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Original Research Article

Clinicopathological evaluation of the terminal end of the distal rectal pouch and fistula site in anorectal malformation

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ABSTRACT

Background: The aim is to assess the clinicopathological evaluation of the terminal end of the distal rectal pouch and fistula site in anorectal malformation (ARM).
Methods: This study was conducted on patients who attended the surgery OPD and included 40 cases of ARMs over a 12-month period. All cases were admitted to the department of surgery at B. R. D. medical college, Gorakhpur, UP.
Results: Out of 40 cases studied 22 cases (55%) were female and 18 cases (45%). Out of total 40 cases, recto-bulbourethral fistula shown by 8 male patient (20%), recto-prostatic fistula shown by 8 male patient (20%), and recto-bladder neck fistula shown by 2 male patient (5%) out of total 18 male patient (45%) of total cases. Recto-vestibular fistula shown by 7 female patient (17.5%), ano-vestibular fistula by 11 male patient (27.5%), low rectovaginal fistula shown 3 female patient (7.5%), and no fistula shown by 1 female patient (2.5%) out of total 22 female patient (55%). In our study wound infection (5 cases) 12.5%, excoriation (5 cases) 12.5 %, constipation and bleeding (2 case) 5% were noted, no complication seen in 65% cases. All of them were treated conservatively followed by anal dilatation.
Conclusions: Histopathological variations in the distal rectal pouch and fistula site of ARMs have led to differing opinions on whether to preserve or excise the fistula site. Our study found that 45% of patients had a smooth muscle layer present in the histopathological examination.

Keywords: Distal rectal pouch, ARM, Fistula site

INTRODUCTION

Anorectal malformations (ARMs) comprise a wide spectrum of diseases, which can affect boys and girls, and involve the distal anus and rectum as well as the urinary and genital tracts.[1] It is congenital anomalies that occur in approximately 1 in 5000 live births. Imperforate anus has been a well-known condition since antiquity which is commonly seen in neonates. The boys are at higher risk than girls in the ratio of 1.3:1.[2,3] Defects range from the very minor and easily treated with an excellent functional prognosis, to those that are complex, difficult to manage, are often associated with other anomalies, and have a poor functional prognosis. ARMs include a wide range of malformations, that not only involves the anus and rectum, but it also involves urinary and genital tract. High type of lesion resulting into rectovaginal, rectovesical or rectourethral fistula, because in these types bowel is placed higher in the pelvis.[4] The etiology and pathogenesis of ARMs is not clear, and may be multifactorial.[5,6]

The terminal end of the distal rectal pouch and fistula region have not been considered worthy of preservation until recently. Some investigators have now found manometric and histological evidence of features of a normal anus in this region, such as the presence of an internal sphincter, transitional epithelium, hypo- or aganglionosis, and anal glands and crypts.[7-9] Further genetic factors, prenatal exposure of parents to nicotine, alcohol, caffeine, illicit drugs, occupational hazards, overweight/ obesity and diabetes mellitus are suspected as environmental risk factors.[10]

The histological analysis of the malformations in human foetus and newborns showed a ventral-ward deviation of the anal canal as the principal deformity. It has been observed that the pelvic floor and the smooth muscle of the terminal rectum in ARMs remain maldeveloped.[11,12]

Histological studies on ARMs showed the immaturity of the enteric nervous system and absence or reduced number of Cajal cells which might be a cause of postoperative dysmotility responsible for constipation, incontinence, soiling etc. after surgical repair.[13] Management of the condition is chiefly surgical. However, very effective surgical procedures need to be found out to avoid any kind of complications related to altered bowel motility. Knowledge of structural alteration from different segments of surgically excised tissue of ARMs might provide important guidelines for preparation of appropriate surgical procedures.[14-16]

Now a days surgical techniques have improved significantly as compare to the last decade still complete anatomotic and functional restoration of congenital ARMs cannot be achieved. Patients with ARMs often present with varying severity of defecation dysfunction (constipation, incontinence, and fecal soiling) following corrective operations. Constipation is one of the most frequent complications after correction of ARMs, occurring in 30% to 60% of patients the detailed mechanism which play a key role in normal functioning of gastrointestinal tract remains unclear.[17,18]

The postoperative bowel function and surgical outcome depends not only on the type of malformation and the muscular and neurologic condition of the pelvic floor musculature, but also on the histology of the blind pouch and surgical procedure. To the best of our knowledge, there is a paucity of literature describing the histopathologic abnormalities of the distal rectum or distal pouch. The aim of the present study is to assess the clinicopathological evaluation of the terminal end of the distal rectal pouch and fistula site in ARM.

METHODS

This study was carried out on patients with ARM attended OPD of the surgery department at B.R.D. medical college, Gorakhpur from the period of Feb 2018 to April 2019. 40 cases of ARMs were included. The duration of the study was 12 months. Subsequently histopathological examination was carried out on surgically resected tissue specimens from the terminal end of the distal rectal pouch and fistula site in ARM sent to department of pathology, B.R.D. medical college, Gorakhpur, UP. Patients with ARMs who provided written informed consent were included in the study. Those who had already undergone a definitive procedure for ARM were excluded. Autolyzed tissue sample

Preparation of tissue for histopathological examination

The tissue was processed for routine histopathological examination as follows:

Fixation

The tissues were subjected to overnight fixation in 10% formalin solution.

Embedding

It involved the removal of water by alcohol dehydration, infiltration of xylene as a solvent for paraffin wax, and the paraffin wax impregnation.

Microtomy

Sections of 2-3 µm thickness were cut from routinely processed paraffin embedded blocks and gently lowered on surface of water bath at 45^0 ºC. These sections were taken on alcohol cleaned glass slides smeared with a thin film of egg albumin. The slides with the sections were warmed on a hot plate at 58^0 ºC for 1 hour, cooled and stored in a box for staining. Wax removal was done in xylene, slides were kept in xylene for 2 minutes and 2 such changes were done. Removal of xylene was done with absolute alcohol. Slide were kept in absolute alcohol for 2 minutes and 2 such changes were made. Treatment with descending grades of alcohol in 90% alcohol for 1 minute and in 70% alcohol for 1 minute was performed. Finally, the sections were brought to the deionized water. Two to three sections of each case so obtained were processed for H and E staining as below.

Hematoxylin and eosin staining procedure

Sections were stained in a solution of Harris haematoxylin for 5-15 minutes, and washed thoroughly in running water for 15-30 seconds. Sections were decolorized with 1% acid alcohol solution for 10-20 seconds. Again, washed with tap water then sections were kept in warm water for 5 minutes. The counter stained with 1% aqueous eosin for 15 minutes. Washed rapidly in water to remove excessive amounts of eosin. Then hydrated by several changes of increasing grade of alcohol. Cleaned in xylene and mounted with dextrin 80 di-butylphthalate xylene (DPX) mount. All the slides were examined under light microscope at different magnification. Result of the staining was as-nuclei-blue,

cytoplasm-pink, muscle fibre-deep pink, RBC's-orange/red, fibrin-deep pink

Data analysis and statistical tools for observation and result of the study

All relevant data was collected and appropriate statistical tools were applied to analyse the data. Analysis was done by data sorting method, classified by tabulation and presentation by pie diagrams, and histograms. Statistical method such as calculation of p value, z test was employed to find out the significance of the study. A statistician`s help was sought for interpretation of the results.

Ethical clearance

The present work has been conducted after getting ethical clearance from the institutional ethical committee.

RESULTS

Out of 40 cases studied 22 cases (55%) were female and 18 cases (45%) were male with male to female ratio of 1:1.22. The age of the patients with ARM ranged from birth month to 20 years. Youngest patient was of 4 month and oldest patient was 18 years old. It was observed that maximum no of cases i. e., 21 cases (52.5%) were in the age group of 1 to 5 years followed by 10 cases (25%) in the age group of 6 to 11 month.

Table 1: Baseline characteristics.

Gender	N	Percentage (%)
Female	22	55
Male	18	45
Age group (in years)		
0 to 5 months	4	10
6 to 11 months	10	25
1 to 5	21	52.5
6 to 10	4	10
11 to 15	0	0
15 to 20	1	2.5

Out of total 40 cases studied, recto-bulbourethral fistula shown by 8 male patient (20%), recto-prostatic fistula shown by 8 male patient (20%), and recto-bladder neck fistula shown by 2 male patient (5%) out of total 18 male patient (45%) of total cases. Recto-vestibular fistula shown by 7 female patient (17.5%), ano-vestibular fistula by 11 male patient (27.5%), low rectovaginal fistula shown 3 female patient (7.5%), and no fistula shown by 1 female patient (2.5%) out of total 22 female patient (55%) (Table 2).

Postoperative complications following corrective surgery of ARM are wound site infection, excoriation, constipation, bleeding. In our study wound infection (5 cases) 12.5%, excoriation (5 cases) 12.5%, constipation and bleeding (2 cases) 5% were noted, no complication seen in 65% cases. All of them were treated conservatively followed by anal dilatation.

Table 2: Sex wise distribution of anomalies in male and female.

Defects	Patient (cases)	Percentage (%)
	Male	
Recto-bulbourethral fistula	8	20
Recto-prostatic fistula	8	20
Recto-bladder neck fistula	2	5
Total	18	45
	Female	
Recto-vestibular fistula	7	17.5
Ano-vestibular fistula	11	27.5
Low rectovaginal fistula	3	7.5
No fistula	1	2.5
Total	22	55

Table 3: Complication following ARM surgery.

Post-op complication	N	Percentage (%)
Wound infection	5	12.5
Excoriations	5	12.5
Bleeding	2	5
No complication	26	65

Table 4: Histological finding in mucosa and sub-mucosa.

Variables	N	Percentage (%)
Mucosa finding		
Eroded mucosa	24	60
Acute and chronic inflammation	32	80
Congestion	28	70
Haemorrhage	18	45
Submucosa finding		
Acute and chronic inflammation	36	90
Congestion	32	80
Haemorrhage	20	50
Fibrosis	25	62.5

In mucosa layer there was inflammation and congestion seen in 32 cases (80%) and 28 cases (70%) of cases. In submucosa layer there was inflammation seen in 36 cases (90% of cases) and congestion seen in 32 (80%) cases.

The finding of smooth muscle layer in samples. Among these hypertrophied smooth muscle layers were observed in 8 cases (20%) in 5 females (12.5 %) and 3 males (7.5%). Thinning of smooth muscle layer was observed in 10 cases (25%) in 6 females (15%), 4 male (10%), absent

of smooth muscle layer was observed in 20 cases (50%) 11 female (27.5%), 9 male (22.5%).

Table 5: Histological finding in smooth muscle layer.

Smooth muscle layer	N		Percentage (%)	
Hypertrophy	Female	5	Female	12.5
	Male	3	Male	7.5
Thinning	Female	6	Female	15
	Male	4	Male	10
Absent	Female	11	Female	27.5
	Male	9	Male	22.5
Inadequate sample	Female	0	Female	0
	Male	2	Male	5

DISCUSSION

ARMs comprise a wide spectrum of diseases, which can affect boys and girls, and involve the distal anus and rectum as well as the urinary and genital tracts. Defects range from the very minor and easily treated with an excellent functional prognosis, to those that are complex, difficult to manage, are often associated with other anomalies, and have a poor functional prognosis. The terminal end of the distal rectal pouch and fistula region have not been considered worthy of preservation until recently. Some investigators have now found manometric and histological evidence of features of a normal anus in this region, such as the presence of an internal sphincter, transitional epithelium, hypo- or aganglionosis, and anal glands and crypts.[19-21]

ARM cases are diagnosed after birth; antenatal diagnosis of ARM is very rare. ARM present with various types of malformations, that not only involves the anus and rectum, but it also involves urinary and genital tract. It has been observed that the pelvic floor muscle and the smooth muscle of the terminal rectum in ARMs remain mal-developed. Several studies on fetal rats showed that there were abnormal innervations of neural plexus in anorectum inanorecal malformation the important point is that despite various advancement in surgical treatments, voluntary bowel control after surgery is frequently poor and post-op complications like fecal incontinence and chronic constipation still common after all types of reconstructive surgery.[22-24] The clinicopathological analysis were done to know whether the distal rectal pouch histology in ARM was normal enough to use this segment for reconstruction of the terminal end and normal function of anorectal defecation can be achieved after anorectoplast.

There has been marked improvement in the diagnosis and management of ARM due to better understanding of anatomy embroyology and physiology of ARM and marked advancement in imaging studies. But still postoperative faecal and urinary incontinence is a big challenge. These complications are attributed to various histomorphological and neuronal dysfunction associated with ARM.[25] The occurrence of constipation in patients whose fistula region is preserved is clearly related to the presence of a distorted internal sphincter, subepithelial fibrosis and aganglionosis. This leads to increased anal resting pressure generated by preservation of this fistula region, together with decreased rectal sensitivity (due to partial sensory denervation of the distal rectum). Congenital rectal dilatation may predispose patients to the development of constipation and anal incontinence.[26]

The internal sphincter is the most important factor in the anorectal resistance barrier and is thus essential for continence. The term fistula seems incorrect and the bowel opening should rather be called an ectopic anus. This was suggested 30 years ago by Gans and Friedman and Bill and Johnson.[26,27] After embryological studies, they presented a theory of incomplete migration of the distal bowel segment that results in the rectal opening not reaching its correct position in the perineum. They also found histological similarities between the fistulous connections in ARMs and the normal anal canal and proposed that no part of the terminal bowel should be resected unnecessarily, which has been contradicted subsequently by Meier Ruge and Holschneider.[28]

In our study, we have observed vast number of histomorphological findings compatible with various studies and few findings contrary to previous studies as stated by Tiwari et al histopathological examination using H and E revealed numerous changes like inflammation, muscular hypertrophy, fibrosis and serositis etc.[39]

Agarwal et al reported hypertrophy of nerve bundles.[1] Yashika et al study also showed nerve bundle hypertrophy (17%), which was confirmed using S-100 as immune-histochemical marker for nerve bundle.[44] Dysganglionosis and aganglionosis have been frequently described in biopsies of the distal bowel in patients with ARM.

There are many factors that may account for the constipation after the procedure of anorectoplasty such as anal stenosis, abnormalities of extrinsic intestinal innervations, rectal denervation during the surgical procedure, and abnormalities of intrinsic intestinal innervation including aganglionosis, hypoganglionsis, and intestinalneuronal dysplasia, it remains controversial whether the distal rectal pouch should be used for reconstruction in anorectoplasty with the treatment of ARM.[2,26]

Gans and Friedman advocated preserving the rectal blind pouch based on its histology.[41] Yokoyama et al showed distinct thickening of the circular and longitudinal muscle layers in the distal rectal pouch in neonates with high anal atresia.[42] Meier-Ruge and Holschneider observed hypoganglionosis of the myenteric plexus proximal to the anal floor, also most commonly in intermediate ARMs, which is therefore strongly recommended to be excised for reconstruction.[42] Lombardi et al realized that the

resection of distal rectum structural abnormalities may be helpful to permit better functional results during radical treatment.[44]

Holschneider et al reported that after corrective surgery in ARM there are higher incidence of chronic constipation which may be consequence of hypoplastic hypoganglionosis or other anomalies of entric nervous system.[30,38] So, in light of the results of reported studies about histology of distal rectum pouch, the ARMs should no longer be considered as simple and short anomalies. It can be concluded that there exist complex structural abnormalities in several centimeters of the distal anorectal canal.[28-31] However, no standard criteria were drafted about the correct length of distal pouch should be excised. The present study shown conducted in our centreis based on the operation done by single surgeon. Biopsy specimen was further processed and histopathology done by single pathologist in this way we avoided ascertainment bias. We found three sets of patients, in one there is no smooth muscle cells layer in the biopsy sample, in second set of patients, biopsy sample of distal end of rectal pouch and fistula region having quite developed, hypertrophied, smooth muscle layer, in third set of patients, biopsy sample, there was thinning of smooth muscle cell layer.

The histo-morphological changes which were noted in our study are focal erosions, smooth muscle hypertrophy, and thinning out smooth muscle. This different histopathological behavior can be attributed to many factors and variable present during surgery: First, the size of the biopsy sample, second, the way it was excised (knife or cautery) and third, with cautery whether it was cutting or coagulation mode.

Limitations

If there is a significant hemorrhage and charring in the excised biopsy tissue there will be difficulty in processing the sample and finding the histopathology accurately so we need to avoid haemorrhage and charring of the tissue while we take sample here comes the role of appropriate mode of cautery. While doing anterior sagittal anorectoplasty or posterior sagittal anorectoplasty distal end of rectal pouch might have thinned out the smooth muscle layer so fecaloma evacuation from the distal pouch and rectal washes before definitive procedure become very important to normalize the rectal diameter. We routinely perform dye study in all ARMs cases to know the rectal dilatation.

CONCLUSION

Although the distal rectal pouch and fistula site in ARM have shown histopathological variations and these variations has led to different opinion regarding preservation and excision of the fistula site. Most of the studies do not advocate preserving fistula site. Our study has found that 45% patients have smooth muscle layer in histopathological examination. Although there are many factors which affect presence of smooth muscle layer in histopathological examination but surgical outcome of our patient without surgical excision of the distal segment have shown overall good result. However, we further need long term follow up and further detailed studies on the histopathology, contractile function, electrophysiology, IHC and biochemical assay, involving a greater number of ARM cases for better understanding and management of these problems. Based on the result of our study we advocate preservation of distal end of rectal pouch and fistula site.

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