

Peer Review File

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Reviewer A

Comment 1: *what was defined as success - no further intervention?
in which case one in each group is on ISC - this should be a failure*

Reply 1: One patient in each group was on intermittent self-catheterization, prior to the surgery of resection and end-to-end anastomosis, therefore not considered as evidence of recurrence. The definition of success was included in the revised version of the manuscript.

Changes in the text: Page 4, lines 82-83 reads: “Surgical success was defined as absence of symptoms of lower urinary tract obstruction and no instrumentation after the resection.”

Page 6, lines 140-142 reads: “One patient in each group was on intermittent catheterization prior to the resection and end-to-end anastomosis.”

Comment 2: *Three patients (11.5%) of the non-recurrent patients and two (20%) of the recurrent patients have had a previous urethroplasty ($p>0.05$). - what was the type of urethroplasty - should they be excluded as previous surgery will make the stricture more dense and fibrotic?*

The final numbers will need to be adjusted accordingly.

Reply 2: These urethroplasties were on a different location of the urethra and therefore, the specimens from the resection and end-to-end anastomosis were not considered recurrences.

Changes in the text: Page 6, lines 137-139 read: “Three patients (11.5%) of the non-recurrent patients and two (20%) of the recurrent patients have had a previous urethroplasty ($p>0.05$) in a different location, and therefore, the current resection was not considered a recurrence.”

Reviewer B

Comment 1: *Surgical information is lacking*

Patients who underwent end-to end anastomosis for urethral stricture were enrolled in this study. Previous reports indicate that stricture length was a predictor of recurrence. Please describe the length of stricture as median (range) in Table 1. If possible, in addition to the length of resected tissue, please describe actual length of stricture measured on preoperative urethrography as well.

Reply 1: This information was added to the revised table 1. The measures reported in the initial submission were indeed the measurements of the urethrography.

Changes to the text:

Size of stricture measured in preoperative urethrography (cm)	1.65 (range 0.5-2.0)	1.5 (range 1.0-2.0)	NS
Length of resected segment measured as part of the pathology report (cm)	2.78 (range 1.3-4.8)	2.59 (range 1.0-5.0)	NS

Comment 2. *Histopathological information of the resection margin is crucial. The authors investigated the histopathological features of the urethral stricture itself, but what matters should be adjacent normal tissue at anastomosis. Please describe histopathological finding at normal urethra adjacent to stricture site. Further, information on resection margin is important. Whether or not the stricture sites were resected with negative pathological margin should be described.*

Reply 2: We thank the reviewer for bringing up this very important point. Unfortunately, this information is not able to be obtained at this point given that the specimens were not grossed with designation of margins. We have included this as a limitation of the study in the revised version of the manuscript.

Changes to the text: Page 10-11, lines 251-253 reads: “Additionally, this study does not include histopathology of the normal urethra adjacent to stricture site and/or information on the resection margins. It is unclear whether fibrosis and/or inflammation, or their absence, have an impact on the postsurgical outcome.”

Comment 3. *Significant difference of uroflowmetry results were observed postoperatively*

There is a significant difference of uroflowmetry between two groups. Importantly, uroflowmetry Qmax did not improve in the recurrent group. (Voided volume is not important in this setting) This could mean that the strictures were not removed by operation in the recurrent group. Please discuss about this in the discussion part. Exclusion of the possibility of incomplete operation is a prerequisite for further histopathological discussion.

Reply 3: This is another excellent point. We have included a paragraph in the discussion

acknowledging this finding and emphasizing the possibility that symptomatic recurrence could be due to incomplete resection of the stricture.

Changes to the text: Page 10-11, lines 241-246 reads: “Notably, the recurrent group did not show an improvement of the postoperative uroflow compared to the pre-surgical measure. This is markedly different to the non-recurrence group that did experience significant increase in the uroflow post-surgically. This raises the possibility that the stricture was not completely removed during the excision. A follow-up study analyzing only patients with significant improvement in the uroflow could help to better determine the importance of histologic evaluation in predicting recurrence.”

Comment 4: *The authors mention that paucicellular fibrotic plaque is a predictor of urethral stricture recurrence. What makes the fibrosis paucicellular? How more collagen deposited in the extracellular matrix? Repeated medical procedure? Insufficient blood supply? Please discuss the cause of this pathology in the discussion part. If the authors believe the deposition of collagen affect the paucicellularity, collagen staining by Masson’s trichrome staining or Sirius red staining would be informative.*

Reply 4: Thank you very much for these questions. The cellularity of the fibrous area was measured as a number of the stromal cells in 5 HPFs (40x magnification) in the areas with the maximal stromal cellularity. The paucicellular fibrotic plaque was defined as dense fibrotic tissue, characterized by the packs of collagen fibers, strongly positive for Trichrome-blue stain (Masson’s trichrome staining), while the average number of the stromal cells was 12 ± 1.8 per HPF in this group. This information was presented in the “methods” and in the “results” sections of the original submission and edited in the revised version emphasizing the points raised by the reviewer.

Changes to the text:

1. Page 4, lines 92-93 reads: “The cellularity of the fibrous area was measured as a number of the stromal cells in 5 HPFs (40x magnification) in the areas with the maximal stromal cellularity.”
2. Page 4, line 97 reads: “Trichrome-blue stain (Masson’s trichrome stain) was performed to evaluate the extent of fibrosis below the...”
3. Page 5, lines 99 reads: “The fibrotic plaque was defined as a dense fibrotic tissue, characterized by the packs of collagen fibers, strongly positive for Trichrome-blue stain, as previously described (11).”

Minor

Comment 5: *Line 130: Abbreviation for DVIU should be spelled out.*

Reply 5: This abbreviation was spelled out as suggested.

Changes to the text: Page 5, now line 136 reads: "...group had at least one direct vision internal urethrotomy (DVIU) or dilatation procedure prior..."

Comment 6: *Line 144 and line 149: stoma → stroma*

Reply 6: These typographical errors have been corrected in the revised manuscript.

Changes to the text: Now lines 152 and 157 read: "number of stromal cells was 42.8 ± 3.0 per HPF"; and "The average number of stromal cells was 12 ± 1.8 per HPF in this group."

Comment 7: Line 226: "which this as a potential confounder when trying to identify predictors of 227 recurrence." Is this sentence grammatically correct? It is difficult for me to understand what the authors mean. Please check the sentence.

Reply 7: We thank the reviewer for this observation. We have decided to exclude this sentence from the revised version of the manuscript.

Changes to the text: The sentence was eliminated from the revised version of the manuscript, which now reads (line 231): "In our study, we only had four patients who were older than 60 years old, including 1 with recurrent disease and 3 with non-recurrent stricture, which is insufficient to arrive at any conclusion as how age could impact the outcome. While there are multiple reports suggesting older individuals have a worse outcome after urethroplasty, these are thought to be related to a higher rate of comorbidities and worse blood supply."

Comment 8: *Table 1: Information of IPSS, uroflowmetry should be added in Table 1, because the authors only mention these results in the main text.*

Reply 8: As suggested by the reviewer, we have added IPSS and uroflowmetry to the revised version of table 1.

Changes to the text: The information can be found at the bottom of the revised table 1:

Functional assessments			
IPSS (average)	17.4	22.14	0.09
Pre-surgical	9.95	11.66	NS

uroflow (mL/s)			
Post-surgical uroflow (mL/s)	28.89	11.67	0.001

Reviewer C

Comment 1: *Follow up period after urethroplasty is very important and it is not very clear about the follow up duration of each group in the study.*

Reply 1: This information can be found in the “results” section of the manuscript, line 123, where it reads: “The average follow-up time was 68.2 months for the recurrent group (range 28-184 months) and 53.0 months for the non-recurrent group (range 25-134 months).”

Changes to the text: None

Comment 2: *The authors themselves noted in their limitations they noted paucicellular fibrosis in a large number of even the non-recurrent stricture disease group. Although the authors demonstrated statistically significant difference of paucicellularity between the recurrent and non-recurrent groups, it is doubtful whether this is true if we study a larger number of these patients. It will be interesting to study the role of cellularity of the fibrosis in all types of urethroplasties (even in those requiring graft substitution).*

Reply 2: Thank you very much for this comment. We have added these points to the discussion in the revised manuscript.

Changes to the text: Page 11, lines 254-258 reads: Our study showed a significant difference between two groups, since all patients with recurrent strictures and only 53.8% non-recurrent cases showed dense paucicellular fibrotic plaques. However, it is not clear whether the same results would be achieved in a larger cohort. Moreover, the occurrence of dense paucicellular fibrotic plaques should be studied in all types of urethroplasty. Therefore, a prospective study with a larger number of patients is necessary to improve methodological approach and test the hypothesis.