initial operations (6, 15, 20, 30, 34) (i.e., Duhamel, Soave, Swenson). These fistulas can be prevented by improving basic surgical skills. In re-do ARM repair patients, acquired fistulas are thought to occur when lacking the benefit of urethral Foley catheter, or when the anterior rectal wall is damaged (5). Trauma-related fistulas are frequently accompanied by pelvic fracture and complicated by urethroplasty (4, 28, 33), which highlights the need for cooperation between urologists and pediatric surgeons. Nerli et al. (13) suggested such pathologic defects should be cured before definitive fistula repair.

A small portion of acquired RVF patients are also diagnosed in the setting of IBD, particularly Crohn's disease. Most pediatric IBD are teenagers (37), and <1% are neonatal or infantile on-set. In the early 20th century, IBD related RVF (27, 31) was only noticed during adolescence but awareness has recently been raised for infantile-onset IBD related RVF (3). The increased diagnosis rate in younger age children (38) can be attributed to growing awareness in physicians (18) and advancing diagnosis techniques, which would explain a recent report (3) about acquired RVF in infantile onset IBD patients. It is reasonable to presume that this trend will continue in the future. Underlying pathology begins with small colonic mucosal ulcerations, followed by transmural penetration, and fistula formation (39). It is noteworthy that infantile onset patients had more severe clinical presentations and a very high mortality (3). Predisposing genetic mutation may play a critical role.

Attitudes toward conservative treatment as an initial plan varied considerably among different etiology groups. In fistulas induced by IBD or HIV infection, it is currently widely accepted (2, 9, 17, 19) that medicine is the first-line treatment; surgery is preserved as an adjunct therapy with very limited indications. One pivotal fact is that retroviral therapy (17) and antibiotics (40) have been proved effective to alleviate local effect. On the other hand, procedures performed to eliminate the fistula in the setting of active disease or infection are disappointing (2, 3, 27). However, with the advancement of conservative treatment to stable the general condition of patients, a potential change is emerging; surgery might only be needed if the RUV persists. More recent updates showed that patients could benefit from definitive repairs (1, 7), although, the timing and type of surgery needs further study. A careful small selection of patients (7) had a diverting fecal stoma to relieve infection and inflammatory process, which ultimately led to successful fistula closure. Yet as most authors (2, 3, 24) would agree, a FD is a last resort to control life-threating sepsis, advance perineal disease, or acute perforation, because poor healing will

cause the situation to deteriorate. RUF induced by IBD or HIV infection were rarely reported (2), and special attention must be paid to their high infection risk, complex anatomy, and additional surgical needs. In summary, patients with asymptomatic RVF require no immediate surgical intervention, whereas, such intervention can be considered if their general conditions are stable.

In contrast with the above, among surgery or trauma induced fistula patients, debate about the need, timing, type of FD, and its role in protecting surgical repairs continue to be controversial. Two reports (11, 22) in this review mentioned spontaneous closure with FD in few patients, and two (11, 33) suggested diversion can be selectively adopted. One series (4) with a large volume of patients ($n = 19$) reported 100% successful closure rate *via* single stage surgery, but emphasized urethral stricture and secondary megacolon must be cured first. In many (13, 20, 22, 34), authors claimed that most patients did not respond well to FD alone and strongly advocated for FD to aid subsequent repair. Commonly, FD is considered based on the experience and opinions of the surgeons with conditions as follows: (1) recurrent fistulas; (2) severe urinary tract or perineum infection; (3) previous failed repairs; (4) dense scar and adhesion in fistula site. FD types are chosen to emphasize effective and complete diversion (20); an end ileostomy allows colon mobilization and coloanal anastomosis if needed, otherwise one can consider a colostomy. Most surgeons tended to wait 3 to 6 months before definitive repair, when scar may be soft and infections disappeared (4), but there is no clear census about appropriate timing at present. Notably, there are no data to support higher closure rates with FD, and <50% of patients in these two etiology groups had temporary FD before definitive repair. However, we believe that usage of FD must be individualized. FD is generally preferred in patients with large fistulas (>2cm), multiple failed repairs, poor general condition, and/or damaged sphincter function. Reversal of FD usually comes after radiologic confirmation of closure, while temporary UD is removed earlier if no clinical sign of leakage or infection.

The trans-sphincteric approach was used in half the patients and is a good initial approach to repair fistulas. The approach facilitates excellent exposure for the fistula and posterior urogenital structure, and provides a direct surgical field and preserves continence function. Successful closure was reported as 100% regardless of etiology. Two studies (15, 30) suggested to combine endorectal pull-through technique in the following situations: (1) extremely difficult fistula resection; (2) concomitant megacolon or anorectal stenosis; (3) large (>5mm), heavy scarred fistulas. Although, transperineal approach has a comparable closure rate (100%), it also involved multiple unspecified prior failures and postoperative urinary complications. Yet this approach is still favored by many urologists (26, 28, 29) to address concomitant urethra and/or vagina defects (i.e., stricture, atresia), and nearly 90% flap interposition was performed *via* this approach. Transanal approach (i.e., Latzko) is seldom used because of limited exposure to dissect and repair fistula. Large volume of blood loss and long surgical hours was also encountered (32). There remains a need for transabdominal approach for fistula proximal to bladder neck, dense pelvic adhesion, omental harvest. Tissue interposition flaps are not used as frequent as in adults (12). Adult fistulas tend to be large, complex and radiation-induced, requiring muscle flap (i.e., gracilis, dartos). Kubota et al. (30) and others (15) thought it was unnecessary and complicated, but Nikolaev et al. (35) claimed that accurate position of flap was the key to achieve success. No higher closure rate was observed with interposition technique in this review, which suggests that surgeons must carefully take all benefits and risks of interposition into account before repair.

Follow-up information suggested that acquired fistulas have substantial chance at healing with good fecal continence, particularly for RUF. RVF were associated with high mortality rate but this was mostly due to underlying pathology. However, follow-up rate in this study was not ideal (15/26) and length of follow-up was also subpar, with only 10 studies ranging over 1-year. Furthermore, aside from two studies (6, 34) that adopted the 2005 Krickenbeck classification to assess postoperative excretory function, all others were descriptive and therefore subjective. Current clinical guidelines for RVF does not include a scoring system to measure functional outcome, but the international continence society recommends using the ICS score (41) for urinary continence in RUF children. Long-term outcomes for RUF have also been studied using the bowel function score, an established qualitative scoring system for benign anorectal disorders (42), and evaluation of the overall success of RUF treatments has been carried out using the Pediatric Quality of Life Inventory (43). We believe that further use of these scoring systems, singularly or altogether, would provide a more detailed and reliable analysis of treatment success rates for both acquired RUF and RVF.

Limitations

The numerous conservative treatments and surgical repairs reflect the variability in surgeon preference and surgical skills, as well as the need for individualized treatment plan. Due to the heterogeneity of the trials and the limited amount of evidence, we were unable to perform any meta-analyses. A main limitation of this study is that all articles were retrospective studies from single institutions without a control group. The small number of patients and vague description of outcomes also precludes objective comparisons, but some conclusions can still be drawn from the existing information. Careful and extensive screening of the articles adds to the reliability of our conclusions, as lone case reports and articles without sufficient reliable information were excluded. The conclusions we have arrived at and the recommendations made for treatment of this rare disease should be but a stepping stone for further more detailed research.

Perspectives and Implication

Acquired RUF and RVF are rare in children. Most RVF are a sign of systematic diseases whereas RUF are likely to result from trauma or surgery. Treatments for RVF focus on medicine but stable patients may benefit from surgery. Further, and thorough investigation of the timing and type of surgery is needed in acquired RUF. An objective function scoring system for long-term follow-up and reporting consensus are needed in the future to address current treatment inconsistence.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors conceptualized and wrote the manuscript.

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Long-Term Renal Transplant Outcome in Patients With Posterior Urethral Valves. Prognostic Factors Related to Bladder Dysfunction Management

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Introduction: To obtain a successful renal transplant (RT) outcome in patients with posterior urethral valves (PUV), it is necessary to accomplish an adequate bladder dysfunction treatment. Our aim was to determine prognostic factors related to bladder dysfunction management in long-term RT outcome in patients with PUV.

Methods: A retrospective review of patients with PUV who received a first RT after 1985 in our institution with at least 5 years of follow-up was performed. Variables analyzed included prenatal diagnosis, age of diagnosis, initial presentation and management, bladder dysfunction treatment, other surgical treatments, pre-transplant dialysis, age of transplantation, type of donor, immunosuppression regimen, vascular and urological complications, rejections episodes, and graft survival.

Results: Fifty-one patients were included in the analysis. Prenatal diagnosis was done in 37.3%. Median age of diagnosis was 0.30 (0–88) months. Initial presentation was vesicoureteral reflux (VUR) in 78% and obstructive ureterohydronefrosis in 35.3%. Initial management was valve ablation (29.4%), pyelo-ureterostomy (64.7%), and vesicostomy (5.9%). In 33.3%, a type of bladder dysfunction treatment was performed: 21.6% bladder augmentation (BA), 15.7% Mitrofanoff procedure, 17.6% anticholinergic drugs, and 27.5% clean intermittent catheterization (CIC). Pre-transplant dialysis was received by 66.7%. Transplantation was performed at 6.28 ± 5.12 years, 62.7% were cadaveric and 37.3% living-donor grafts. Acute rejection episodes were found in 23.6%. Urological complications included recurrent urinary tract infections (UTIs) (31.4%); native kidneys VUR (31.4%); graft VUR (45.1%); and ureteral obstruction (2%). Vascular complications occurred in 3.9%. Mean graft survival was 11.1 ± 6.9 years. Analyzing the prognostic factor that influenced graft survival, patients with had CIC or a Mitrofanoff procedure had a significant better long-term graft survival after 10 years of follow-up ($p < 0.05$), despite

of the existence of more recurrent UTIs in them. A better graft survival was also found in living-donor transplants ($p < 0.05$). No significant differences were observed in long-term graft survival regarding native kidneys or graft VUR, BA, immunosuppression regimen, or post-transplant UTIs.

Conclusion: Optimal bladder dysfunction treatment, including CIC with or without a Mitrofanoff procedure, might result in better long-term graft survival in patients with PUV. These procedures were not related to a worse RT outcome in spite of being associated with more frequent UTIs.

Keywords: posterior urethral valves, pediatric renal transplant, bladder dysfunction, long-term renal transplant outcome, lower urinary tract obstruction

INTRODUCTION

Posterior urethral valves (PUV) are a major cause of end stage renal disease (ESRD) in the pediatric population and constitute one of the most common causes of pediatric renal transplant (RT) (15.3%) (1). Patients with PUV are associated with some kind of bladder dysfunction, which has been called "valve bladder" (2). Manifestations of bladder dysfunction in PUV are variable, ranging from high-pressure low-compliant bladders and overactive bladders to myogenic-failure high-capacity bladders (3). In spite of bladder dysfunction, it has been demonstrated that RT outcome in patients with PUV is comparable to patients transplanted due to a non-urological anomaly in the mid and long-term (4–10). These favorable outcomes in graft survival are due to an adequate bladder dysfunction treatment before and after transplantation, but there is no consensus about what is considered optimal bladder management.

The aim of this study was to determine the prognostic factors related to bladder dysfunction management in long-term renal transplant outcome and to attempt to identify the best strategies to improve graft survival in these patients.

MATERIALS AND METHODS

This study was approved by the ethical committee of our center. A retrospective review of patients who received an RT between 1985 and 2020 with the diagnosis of PUV in our institution was performed. Patients who had a graft failure within the first 15 days post-transplantation and a post-transplant follow-up lower than 5 years were excluded from the analysis. In patients who received more than one renal transplant, only the first graft was considered for the analysis.

Variables analyzed included **data related to the PUV disease**: prenatal diagnosis and treatment, age at diagnosis, initial presentation (vesicoureteral reflux, obstructive ureterohydronefrosis), initial management (valve ablation, ureterostomy, vesicostomy), bladder dysfunction management (urodynamic findings, anticholinergics, clean intermittent catheterization, bladder augmentation, Mitrofanoff procedure), other surgical treatments (ureteral reimplantation, endoscopic reflux treatment, nephrectomy), and age of end stage renal disease settlement. The other variables analyzed included **data related to the renal transplant**: pre-transplant dialysis, type of donor, immunosuppression regimen applied, vascular and urological complications, rejections episodes, and graft and patient survival. We studied these variables to identify a prognostic factor that may have influence on long-term graft survival.

Bladder dysfunction diagnosis was achieved by performing an initial pre-transplant urodynamic study in all patients. After transplantation, all patients had a close follow-up that included a renal ultrasound and a voiding diary associated with urine culture if presenting urinary tract infection (UTI) symptoms. In cases with an increase of dilation of the urinary tract in the renal ultrasound, alteration of the voiding diary or symptomatic UTI, a urodynamic study was achieved to confirm bladder function status.

The criteria used for bladder dysfunction management was as follows: Anticholinergics were indicated when patients presented an overactive bladder in the urodynamic studies or patients with a low-compliant bladder. Clean intermittent catheterization (CIC) was indicated in patients with a myogenic failure in the urodynamic studies with a post-void residual volume of >10%. The Mitrofanoff procedure was indicated in the same cases of CIC but when urethral catheterization was painful or difficult to the patient. Bladder augmentation (BA) was indicated in patients with a urodynamic study with a low-compliant bladder that had not responded to anticholinergic drugs or a detrusor Botox injection.

Data were analyzed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). Categorical data were compared using the Chi-squared test. Continuous data were presented as median and standard deviation and compared using Student's *t*-test. Graft and patient survival were analyzed by actuarial methods. Differences between the Kaplan-Meier survival curves were tested by log-rank tests. Differences were considered statistically significant at *p*-values of <0.05.

RESULTS

From a total of 501 RTs, 65 patients had a primary diagnosis of PUV, and 51 patients met the inclusion criteria for the analysis.

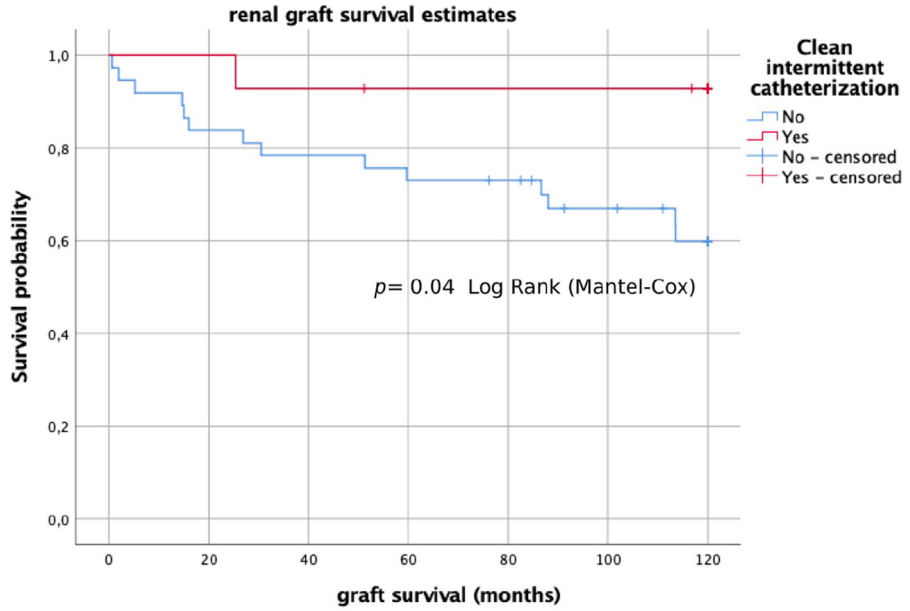

FIGURE 1 | Long-term graft survival (Kaplan–Meier analysis) in patients with CIC and without CIC.

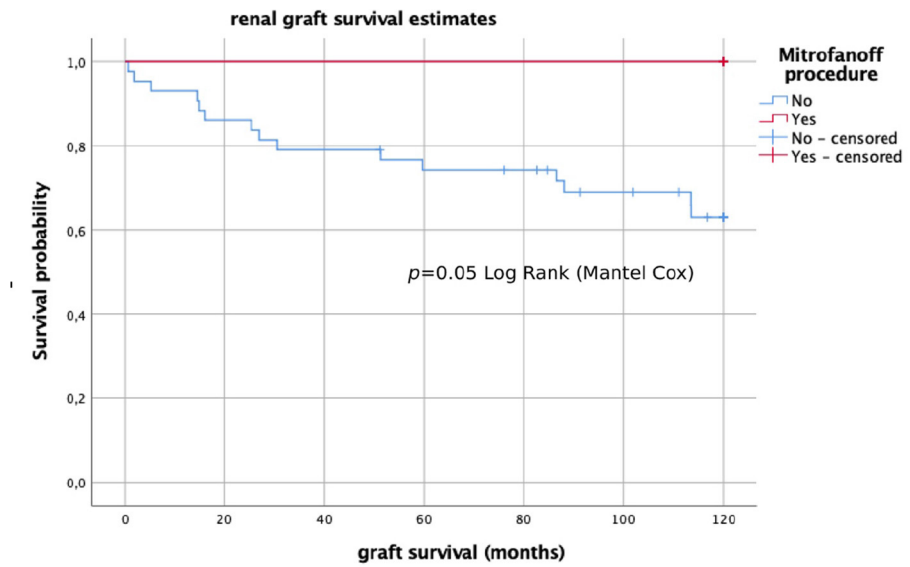

FIGURE 2 | Long-term graft survival (Kaplan–Meier analysis) in patients who did and did not undergo the Mitrofanoff procedure.

Prenatal diagnosis was found in 19 patients (37.3%), while two patients (3.9%) received prenatal treatment (fetoscopic valve ablation). Median age of diagnosis was 0.30 (0–88) months (mean age 4.1 ± 13.8 months). Initial vesicoureteral reflux (VUR) was found in 39 boys (78%) (62% bilateral, 16% unilateral) and initial obstructive ureterohydronefrosis (UHN) in 18 (35.3%) (21.6% bilateral, 13.7% unilateral). Initial treatment consisted in valve ablation in 15 cases (29.4%), pyelo-ureterostomy in 33 (64.7%), and vesicostomy in 3 (5.9%). Seventeen patients (33.3%) had lower urinary tract dysfunction with an altered pattern in the urodynamic studies: 11 (21.6%) low-compliant bladders, 4 (7.8%) myogenic failures, and 2 (3.9%) overactive bladders. The rest of the patients did not present a significant bladder dysfunction in the pre-transplant assessment that required a specific pre-transplant treatment nor in the follow-up. Bladder dysfunction treatment consisted of 11 (21.6%) BAs (nine with ureter and

two with intestine); 14 (27.5%) CICs; 8 (15.7%) Mitrofanoff procedures (six with ureter, two with **Supplementary Material**); and nine (17.6%) cases with anticholinergic drugs. Combination of treatments were achieved in most of the patients: BA+ CIC in 2 patients, BA+ CIC + anticholinergic drugs in 2; BA+ CIC + Mitrofanoff procedure in 4; BA+ CIC + Mitrofanoff + anticholinergic drugs in 2; CIC + Mitrofanoff procedure + anticholinergic drugs in 2; and CIC + anticholinergic drugs in 1. Two patients received only anticholinergic drugs, and one patient only CIC. In nine boys (17.6%) ureteral reimplantation was performed, five (9.8%) received reflux endoscopic treatment, and in 39 (76.5%) nephrectomy was carried out (35.3% unilateral, 41.2% bilateral).

From the total of 51 patients, 34 (66.7%) received pre-transplant dialysis (17.6% peritoneal, 49% hemodialysis), with a mean time in dialysis of 7.1 ± 8.6 months. Thirty-two patients (62.7%) received cadaveric transplants and 19 (37.3%) received living-related donor transplants. Mean age of transplantation was 6.28 ± 5.12 years. Episodes of acute rejection were found in 12 patients (23.6%). Post-transplant urological complications included recurrent urinary tract infections (UTIs) in 16 cases (31.4%); VUR to native kidneys in 16 (31.4%); VUR to the kidney graft in 23 (45.1%); and 1 (2%) ureteral obstruction after catheter extraction. Vascular complications occurred in 2 patients (3.9%): 1 had venous thrombosis with graft recovery after graft immediate re-transplantation, and 1 had an arterial hemorrhage with resolution after surgical exploration. Regarding the immunosuppression regimen, 26 patients (51%) received the initial immunosuppression protocol that consisted of induction with basiliximab or antithymocyte globulin, and triple therapy with mycophenolate mofetil, cyclosporine, and steroids; and 25 patients (49%) received the current protocol in which the cyclosporine was substituted by tacrolimus.

During the follow-up 18 grafts were lost. Mean graft survival was 133.43 ± 83.35 months (11.1 ± 6.9 years). Causes of graft lost were chronic rejection in 14 (27.5%) cases, chronic glomerulopathy in 2 (3.9%), chronic toxicity to calcineurin inhibitors in 1 (2.0%), and death (with graft function) in 1 (2.0%). In analyzing the prognostic factors that may have an influence on long-term graft survival, we identified that patients who underwent CIC or had the Mitrofanoff procedure had a significantly better long-term graft survival after 10 years of follow-up ($p = 0.05$; $p = 0.04$) (**Figures 1**, **2**). To analyze the possible confounders, we categorized patients into groups of CIC and no CIC patients, as well as Mitrofanoff and no Mitrofanoff patients, and we compared all the clinical variables collected in the study (**Tables 1**, **2**). In these comparative analyses, we identified no significant differences in almost all the clinical variables, except for bladder dysfunction in urodynamics, bladder augmentation, anticholinergic drugs, and recurrent symptomatic UTIs. We found more cases of bladder dysfunction (100 vs. 8.1%), more cases with augmentation cystoplasty (71.4 vs. 2.7%; $p = 0.00$), more uses of anticholinergics (50 vs. 5.4%; $p = 0.00$), and more recurrent UTIs (85.7 vs. 10.8%; $p = 0.00$) in patients who underwent CIC compared to without CIC. We also found more cases of bladder dysfunction (100 vs. 20.9%), more cases of augmentation cystoplasty (75 vs. 11.6%; $p = 0.00$), more uses of anticholinergics (50 vs. 11.6%; $p = 0.03$), and more recurrent UTIs (87.5 vs. 20.9%; $p = 0.00$) in patients who had had the Mitrofanoff procedure compared to those who had not.

TABLE 1 | Comparative analysis of clinical characteristics in patients with CIC and without CIC.

Clinical variable	CIC ($n = 14$)	No CIC ($n = 37$)	*P*-value
Prenatal diagnosis	5 (35.7%)	14 (37.8%)	0.89
Prenatal treatment	1 (7.1%)	1 (2.7%)	0.48
Initial VUR	12 (85.7%)	27 (75%)	0.71
Initial obstructive UHN	4 (28.6%)	15 (42.9%)	0.35
Initial valve ablation (no urinary diversion)	3 (21.4%)	12 (32.4%)	0.51
Nephrectomy	13 (92.9%)	26 (72.3%)	0.22
Bladder dysfunction in the urodynamics	**14 (100%)**	**3 (8.1%)**	**0.00**
Bladder augmentation	**10 (71.4%)**	**1 (2.7%)**	**0.00**
Anticholinergic drugs	**7 (50%)**	**2 (5.4%)**	**0.00**
Mitrofanoff	**8 (57.1%)**	**0 (0%)**	**0.00**
Pre-transplant dialysis	8 (57.1%)	26 (70.3%)	0.51
Age of transplantation	7.24 ± 4.83	6.92 ± 4.90	0.84
Living-donor transplantation	5 (35.7%)	14 (37.8%)	1.00
Acute rejection episodes	2 (14.3%)	10 (27.1%)	0.55
Recurrent UTIs	**12 (85.7%)**	**4 (10.8%)**	**0.00**
VUR to native kidneys	5 (35.7%)	11 (32.4%)	0.89
VUR to the kidney graft	7 (53.8%)	16 (51.6%)	1.00
Other post-transplant urological complications	0 (0%)	1 (2.7%)	1.00
Post-transplant vascular complications	1 (7.1%)	1 (2.7%)	0.48
Initial immunosuppression protocol	8 (57.1%)	18 (48.6%)	0.59
Graft lost at the end of the study	**1 (7.1%)**	**17 (45.9%)**	**0.00**

Bold values represent the clinical characteristics with a significant difference in the comparison.

Another factor that influenced long-term graft survival was the type of transplant, with better graft survival in living-donors ($p = 0.03$) (**Figure 3**). We did not identify differences in long-term graft survival regarding other factors such as pre-transplant native kidneys VUR ($p = 0.50$), graft VUR ($p = 0.86$), BA ($p = 0.47$), pre-transplant dialysis ($p = 0.51$), immunosuppression regimen ($p = 0.40$), or post-transplant UTIs ($p = 0.07$).

Only one patient died due to septic shock secondary to a respiratory infection 60 months after transplantation.

DISCUSSION

RT is the therapy of choice in patients with ESRD (11). Most previous studies have demonstrated favorable RT outcomes in

TABLE 2 | Comparative analysis of clinical characteristics in patients who did and did not undergo the Mitrofanoff procedure.

Clinical variable	Mitrofanoff ($n = 8$)	No Mitrofanoff ($n = 43$)	*P*-value
Prenatal diagnosis	3 (37.5%)	16 (37.2%)	1.00
Prenatal treatment	0 (0%)	2 (4.7%)	1.00
Initial VUR	8 (100%)	31 (73.8%)	0.17
Initial obstructive UHN	1 (12.5%)	18 (43.9%)	0.13
Initial valve ablation (no urinary diversion)	2 (25%)	13 (30.2%)	1.00
Nephrectomy	7 (87.5%)	32 (76.2%)	0.67
Bladder dysfunction in the urodynamics	**8 (100%)**	**9 (20.9%)**	**0.00**
Bladder augmentation	**6 (75%)**	**5 (11.6%)**	**0.00**
Anticholinergic drugs	**4 (50%)**	**5 (11.6%)**	**0.03**
Clean intermittent catheterization	**8 (100%)**	**6 (14%)**	**0.00**
Pre-transplant dialysis	4 (50%)	30 (69.8%)	0.42
Age of transplantation	6.90 ± 4.09	7.03 ± 5.00	0.94
Living-donor transplantation	4 (50%)	15 (34.9%)	0.45
Acute rejection episodes	2 (25%)	10 (23.6%)	0.92
Recurrent UTIs	**9 (87.5%)**	**7 (20.9%)**	**0.00**
VUR to native kidneys	5 (62.5%)	11 (27.5%)	0.10
VUR to the kidney graft	4 (57.1%)	19 (51.4%)	1.00
Other post-transplant urological complications	0 (0%)	1 (2.3%)	1.00
Post-transplant vascular complications	0 (0%)	2 (4.7%)	1.00
Initial immunosuppression protocol	4 (50%)	22 (51.2%)	1.00
Graft lost at the end of study	**1 (12.5%)**	**17 (39.53%)**	**0.01**

Bold values represent the clinical characteristics with a significant difference in the comparison.

patients with PUV comparable to non-uropathic patients, with no major impact of bladder dysfunction associated with PUV in long-term graft survival (4–10). However, the surgical procedures that may be performed to optimize bladder function are still a controversial topic. Some authors have described better RT outcomes in patients with limited surgical procedures prior to transplantation (early vesicostomy and delay valve ablation, or primary valve ablation), and worse results in those patients with extensive surgical interventions (nephrostomy, pyelostomy, ureterostomy, urethral dilatation, ureteral reimplantation, or BA) (10, 12). On the other hand, Lopez Pereira et al. did not find any significant differences in RT outcome in patients with or without BA (13), and other studies found good RT outcomes in patients with augmented bladders (14–18). Also, Rickard et al. (19) identified similar RT outcomes in patients who did and did not undergo a Mitrofanoff procedure.

In the present study it has been found that patients with PUV that received a Mitrofanoff procedure or that accomplished CIC as treatments of their bladder dysfunction had a better long-term RT outcome. It is well-known that bladder dysfunction contributes to native renal function deterioration in patients with PUV (9, 20). Furthermore, bladder dysfunction characteristics tend to change over time as the patient grows, turning from overactive bladders in the first years of life to a myogenic failure pattern with an increase in post-voiding residuals (21, 22). It is probable that patients that accomplish CIC *via* urethra or *via* the Mitrofanoff procedure have fewer possibilities to develop these behavior changes and to have a better urodynamic pattern in the follow-up (9). Rickard et al. (19) found that implementing CIC *via* Mitrofanoff was associated with a delay in native renal function deterioration for a longer period, and that dialysis onset occurred at an older age compared to patients without Mitrofanoff. They postulated that this delay in renal failure was due to an improved compliance with CIC *via* Mitrofanoff and not *via* a sensate urethra. This argument may also be inferred to graft function, in which deterioration may be diminished with a better CIC compliance. However, in our study in most patients without Mitrofanoff and without CIC that had a graft failure, the cause of lost was mainly a chronic graft rejection. Despite the fact that bladder dysfunction was not identified as the main cause of the graft lost, it is probable that it may have contributed to renal function worsening in the very long-term. When analyzing the possible confounder factors, we did not identify any significant difference for most of the variables, except for BA, need of anticholinergic drugs, or recurrent UTIs, which were more frequent in the groups of CIC and Mitrofanoff procedure. These variables are considered unfavorable and we would have expected worse results in these group of patients. But we found the opposite, favorable results in these groups of patients with better long-term graft survival.

Regarding BA, in our study we did not find significant differences in long-term graft survival comparing patients with and without augmentation cystoplasty. This result is concordant with some previous studies about this topic (23). In the literature, there are not clear criteria to indicate BA in PUV patients (7, 14, 17, 24). Some authors recommend BA before RT to reduce the risk of graft function deterioration due to valve bladder syndrome, and also for technical reasons, to avoid the risk of graft pedicle lesions (14, 16). Other authors recommend performing BA after transplantation, because they argue that bladder dynamics may change after transplantation and BA may not be needed in some cases, also there is a potential increased risk of UTIs, and BA may preclude peritoneal dialysis (18, 25, 26). In our study, the number of patients that required BA was small. It may be due to the fact that early diagnosis and treatment of patients with posterior urethral valves in the recent years has diminished the need to performed a BA procedure, and that high-pressure low-compliant bladders treated early in life usually improve with an adequate CIC.

In respect of UTIs, no significant differences was found in 10-year graft survival in patients with and without post-transplant UTIs. But a tendency toward better results was

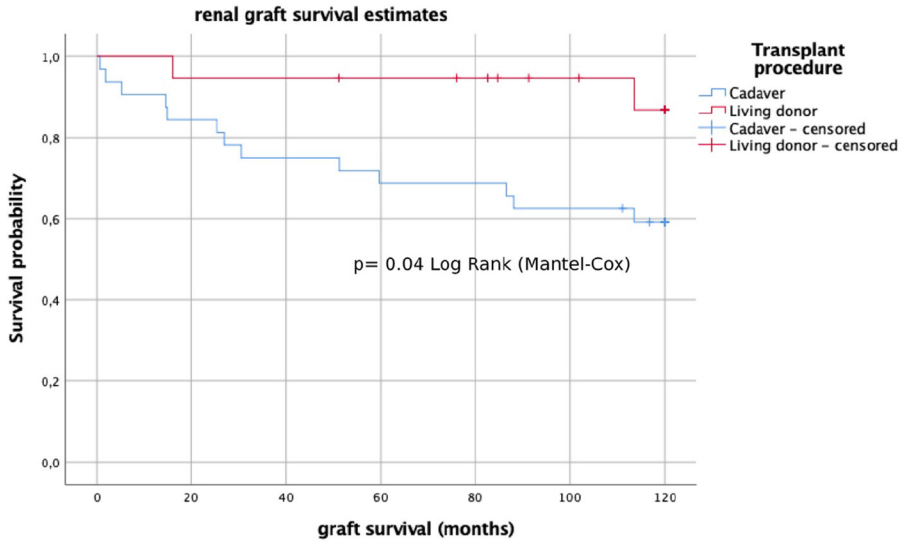

FIGURE 3 | Long-term graft survival (Kaplan–Meier analysis) of living-donor and cadaveric transplants.

found in patients with UTIs. This surprising finding could be explained by the fact that patients with UTIs had a closer follow-up with frequent reviews in the outpatient clinic, which may have optimized bladder dysfunction treatment and immunosuppression treatment. Several authors found increased prevalence of UTIs in transplanted patients with PUV, especially in patients with BA (13, 18, 27), but despite the increased risk of recurrent UTIs in these patients, most studies reported no direct contribution of UTIs in graft loss (10, 13, 23, 28, 29).

Another important finding of our study was that living-donor transplants achieved better long-term graft survival compared to cadaveric grafts. This is concordant with previous literature about this topic, in which it has been found that a living donation has a better RT outcome due to different reasons: this type of transplant involves better quality grafts, usually better cold ischemia times and better HLA matching, and allows for preemptive transplantation (30–34).

Limitations of the study include the retrospective nature of it and the small size of the patients analyzed. The groups of patients treated with CIC and the Mitrofanoff procedure were reduced and their favorable results could be obtained due to other confounding factors not identified in this study. Nevertheless, in spite of these limitations, some facts can be underlined: procedures to optimize bladder function in PUV, such as CIC (with or without the Mitrofanoff procedure) and BA, were not related to a worse long-term graft survival despite of being associated with more frequent UTIs. However, to confirm these findings, further multicenter studies with a higher number of patients must be undertaken.

CONCLUSIONS

Optimal bladder dysfunction treatment, including CIC with or without a Mitrofanoff procedure, might result in a better long-term graft survival in patients with PUV. These procedures were not related to a worse RT outcome in spite of being associated with more frequent UTIs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Ética Hospital Universitario La Paz. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MA performed the study design, achieved the restrospective review of patients, and wrote the manuscript. CG-V performed the statistical analysis and help with the result interpretation and manuscript writing. LE helped to collect the data from the clinical charts of patients and helped in the manuscript writing. MM-U help to collect the data from the clinical charts and supervised the manuscript writing. PL-P is the senior and last author and he helped with the study design and supervised the manuscript writing. All authors discussed the results and commented on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.646923/full#supplementary-material

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