

Rethinking Fistula Preservation in Anorectal Malformation Surgery: A Histopathological Perspective

Preeti Agarwal¹ Shubhajeet Roy² Anand Pandey³ Nirpex Tyagi³ Nitin Pant³ Piyush Kumar³
Jiledar Rawat³

¹ Department of Pathology, King George's Medical University, Lucknow, Uttar Pradesh, India

² Faculty of Medical Sciences, King George's Medical University, Lucknow, Uttar Pradesh, India

³ Department of Pediatric Surgery, King George's Medical University, Lucknow, Uttar Pradesh, India

Address for correspondence Anand Pandey, MS, MCh, Department of Pediatric Surgery, King George's Medical University, Lucknow 226003, Uttar Pradesh, India (e-mail: dranand27@rediffmail.com).

Eur J Pediatr Surg 2025;35:15–21.

Abstract

Objective This study aims to assess the histopathological characteristics of the fistulous tissue in males with anorectal malformation (ARM) to determine its suitability for use in constructing a neanus.

Methods This prospective observational study included male ARM patients with recto-urethral fistula. All other types were excluded. The tissue specimens comprised 0.5 to 2.0 cm of the most distal part of the rectal pouch and the fistulous tissue. Hematoxylin and eosin-stained sections were prepared. Histological features, viz. internal sphincter, anal gland and crypts, ganglion cells, presence of subepithelial fibrosis, thickened nerve trunks, and metaplasia, were evaluated.

Results Of 65 patients of ARM admitted, 24 met the inclusion criteria. Gross and microscopic internal sphincter was not found in any sample. The anal mucosa was visualized in all samples; however, crypts were irregular and distorted architecture was seen in 4 (16.67%) samples. Urothelial metaplasia was identified in 10 (41.67%) samples. Adequate ganglion cells were seen only in 6 (25%) samples. Significant submucosal fibrosis was seen in all samples. Thickened nerve trunks were identified in 4 (16.67%) samples.

Conclusions All normal anal histological features could not be found together in the fistula tissue. With the absence of normal features, such as internal anal sphincter muscles, and the presence of abnormal histopathological features, such as subepithelial fibrosis, thickened nerve trunks, and metaplasia, there are high chances of abnormal bowel function like constipation if the fistulous tissue is used for neanus construction. It may have an impact on the quality of life of patients.

Keywords

- ▶ anorectal malformation
- ▶ fistula
- ▶ histopathology
- ▶ PSARP
- ▶ surgery

received
March 20, 2024
accepted after revision
August 13, 2024
accepted manuscript online
August 16, 2024
article published online
September 9, 2024

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Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI <https://doi.org/10.1055/a-2389-7550>.
ISSN 0939-7248.

Introduction

Anorectal malformation (ARM) is an important congenital condition having a wide range of clinical presentations.¹ Among its varied clinical presentations in male patients, communication between a partly formed anorectum and urethra known as rectourethral fistula is considered to be the most common clinical presentation.¹ A clear understanding of its various types and the surgical intervention for them is very important. A poorly performed surgery without understanding the type of presentation may lead to bowel incontinence, constipation, and even urinary problems. These problems may persist for life.

The surgical procedure for ARM with rectourethral fistula is called posterosagittal anorectoplasty (PSARP) in which the fistulous communication—the rectourethral fistula—is separated from the urethra and anorectoplasty is performed. There are divergent opinions on using the fistula part for anoplasty. Some consider the fistula to be an anal canal having a normal physiological or anatomical feature.^{2–5} These studies believe that the fistula having features of an anal canal when incorporated into the anoplasty will make the neoanus like a normal one that will be able to work like the normal anal canal, which can sense liquid or gas. Thus it may help in achieving a continence like a normal child.

The fistula is believed to be an abnormal tissue by other workers.^{6–8} Based on their studies, they found fibrosis and other abnormal histopathological features in the fistula and believed that using it for anoplasty causes constipation in these children. Since the number of studies in this field is limited, the researchers still face a dilemma in preserving the fistula.

Good and fair continence is the main surgical outcome objective which is intended. The use of the fistulous tract for reconstructive proposes in the first case scenario, where the fistula is considered to be the unremarkable physiological and anatomical anal structure, makes the tract tissue appropriate for reconstruction purposes and theoretically leads to the desired outcome. If the tissue is not normal either anatomically or physiologically, it will lead to momentous postsurgical outcomes if utilized for reconstructive purposes. Anatomical and histomorphological examination can provide us with significant information in this regard.

This study was conducted to assess the histopathological characteristics of the fistulous tissue in males with ARM to determine its suitability for use in constructing a neoanus.

Materials and Methods

It was a prospective observational study conducted in the department of pediatric surgery in collaboration with the department of pathology from August 2022 to January 2024 of a medical university. It was approved by the Institutional Ethics Committee (Reference code- 117th ECMIIA/P25). We followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines endorsed by the EQUATOR Network for conducting this study.

We followed the Krickenbeck classification system of ARM in this study. All male ARM patients having rectourethral fistula of bulbar or prostatic type were included in it. They all underwent the PSARP procedure. The patients undergoing other procedures or other clinical variants of ARM were excluded.

Evaluation of the Patients

During the study period, all patients of ARM were evaluated. Of these, perineal fistula or those having bladder neck fistula with or without associated pouch colon were excluded. Perineal fistula was managed by anoplasty. Bladder neck fistula without pouch colon was managed by abdominal perineal pullthrough, and associated pouch colon was managed by coloplasty and abdominal perineal pullthrough. The patients having rectourethral fistula of bulbar or prostatic type were evaluated for their age at presentation and body weight. After anesthetic fitness, they underwent PSARP.

Operative Procedure and Sample Collection

During the PSARP, the fistula site was identified. After careful dissection and separation of the rectum from the urethra, the tissue specimens consisting of 0.5 to 2.0 cm of the most distal part of the rectal pouch up to the recto-urethral connection were obtained. For easy identification, the proximal end was marked by a silk suture. If a sample got autolyzed due to any reason, that patient was excluded from the study.

Histopathological Analysis of the Samples

The properly threaded and labeled received specimen was fixed in 10% neutral formalin and sectioned carefully. Transverse sections from the proximal end and distal end along with at least two longitudinal sections from the fistulous tract were processed. These sections were objectively evaluated for the type of lining mucosa, the extent of inflammation, and fibrosis in the submucosa and muscularis propria. Morphological presence or absence of sphincteric muscle and ganglion cells in submucosal and myenteric plexus was evaluated. Status of nerve trunks whether it was thickened or not was also included. Its criteria considered for evaluation was more than two submucosal nerves and more than 40 μm diameter in a 400 \times field.⁹ The anorectal mucosa if present was observed for its architecture, type and extent of laminar inflammation, and presence or absence of cryptitis or abscesses. Any other significant morphological finding if seen was also recorded in the Excel sheet. Both inflammation and fibrosis were graded in mild, moderate to marked.¹⁰

The parametric data are presented as mean and standard deviation.

Results

The total duration of the study was 18 months. During this period, 64 patients of ARM were managed. Of these, 21 had perineal fistula, 12 patients had bladder neck fistula, and 4 had associated pouch colon. Twenty-seven patients having rectourethral fistula of bulbar or prostatic type (according to the Krickenbeck classification) underwent PSARP. The

Table 1 Details of the histopathological findings in patients operated during the course of the study

Type of ARM	Age (in months)	Length of tissue specimen	Length of tissue specimen	Ganglion cells present	Transitional epithelium	Anal glands	Anal crypts	Subepithelial fibrosis	Thickened nerve trunks ^a	Miscellaneous aberrations in distal rectal pouch and fistula region
RPUF	13	2 cm	2 cm	No	+	+	-	+++	Not seen	Section shows hemorrhage necrosis
RBUF	30	1.2 cm	1.2 cm	No	+	+	-	++	Present	Mixture lining with base as transitional and upper layer of columnar cells seen
RPUF	24	0.8 cm	0.8 cm	No	-	+	-	++	Not seen	Mixture lining with base as transitional and upper layer of columnar cells seen
RPUF	84	0.5 cm	0.5 cm	No	-	+	-	+	Not seen	Largely denuded mucosa with more of edema
RPUF	8	2 cm	2 cm	Yes	-	+	-	+++	Not seen	Lymphoid aggregates multiple
RBUF	18	0.5 cm	0.5 cm	No	-	-	-	+	Not seen	Eroded mucosa
RPUF	8	1 cm	1 cm	Yes	+	+	-	+	Not seen	Lymphoid aggregates multiple
RPUF	12	2 cm	2 cm	No	+	+	Few and irregular	++	Not seen	Mixture lining with base as transitional and upper layer of columnar cells seen
RPUF	12	1.5 cm	1.5 cm	No	+	+	Few and irregular	++	Not seen	Mixture lining with base as transitional and upper layer of columnar cells seen
RPUF	36	1 cm	1 cm	Yes	-	+	-	++	Present	Eroded mucosa
RPUF	14	0.5 cm	0.5 cm	No	-	+	-	++	Not seen	Largely denuded mucosa with more of edema
RBUF	66	0.5 cm	0.5 cm	No	-	+	-	++	Not seen	eroded mucosa
RBUF	10	1 cm	1 cm	No	-	+	-	+++	Not seen	Section shows hemorrhage necrosis
RPUF	11	1.5 cm	1.5 cm	No	-	+	-	+++	Not seen	Section shows hemorrhage necrosis
RPUF	12	2 cm	2 cm	No	-	+	-	++	Not seen	Section shows hemorrhage necrosis
RPUF	9	0.9 cm	0.9 cm	Yes	+	+	Few and irregular	+	Not seen	Mixture lining with base as transitional and upper layer of columnar cells seen
RBUF	60	1.5 cm	1.5 cm	No	+	+	Not seen	+++	Present	Mixture lining with base as transitional and upper layer of columnar cells seen
RPUF	18	1 cm	1 cm	No	-	+	-	+	Not seen	Lymphoid aggregates multiple
RPUF	24	0.8 cm	0.8 cm	No	-	+	Few and irregular	+	Not seen	Section shows hemorrhage necrosis
RPUF	20	2 cm	2 cm	No	+	-	-	+	Not seen	Lymphoid aggregates multiple
RPUF	9	1.5 cm	1.5 cm	Yes	-	+	-	++	Present	Section shows hemorrhage necrosis

(Continued)

Table 1 (Continued)

Type of ARM	Age (in months)	Length of tissue specimen	Length of tissue specimen	Ganglion cells present	Transitional epithelium	Anal glands	Anal crypts	Subepithelial fibrosis	Thickened nerve trunks ^a	Miscellaneous aberrations in distal rectal pouch and fistula region
RBUF	10	1 cm	1 cm	No	-	+	-	++	Not seen	Mixture lining with base as transitional and upper layer of columnar cells seen
RBUF	14	1.2 cm	1.2 cm	Yes	-	+	-	+++	Not seen	Mixture lining with base as transitional and upper layer of columnar cells seen
RPUF	12	1 cm	1 cm	No	-	+	-	++	Not seen	Mixture lining with base as transitional and upper layer of columnar cells seen

Abbreviations: RBUF, rectobulbar urethral fistula; RPUF, rectoprostatic urethral fistula.

^aThickened nerve trunk if the diameter was more than 40µm in a 400× field.

specimen was autolyzed in three of them; hence, they were excluded from the study. Thus, 24 patients were included in the study. Of these, 17 had prostatic fistula while the remaining 7 had bulbar fistula (→Table 1). The mean age of the patients was 22.25 ± 20.08 months (range: 8–84 months). All these patients were managed by PSARP during this stage of surgery. The mean weight of the patients was 12.12 ± 3.65 kg (range: 8.5–24 kg). The length of the resected specimen was 1.20 ± 0.52 cm (range: 0.5–2 cm).

Both on gross examination and microscopy, internal sphincter was not identified in any case. Anorectal mucosa was seen in all cases at the proximal end. Unremarkable crypt architecture was seen in 20 (83.33%) samples; however, distorted architecture was identified in 4 (16.67%) samples. Mucosal erosions and large lymphoid aggregates were also seen in 2 (8%) and 4 (16.67%) cases, respectively. Adequate ganglion cells were seen in only 6 (25%) samples.

Fibrosis of the submucosa and muscularis layer in the form of the presence of spindle cell proliferation and deposition of hyaline-type material along with splaying of muscle fibers was identified in all the studied samples (→Fig. 1A). The fibrosis was moderate to marked in 16 (66.67%) samples. Fibrosis was seen in the proximal, distal, and even the middle part of the resected tissue, which were sampled separately.

Overall, the anorectal mucosa seen in all cases displayed one or the other morphological abnormality, fibrosis, and no identifiable sphincter was seen in all with added features of either crypt architecture distortion, aganglionosis, or hyperplastic nerves. There was metaplastic epithelium that had the features of urothelium and not like the stratified columnar epithelium (→Fig. 1B, C) of the anal canal. There was the presence of thickened nerve trunks in 16.67% (4/24) at the distal end of the studied samples (→Fig. 1D). Interestingly, we noted urothelial metaplasia in the anorectal mucosa in 10 (41.67%) samples (→Fig. 2). Overall, the fistula-site histopathology lacked a well-formed anal canal in any of the samples (→Table 1). The presence of fibrosis, abnormal muscle architecture, metaplasia, and hyperplastic nerves suggests that the fistulous tissue is not normal, and using it for anoplasty may not fetch the desirable results for future normal bowel habits.

The proximal end of the specimen had the histopathology of normal rectal mucosa in all those specimens where the total length was equal to or more than 1.5 cm ($n = 9$, 37.5%). This finding suggests that the fistula up to proximal 1.5 cm of bowel tissue may be abnormal, and it may need excision for normal bowel to be used for anoplasty.

Discussion

Incorporating the fistula site in anorectoplasty has been a matter of debate for a long time. The normal anal canal has three types of epithelium⁵—simple columnar epithelium (colorectal zone), stratified columnar epithelium (anal transitional zone), and stratified squamous epithelium (squamous zone). There is a transition of epithelium from proximal to distal ends.

Since 1980, various studies have claimed to note normal anal canal histology in the fistula region. In the animal and

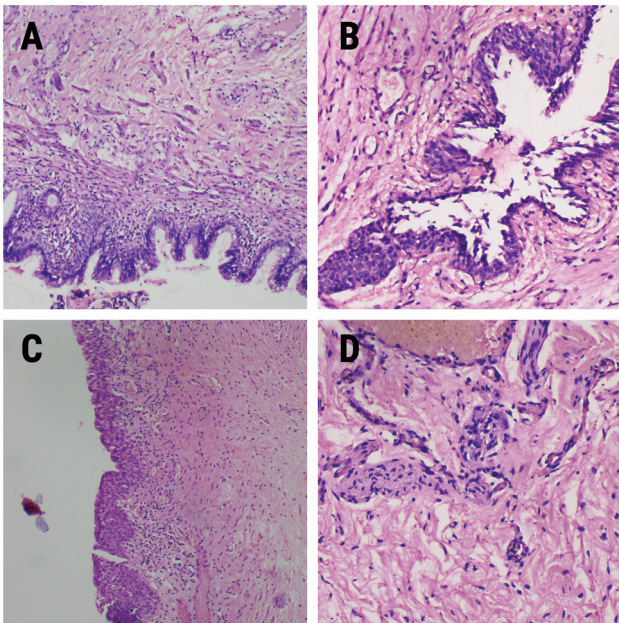


Fig. 1 Section shows fibrosis of the wall of the rectal area with splaying of muscle fibers in (A), with urothelial lining displaying focal columnar metaplasia in (B). Urothelial lining is seen in section (C). In section (D) a thickened nerve is seen with ganglion cells in the submucosal plexus (H&E; A and C $\times 100$; B and D $\times 200$).

human experiments, it was suggested that it should be regarded as an ectopic anal canal.^{2,4} There are studies where claims of manometric assessment of the fistula have been reported.^{3,11,12} It is important to note that most of these studies had predominantly female patients with a vestibular fistula or rectoperineal fistula.^{11,12} One study has no description of the type of ARM evaluated.² It has used the term “rectourgenital fistula.” Hence, the study population is not clear. Only one study evaluated six patients with rectourethral fistula to suggest the presence of a sphincter in the fistula region.³ In a recent study,⁵ the authors evaluated histopathology of the fistula region in both male and female patients. They found columnar and stratified columnar epithelium in the rectourethral fistula ($n = 17$) patients.

The opponents of preserving the fistula rely on the absence of normal histopathology in the fistula region.^{6–8,13,14} In these studies, the authors could not find evidence of good internal sphincter or ganglion cells. The absence of ganglia in the rectourethral fistula and its use for anoplasty was found to be associated with constipation.¹⁵ Besides, the presence of fibrosis and hypertrophic nerve bundles suggests against the preservation of the fistula. One study assessing rectoperineal fistula also noted that the distal 3 cm of the fistula has abnormal histopathology and suggested its excision.¹⁶ A study, specifically analyzing

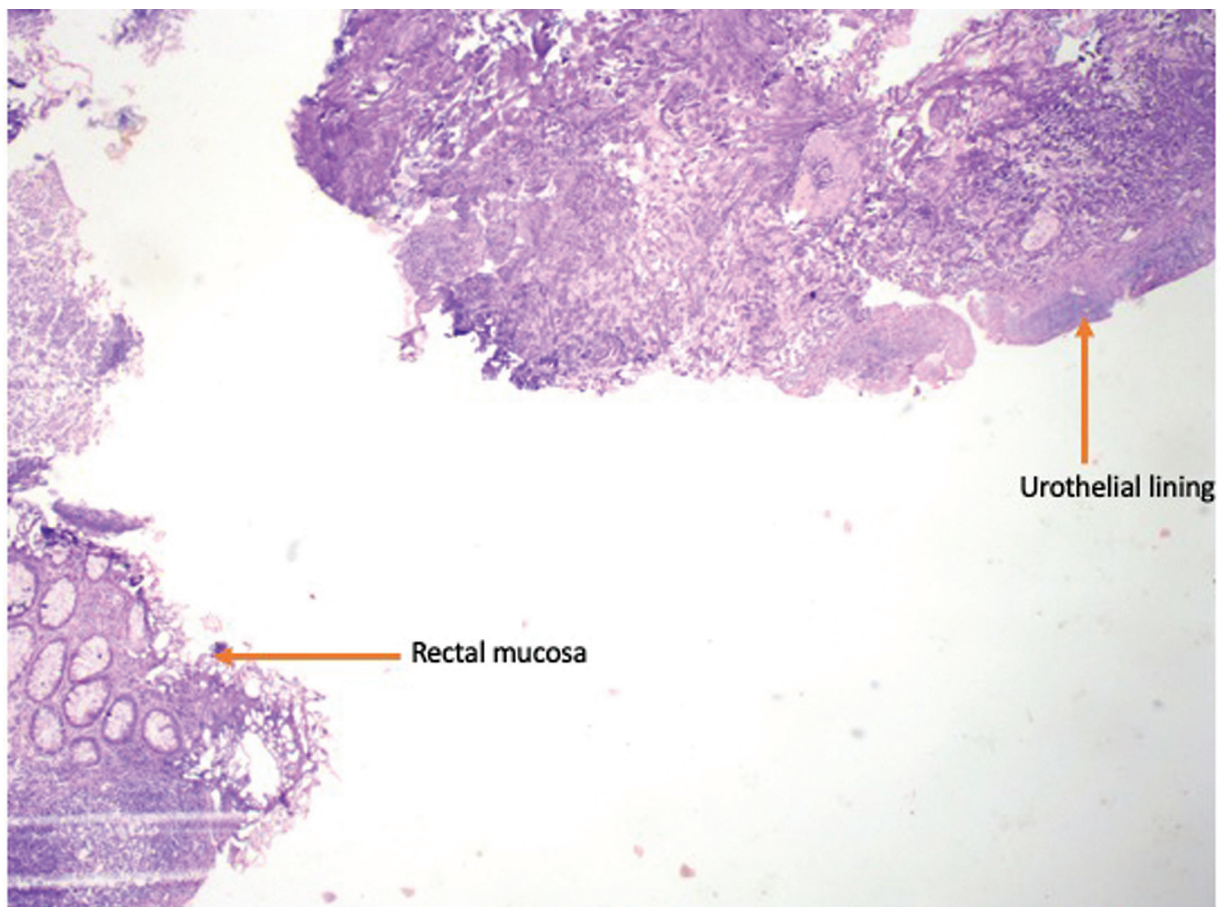


Fig. 2 Section shows scanner view of the biopsy tissue displaying rectal mucosa at one end along with urothelial mucosa at another end both marked with an arrow (H&E $\times 40$).

rectourethral and rectobulbar fistula, noted that the fistula has abnormal histological as well as immunohistochemical findings.¹⁷

Our study is more in line with those who did not find normal histopathology. From the point mentioned above, many papers claiming normal anal histopathology had predominantly female patients having vestibular fistula or rectoperineal fistula. Our study population was exclusively male patients with rectourethral fistula of prostatic or bulbar type. Some studies where manometric or histopathological evidence was found had pigs as their study population.^{4,18} Extrapolating these findings to the human population may not have the same results.

In the present study, there was the presence of urothelium in 10 patients. We believe that it was a metaplastic change in the fistula due to exposure to the urine via urethral communication. Besides, subepithelial fibrosis was uniformly present in all the samples, which is not present in normal tissue. Metaplasia is an abnormal change in a tissue. Taking metaplasia and fibrosis together, it seems unlikely that the neanus having these abnormalities will function normally. Constipation has been found in the patients where this tissue was used in anoplasty.¹⁴ It is pertinent to note that the fistula communication to the urethra is a narrow channel. How this narrow channel adds to the continence if used for creating a wide anus at the proposed anal site during PSARP is a matter of imagination. Since this study had a specific set of patients, the generalization of its outcome on other subtypes of ARM would be difficult at this point.

From the discussion made above, a query may arise that metaplasia may not occur if PSARP is performed early in life or if primary PSARP (single-stage surgery)¹⁹ is performed. There is one study where PSARP was performed as either staged or as a primary procedure.⁶ All the abnormal changes including metaplasia were noted in this study also. It suggests that metaplastic changes can be seen in the early part of life.

The present study may be used as a template for future studies including analysis of the rectovesical fistula in whom the abdominoperineal pull-through procedure is used. Besides, using immunohistochemistry may throw additional light on the histopathological characteristics of the fistulous tissue. We were not able to see any dentate line in our specimens. Other studies may work on this line too.

The limitation of this study is no comparison with single-stage surgery to confirm the claim made above; however, since we prefer three-stage surgery in our center, it was not possible to get this subset of patients. For the same reason as performing three-stage surgery, we are not able to comment upon the functional outcome of the patients during this study. The sample size was small due to the single-center study. We avoided selection bias by including a particular group of patients undergoing a specific procedure. We did not look for the presence of Hirschsprung's disease (HD) in the study population. The association is very rare. As per the literature, 2.4% of HD patients may have an associated ARM. In contrast, only 0.17% of ARM patients have HD.²⁰

In conclusion, all normal anal histological features could not be found together in the fistula tissue. With the absence

of normal features, such as internal anal sphincter muscles, and the presence of abnormal histopathological features, such as subepithelial fibrosis, thickened nerve trunks, and metaplasia, there are high chances of abnormal bowel function like constipation if the fistulous tissue is used for neanus construction. It may have an impact on the quality of life of the patients. Further studies in this field may substantiate our efforts.

Conflict of Interest

None declared.

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