Histopathologic profile of esophageal atresia and tracheoesophageal fistula

Mubarak M. Al-Shraim[a], Ashraf H.M. Ibrahim[b], Talal A. Malki[c] and Nader A. Morad[d]

Purpose **Few reports are available in the literature on the histology of the congenital atretic esophagus in humans. Histologic abnormalities including congenital esophageal stenosis (CES) may contribute toward the abnormal esophageal motility after successful repair of esophageal atresia (EA) and tracheoesophageal fistula (TEF). The main aim of this study is to document the histopathologic profile in cases of EA.**

Methods **One hundred and nineteen surgical specimens were collected from 69 consecutive EA patients who underwent surgical repair at the Aseer Central Hospital, Abha, and Armed Forces Hospital Southern Region, Saudi Arabia, from May 1999 through May 2009. This included 62 cases with EA and distal TEF, five cases of pure EA, and two cases of N-type TEF. Samples from tips of the upper pouch (UP), lower pouch (LP), and mid portion of the TEF were preserved in 10% formalin, sectioned, and stained with hematoxylin and eosin.**

Results **The combined three elements of tracheobronchial tissue were observed in only three LP specimens. Gastric-type mucosa was seen in one UP and one LP specimen. Except for one N-type fistula, all sections showed full-thickness muscle coats. Distortion of muscles by fibrosis was most commonly seen in the UP. The muscle layer in the LP was more commonly distorted by glands with or without cartilage. Fourteen samples (10.8%) showed a histological picture consistent with CES.**

Conclusion **Glands in the submucosa may be abnormal in number and type, and may extend to different esophageal coats. Muscle distortion by fibrosis, glands, or cartilage and associated CES may contribute toward esophageal dysmotility and stricture after surgery. Cutting the TEF ~3–5 mm distal to its origin from the trachea is adequate histologically for primary anastomosis of the atretic esophagus. The histological changes associated with the TEF need to be revised.** ***Ann Pediatr Surg*** **10:1–6 © 2014 Annals of Pediatric Surgery.**

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[a]Department of Pathology, College of Medicine, King Khalid University, Abha, [b]Department of Surgery, Division of Pediatric Surgery, Armed Forces Hospital Southern Region, Khamis Mushait, [c]Department of Pediatric Surgery, College of Medicine and Medical Sciences, Taif University, Taif, Saudi Arabia and [d]UMAss Memorial Pathology, UMass Memorial Medical Center, Worcester, Massachusetts, USA

Correspondence to Ashraf H.M. Ibrahim, FRCSI, MD, Department of Surgery, Division of Pediatric Surgery, Armed Forces Hospital, Southern Region, King Fasial Military City, PO Box 5062, Khamis Mushait, Saudi Arabia
Tel: +96 6172511180; e-mail: ash_ib@hotmail.com

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Introduction

Postoperative abnormal esophageal peristalsis in cases of esophageal atresia (EA) and/or tracheoesophageal fistula (TEF) is well documented in the literature [1,2]. There is controversy over whether these abnormalities are secondary to vagal disruption, ischemia, or increased traction on the lower esophagus during the surgical procedure [3], or because of congenital architectural abnormalities [4–7].

Few histopathologic studies have been carried out in humans [6,7]. High incidence of tracheobronchial remnants (TBR) had been reported in the lower pouch (LP) in autopsy cases [8], in animal models [9,10], and surgical specimens [8]. The fistulous end may lack the muscle coat that may extend down to a variable distance [8,10]. Those findings may contribute toward the postoperative esophageal dysmotility. Furthermore, congenital esophageal stenosis (CES) may be associated with EA and may contribute toward esophageal dysmotility as well [7,11]. The aim of this report is to study the histopathologic profile in the distal portion of the upper pouch (UP), the fistulous segment of the LP, and in the N-type TEF. Also, we aim to document whether the transected fistulous end is adequate histologically for primary anastomosis.

Patients and methods

One hundred and nineteen surgical specimens were subjected to a histologic examination, where they were collected during surgical repair of 69 consecutive patients with EA admitted to Aseer Central Hospital, Abha, and Armed Forces Hospital Southern Region, Saudi Arabia, over a 10-year period. There were 54 specimens from the UP and 63 from the LP. Only five patients had pure EA whereas 62 patients had classical EA with distal TEF and two had N-type TEF. During surgery, specimens were collected from the tips of the UP and LP and ~3–5 mm distal to the TEF origin when present. The mid portion of the N-type TEF was collected for histologic examination. All specimens were fixed in 10% formalin, processed, and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and evaluated for the presence of any abnormal epithelium, for example, respiratory or gastric. The muscularis mucosa was noted for its thickness and contents. The submucosa was examined for the presence of fibrosis, glands, ducts, or cartilage. Glands were examined for their type, abundances, site, and extension outside the submucosa. The type of muscle fibers was identified. Muscles were examined for fibrosis and/or abnormally seated glands, ducts, or cartilage. Sections were also examined for the

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presence of ganglia and hypertrophied nerve trunks. After consent, seven control non-EA cases were subjected to a punch biopsy through a rigid esophagoscope from the upper and lower thirds of the esophagus immediately after death because of severe cardiac anomalies.

Results

The results of histological studies from seven control cases are shown in Table 1. All the cases showed normal arrangements of different layers of the esophagus. Histological results of 54 specimens from the UP, 63 specimens from the LP, and two N-type TEF are shown in Table 2. Only five patients had nonesophageal epithelial lining, that is, two with gastric alternating with esophageal epithelium (Fig. 1) and three with respiratory epithelium. One UP specimen with gastric epithelium was a case of pure EA with a very long UP reaching down to the 10th thoracic vertebra. The other specimen with gastric epithelium was taken from the LP of a case of EA and obliterated TEF. This patient was diagnosed as a case of pure EA with a long gap and was operated at the age of 8 weeks. A third patient with pure EA had delayed gastric pull-up. The entire LP showed TBR with respiratory epithelium, cartilage, and seromucus glands down to the cardia. Muscle distortion by fibrosis was more commonly seen in the UP specimens (16/54 vs. 3/63 for the LP) (Fig. 2). However, glands were more commonly seen in the submucosa of the LP than in the UP. The glands were seen extending to the muscle layer, causing much distortion only in the LP specimens (Fig. 3). Ganglia were seen in all cases excluding the two N-type TEF. The most common muscle arrangement in the UP was the inner smooth and outer skeletal muscle fibers (35/54), whereas that of the LP was smooth muscle fibers (61/63). Skeletal muscle fibers were rarely seen in the LP (Fig. 4). Fibromuscular disease (FMD) with hypertrophied muscle fibers and fibrosis was noted in two UP specimens and one LP specimen (5.5%) (Fig. 5). Eleven out of 63 LP specimens (17.5%) showed a picture of TBR, eight without cartilage and three with cartilage (Figs 6 and 7). In both N-type fistulae, the lining epithelium was esophageal and one of the fistulae had smooth muscle muscularis propria.

Table 1 Histologic profile in seven control cases

Histopathologic features	Upper esophagus ($n=7$)	Lower esophagus ($n=7$)
Lining epithelium		
Stratified squamous	4	4
Not seen	3	3
Muscularis mucosa		
Thick	1	5
Thin	5	–
Not seen	1	2
Submucosa		
Few glands	–	2
Numerous glands	–	–
Muscular layer		
Only skeletal fibers	3	–
Only smooth fibers	–	7
Inner smooth and our skeletal	3	–
Not seen	1	–
Muscle distortion	–	–
Well-developed muscle coat	6	7
Glands/ducts	–	–
Ganglia	7	7

Table 2 Histologic profile in 69 patients with esophageal atresia

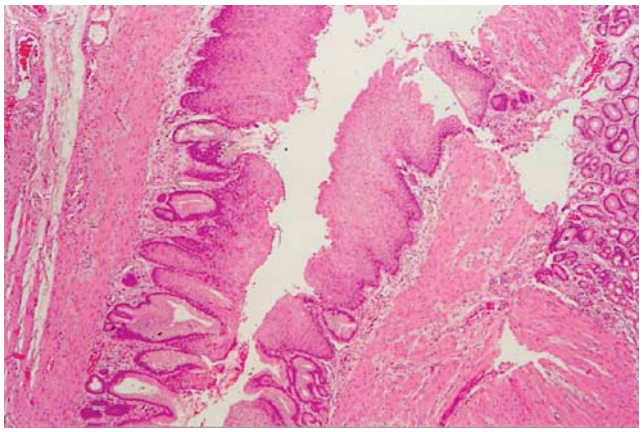
Histologic features	Upper esophagus ($n=54$)	Lower esophagus ($n=63$)	N-type TEF ($n=2$)
Lining epithelium			
Esophageal (stratified squamous)	35	43	2
Respiratory		3	
Gastric/esophageal	1	1	
Not seen	18	16	
Muscularis mucosa			
Well developed	22	37	
Thin	17	15	2
Not seen	15	11	
With mucous glands	2	13	2
With seromucus glands	–	2	1
Submucosa			
No glands or few mucus glands	43	43	
Both mucus and seromucus	–	2	1
Numerous mucous glands	9	11	2
Numerous seromucus glands	–	5	
Not seen	2	2	
Muscle layers			
Absent	–	–	1
Only skeletal	7	–	
Only smooth muscle fibers	4	61	1
Inner smooth/outer skeletal	35	1	
Inner smooth/outer mixed	4	1	
Inner skeletal/outer smooth	4	–	
Well-arranged muscle fibers	36	48	
Distorted muscle fibers	18	15	
Distortion by mucous glands	2	4	
Distortion by seromucus glands	–	5	
Distortion by cartilage and seromucus glands	–	3	
Distortion by fibrosis	14	2	
Hypertrophied fibers with fibrosis	2	1	
Ganglia present	54	63	–
Hypertrophied nerve trunks	–	2	–

TEF, tracheoesophageal fistula.

Discussion

Few reports are available in the literature on the histology of the LP of the atretic esophagus. Most of the reports were on autopsy specimens [8,12,13], experimental animal models [9,10], and very few in humans [6–8]. Histology of the UP is even more rare [12].

Fig. 1



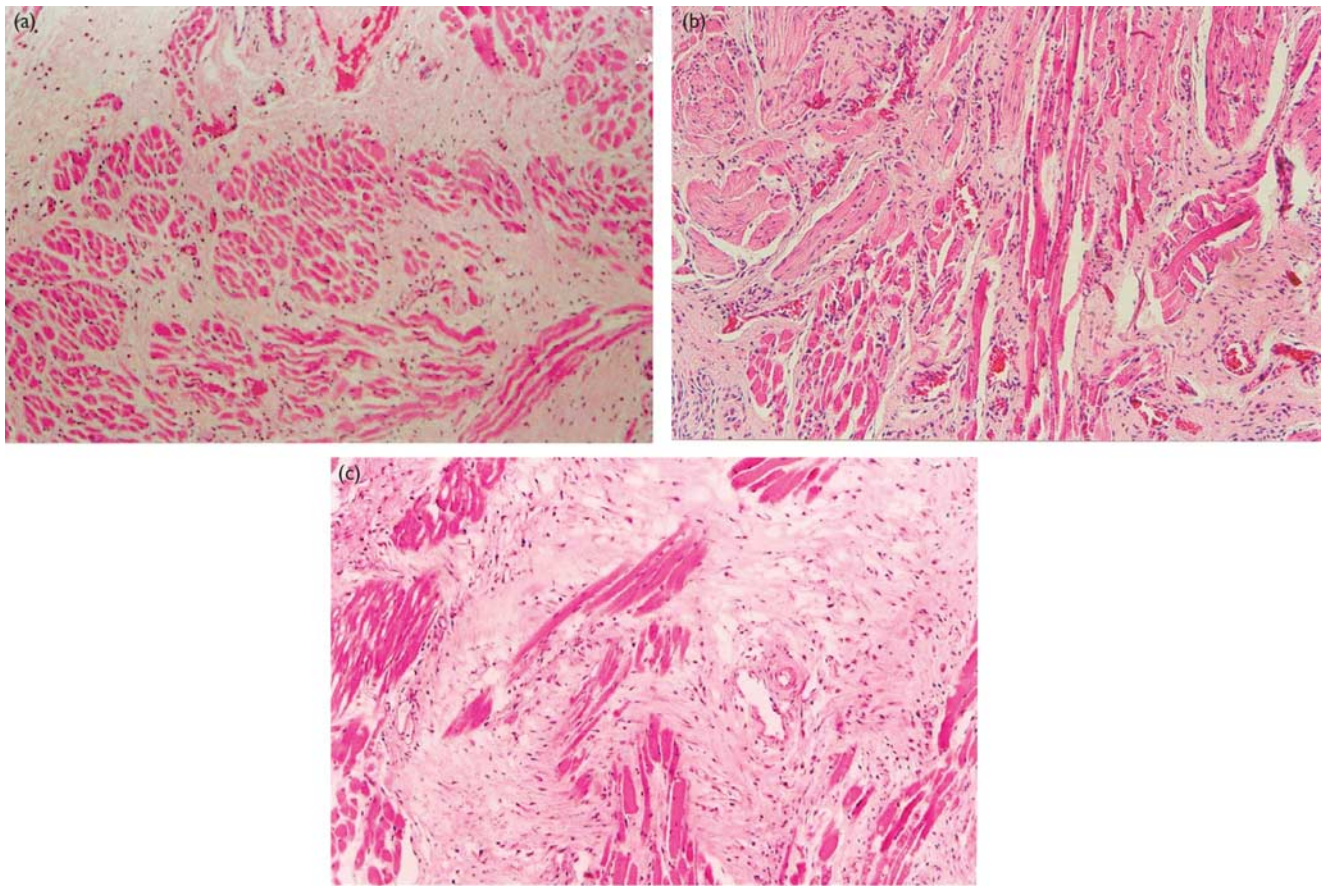
A photomicrograph of the upper pouch showing esophageal alternating with gastric mucosa (hematoxylin and eosin stain, original magnification, ×180).

Nakazato *et al.* [13] found gastric-type epithelium in one distal esophagus in five autopsy cases with EA. However, Emery and Haddadin [14] reported a high incidence of a gastric-type epithelium in the UP (34%, 12/35 patients). Gastric-type epithelium alternating with normal esophageal mucosa was reported in one case from one of our institutions [15].

A high incidence of a ciliated respiratory epithelium was reported in the LP in autopsy cases [8], animal models [9,10], and in surgical specimens [8]. However, no ciliated respiratory epithelium could be seen in 38 surgical specimens of the lower esophageal segment in a previous study [6].

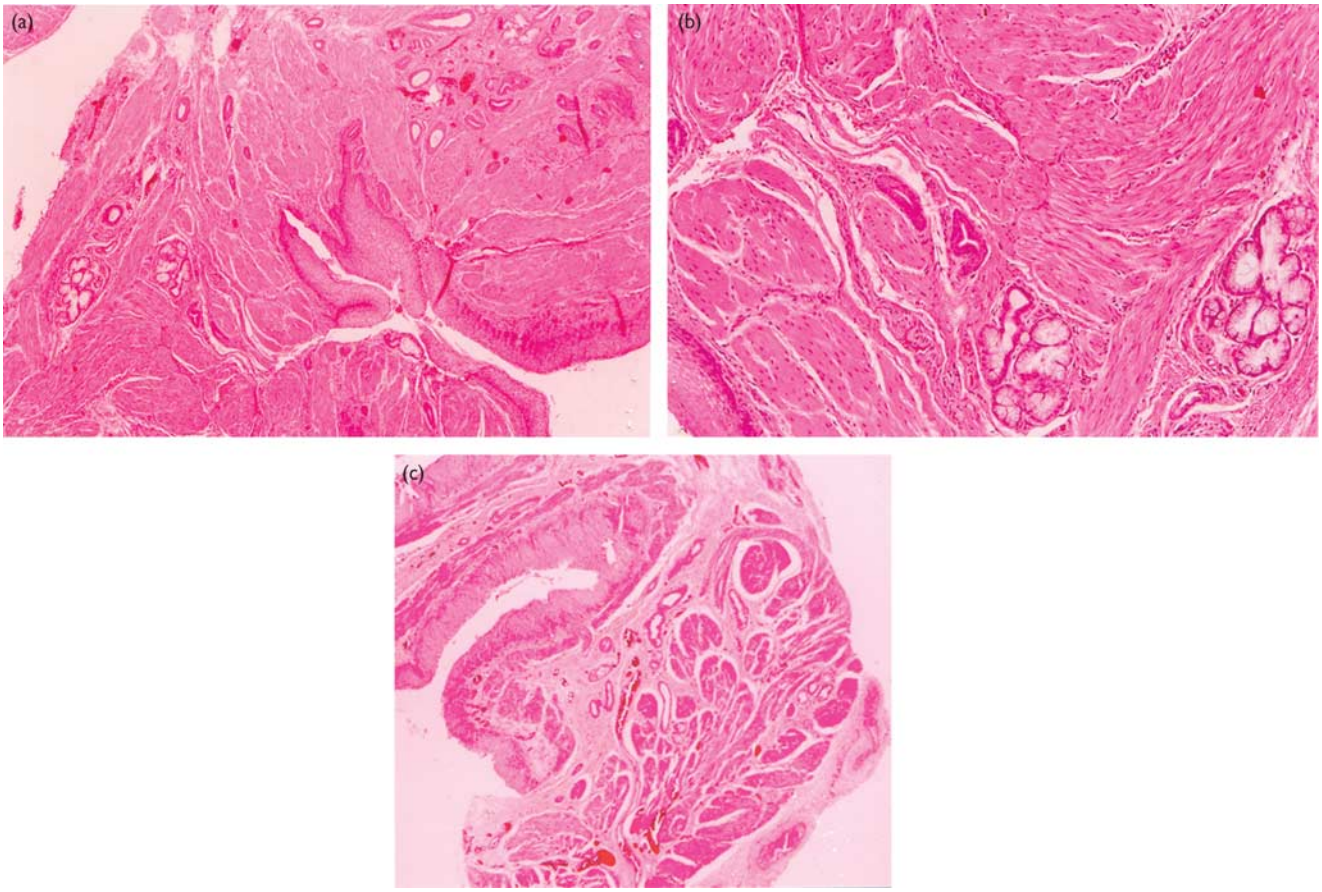
Esophageal glands are considered abnormal if they are increased in number [6], located outside the submucosa, or seromucus in the lower esophagus [8]. Tracheobronchial elements found in the lower esophagus are defined as ciliated pseudostratified columnar epithelium, seromucus glands, or cartilage, alone or in combination [8]. These elements are frequently found together with irregular smooth muscles. Seromucus glands may be seen among the muscle bundles, causing distortion. The plates of cartilage or glands may extend well to the adventitia [7,8,10]. The muscular layers may be absent from the

Fig. 2



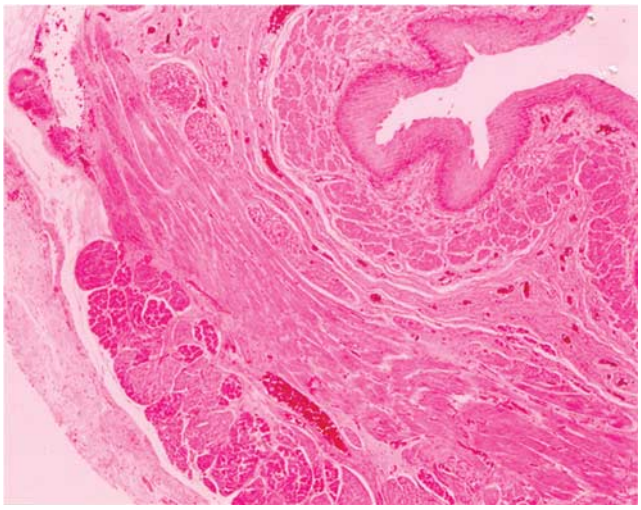
(a) A photomicrograph of the upper pouch (UP) showing skeletal muscle fibers moderately distorted by fibrosis. (b) A photomicrograph of the UP showing skeletal and smooth muscle fibers distorted by fibrosis. (c) A photomicrograph of the lower pouch showing smooth muscle fibers markedly distorted by fibrosis.

Fig. 3



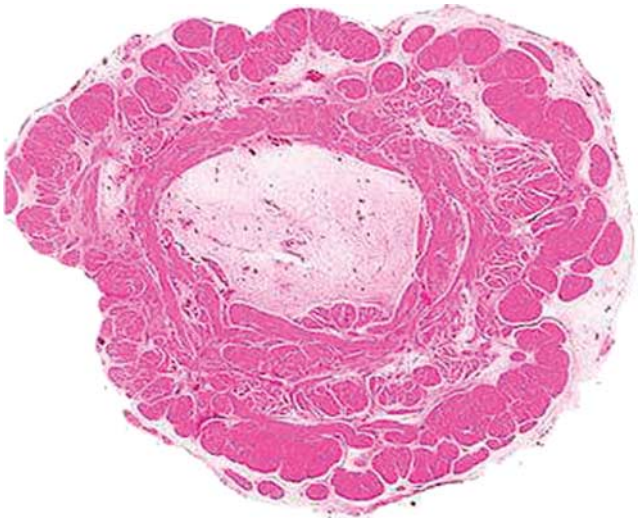
(a) Mucus glands extending to the muscle layer of the lower pouch (LP) causing significant distortion (hematoxylin and eosin stain, original magnification, × 80). (b) Significant muscle distortion in the LP by mucus glands (high power). (c) LP showing mucus glands in the submucosa with few extending to the muscle layer with little distortion (arrow).

Fig. 4



A photomicrograph of the lower pouch showing rare skeletal muscle fibers.

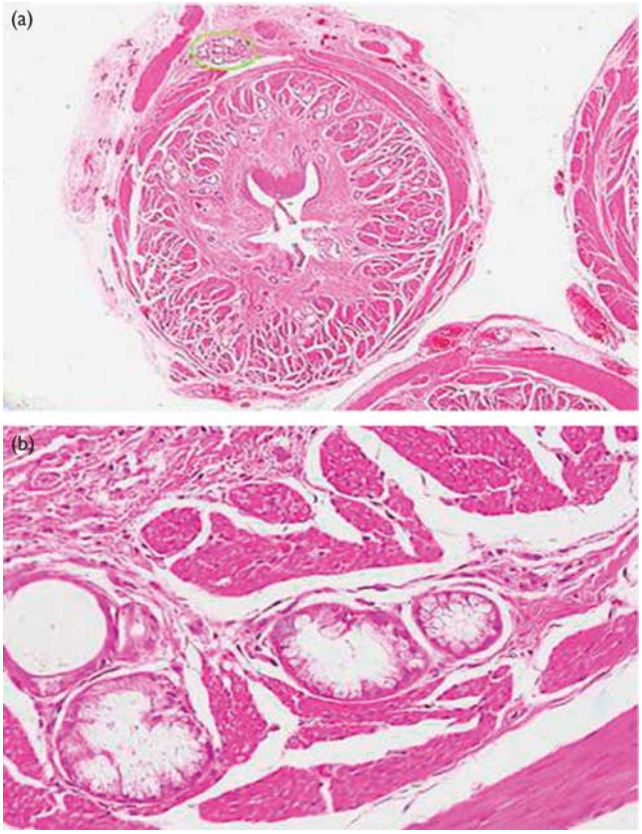
Fig. 5



A photomicrograph of the upper pouch showing hypertrophied muscle fibers with fibrosis, an example of fibromuscular disease.

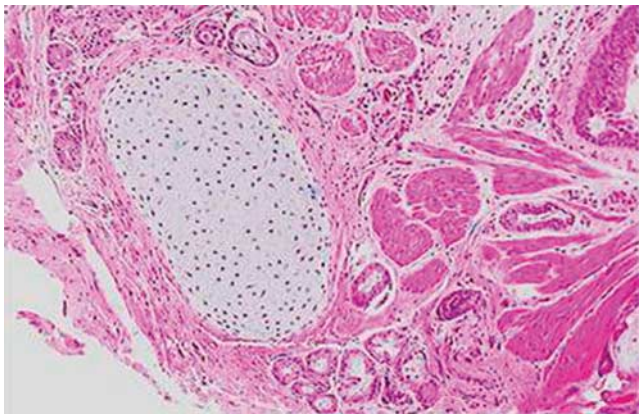
fistulous origin. Later, it develops to form irregular smooth muscle fibers, which are not properly arranged into normal esophageal layers. After transition to the normal esophageal epithelium, they become regular [10]. The proximal pouch has striated muscles and the distal pouch has no striated muscles. Transition from striated to smooth muscles is not abrupt [16,17]. Mahnke and Bennek [12], reported his studies of the UP in 10

Fig. 6



Lower pouch photomicrograph showing (a) mixed respiratory glands extending even to the adventitia and (b) high power; an example of tracheobronchial remnants without cartilage.

Fig. 7



(a) A photomicrograph of the lower pouch showing respiratory epithelium, respiratory glands, and cartilage, an example of tracheobronchial remnants with cartilage.

patients of unoperated EA cases. They found aplasia and hypoplasia in the muscle layers. There were deficiencies and texture disturbances of the intramural ganglia.

Using the microdissection technique and point-count morphometric studies, Nakazato *et al.* [13], examined five autopsy cases with EA and TEF who were not operated upon. They found looser than normal Auerbach plexus in the distal esophagus and to a lesser extent in the proximal esophagus and stomach fundus. The distal esophagus showed a markedly lower relative amount of neural tissue. The proximal esophagus showed ganglia that were larger than normal. Their histological sections did not show any structural abnormalities of the smooth muscle layers.

In the present study, all sections contained muscle layers even at the fistulous end cut ~3–5 mm distal to the origin from the trachea. One of the two N-type fistulae was devoid of the muscle layer. This contrasts with what has been reported by Hokama *et al.* [8] and Merei *et al.* [10] on the absence of the muscle layers at the fistulous end. In the present study, we found that the most common epithelial lining was esophageal stratified squamous epithelium (Table 2). Gastric mucosa alternating with esophageal mucosa was seen in one UP and another LP specimen. Ciliated respiratory epithelium could be seen in three LP specimens. This contrasts with the findings of other investigators [8,10]. Merei *et al.* [10], using their animal model, found that all the fistulae were lined with ciliated respiratory epithelium extending to a variable distance from the origin and in some instances as far as the stomach. The transition from the ciliated epithelium to be stratified squamous occurred either abruptly or by partial replacement [10].

Numerous seromucus glands with dilated ducts in the submucosa and extending to the muscle layer and adventitia were seen in five of our LP specimens. Seromucus glands are considered a component of a respiratory epithelium abnormally found in the LP [8].

The most common arrangement of the muscularis propria was the inner smooth and outer skeletal for the UP (35/54) and smooth muscle fibers only for the LP (61/63). This study is in agreement with Davis [16,17] that the transition from striated to smooth muscle is not abrupt. However, two patients with overlapping pouches showed skeletal muscle fibers in the LP (Fig. 4). The muscle layer was distorted in 18 UP specimens and 15 LP specimens. In the UP, it was distorted by mucus glands in two and by fibrosis in 16. In the LP, it was distorted by mucus glands in four, by seromucus glands in five, by seromucus glands and cartilage in three, and by fibrosis in three. Most previous studies focused on the histopathologic changes only of the lower esophageal segment. This study also shows that the UP may show abnormalities as well. Muscles were distorted in the LP mainly by glands (12/63) whereas in the UP, they were mainly distorted by fibrosis (16/54). CES can be associated with EA at the anastomotic site [7]. In the present study, TBR was found in 17.5% of the LP specimens and FMD in two UP and one LP specimens. The clinical significance of CES associated with EA has been reported before [7]. Cases with TBR without cartilage show excellent long-term clinical outcome despite the persistence of radiological minor dysmotility. They respond well to medical antireflux measures together with balloon dilatation if stricture develops. Cases with TBR with cartilage present with anastomotic stricture or major esophageal dysmotility. Surgical resection is usually indicated if initial dilatation is not achieved or symptoms recur very soon after

dilatations. FMD present with stricture or esophageal dysmotility and usually respond to balloon dilatations. Surgical resection may be required if dilatation fails.

Conclusion

The histological changes associated with the TEF need to be revised. Muscle distortion of the atretic esophagus by fibrosis is more common in the UP whereas muscle distortion by abnormal glands with or without cartilage is more common in the LP. Out of 119 specimens, 14 (10.8%) showed a histopathologic picture of CES. Also, we conclude that transecting the TEF ~3–5 mm distal to its origin from the trachea is quite adequate for the presence of a relatively normal distal LP to allow for a primary anastomosis. Further studies are required to correlate these histopathologic abnormalities with the clinical course and radiological findings.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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