Original Article

HISTOLOGICAL STUDY OF NEONATAL BOWEL IN ANORECTAL MALFORMATIONS


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ABSTRACT

Anorectal malformations are the congenital condition, seen in approximately 1 in 5000 live births. It affects male and female in the ratio of 1.3:1. Anorectal malformations include a wide range of malformations, that not only involves the anus and rectum, but it also involves urinary and genital tract.

Aims and objectives of the study, was to understand the structures involved in anorectal malformations by histological study of surgically excised segments of involved part of neonatal intestine and to understand the degree and cause of possible structural impairment in different segments of involved parts of neonatal bowel that may help in the surgical management of anorectal malformations. Present study was conducted on surgically excised segments of fifteen cases of anorectal malformations, that have been collected from Department of Paediatrics Surgery, IMS, BHU. After that processing of the samples have been done and blocks have been prepared. Then after sectioning and staining with Hematoxyline and Eosin, findings have been noted under the microscope. Histopathological examination revealed the abnormalities of varying degrees. To conclude this study supports that the malformed segments should be excised, regarding controversial issue of preserving or excising the distal segment of anorectum for better functional outcome.

KEYWORDS: Anorectal malformations, neonatal bowel, histological study.

INTRODUCTION

Anorectal malformations have been recognised in the animals since the time of Aristotle. Amussat, in 1835, first sutured the rectal wall to the skin edges, which could be considered the first anoplasty.

Anorectal malformations are commonly seen in neonates. The boys are at higher risk than girls in the ratio of 1.3:1[1] and the incidence of anorectal malformations ranges from 1 in 2,500 to 1 in 5,000 [2]. Anorectal malformations 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. However, in low type lesion there may be stenosis of anus and rectum or they may end in a blind pouch because, the terminal bowel is lower in situation [3]. The etiology and pathogenesis of anorectal malformations is not clear, and may be multifactorial [4, 5]. Various studies were carried out on functional as well as histopathological aspects of the condition on human and animals [6, 7, and 8]. The studies carried out in human that mainly focused on histology and immunohistochemistry which suggests the structures involved in anorectal...
malformations, however, the genetic cause was also suggested. It has been observed that there is an increased risk for a sibling of a patient with anorectal malformations to be born with a malformation, as much as 1 in 100, compared with the incidence of about 1 in 5000 in the general population. 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 [9]. The histological analysis of the malformations in human foetuses and newborns showed a ventralward 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 anorectal malformations remain maldeveloped. Studies on fetal rats showed that there were abnormal innervations of neural plexus in anorectum in anorectal malformations [10, 11]. Histological studies on anorectal malformations 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 [12]. Management of the condition is chiefly surgical. However, very effective surgical procedures needs 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 anorectal malformations might provide important guidelines for preparation of appropriate surgical procedures [13, 14, and 15].

MATERIALS AND METHODS
The samples were collected from the department of Pediatric surgery, Sir Sunder Lal hospital, Banaras Hindu University, Varanasi. Histopathological analysis was performed in the Department of Anatomy and Pathology at S.S. Medical College, Rewa respectively.

- Number of Cases: 15
- Inclusion criteria: Newborns with anorectal malformations.
- Exclusion criteria: Newborns > 1 month age and all normal newborns.
- Ethical Clearance: The study was carried out with the guidelines and following approval of Institutional ethical committee.
- Collection of Sample: initially collected in a wide mouth bottle containing ice-cold, Krebs Ringer solution having the composition (mM): NaCl-119, KCl-4.7 CaCl$_2$·2H$_2$O-25, KH$_2$PO$_4$-1.2, MgSO$_4$-7, NaHCO$_3$-5, H$_2$O-1.2, and Glucose-11. Finally the specimens were stored in 10% formalin solution for fixation.
- Gross examination
- Preparation of tissues
- Microtomy: We used rotatory microtome for section cutting. We prepared sections of 3-5 micrometer thickness with the help of microtome.
- Preparation of H & E stained slides
- Microscopic examination: The findings were noted in the mucosa, submucosa, muscle layer, myenteric plexus and adventitia of the bowel wall.

RESULTS
Histopathological examinations of proximal and distal segments of all cases of anorectal malformations were performed to know structural abnormalities. After observing the histological findings of all specimens, we observed that there was congestion with infiltration in mucosa and submucosa in most of the specimens in both segments (Graphs 1 and 2). In the muscle layer we found that, hypertrophy of the both muscle layers (inner circular and outer longitudinal) were seen but the number of cases with hypertrophy in inner circular layer was more (Graphs 3, 4, 5 and 6). Also in proximal segments, myenteric plexus were present in good number while in distal segments myenteric plexus were less in number (Graphs 7 and 8).

After examination under microscope we have found various types of abnormalities in anorectal malformations, like thinning of muscle layer (case no. 1), degeneration and fibrosis of muscles (case no. 2), constriction band (case no. 3), irregular muscle bundles (case no. 6), extensive hypertrophy of muscle layer (case no. 12) and many other findings. Serosa in most of the
specimens was oedematous because of congestion and inflammatory infiltrate.

In our present study we have found that all structural abnormalities were more pronounced in distal segments as compared to proximal segments. The details of findings, comparative analysis and various photographs displaying the histopathological abnormalities are as following (Fig1 - 9).

**Fig 1:** Case No. 1 Distal segment, arrow indicating, thinning of inner circular muscle layer.

**Fig 2:** Case No. 2 Proximal Segment.

**Fig 3:** Case No. 3 Proximal segment, arrow indicating, constriction band.

**Table 1:** Mucosal findings.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chadha et al</td>
<td>1998</td>
<td>Normal mucosa in all cases.</td>
</tr>
<tr>
<td>Agrawal et al</td>
<td>2005</td>
<td>Acute and chronic inflammation of the mucosa was most frequent finding.</td>
</tr>
<tr>
<td>Present study</td>
<td>2013</td>
<td>Abnormal mucosa in 60%, acute and chronic inflammation in 60% Disrupted muscularis mucosae in 53.3%, and hemorrhage in 40% of cases.</td>
</tr>
</tbody>
</table>

Arrows indicating: red= mucosal hyperplasia, green= inner circular muscle layer hypertrophy, yellow= fibrosis and degeneration of inner circular muscle layer.

**Fig 4:** Case No. 6 Proximal segment.

Arrows indicating: red= inner circular muscle layer hypertrophy, green= fibrosis and degeneration of muscle layer.

**Fig 5:** Case No. 6 Distal segment.

Arrows indicating, green = myenteric plexus, red= muscle disruption with irregular muscle bundles.
**Fig. 6:** Case No. 7 Proximal segment, red arrow showing disrupted inner circular and outer longitudinal muscle layer.

**Fig. 7:** Case No. 8 Proximal segment. Arrows indicating: red = inner circular muscle layer hypertrophy, green = myenteric plexus

**Fig. 8:** Case No. 9 Proximal segment. Arrows indicating, red = extensive haemorrhage and congestion in submucosa, green = hypertrophy and splaying of inner circular muscle layer, blue = myenteric plexus

**Fig. 9:** Case No. 12 Distal segment. Red arrow showing extensive hypertrophy in inner circular muscle layer.

### Table 2: Submucosal findings.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarwal et al</td>
<td>2005</td>
<td>Acute and chronic inflammation in most cases.</td>
</tr>
<tr>
<td>Present study</td>
<td>2013</td>
<td>Acute and chronic inflammation in 80% of cases, thickening in 66.6% of cases and hemorrhage in 60% of cases.</td>
</tr>
</tbody>
</table>

### Table 3: Muscle layer findings.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chadha et al</td>
<td>1998</td>
<td>Thinning of the muscle layers in 48% of specimens and hypertrophy of the muscle layers in 8% of specimens.</td>
</tr>
<tr>
<td>Agarwal et al</td>
<td>2005</td>
<td>Focal or generalized thinning of muscle layers, especially of the outer muscle coat and disorganized muscle layers.</td>
</tr>
<tr>
<td>Gupta DK</td>
<td>2007</td>
<td>Presence of criss-cross pattern of decussating fibers in the muscle coat.</td>
</tr>
<tr>
<td>Gupta and Sharma</td>
<td>2007</td>
<td>Incomplete inner circular layer in 50% of cases.</td>
</tr>
</tbody>
</table>

**Present study** 2013

Hypertrophy of inner circular layer in 86.6% of proximal segments and 46.6% of distal segments of cases, hypertrophy of outer longitudinal layer in 20% of proximal segments and 66.6% of distal segments of cases. Thinning of inner circular layer in 26.6% of proximal segments and 20% of distal segments of cases, thinning of outer longitudinal layer in 73.3% of proximal segments and 33.3% of distal segments of cases. Some other findings were irregular muscle bundles (20% of cases), incomplete inner circular layer (40% of cases) and constriction band (13.3% of cases).

### Table 4: Myenteric plexus findings.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yadav and Rao</td>
<td>1983</td>
<td>Decreased number of myenteric plexus.</td>
</tr>
<tr>
<td>Agrawal et al</td>
<td>2005</td>
<td>Decreased number of myenteric plexus.</td>
</tr>
<tr>
<td>Gupta &amp; Sharma</td>
<td>2007</td>
<td>Mature ganglion cells in all cases.</td>
</tr>
<tr>
<td>Present study</td>
<td>2013</td>
<td>Myenteric plexus present in 53.33% of cases (40% in proximal segments and 13.3% in distal segments). Decreased number of myenteric plexus were present in 40% of cases.</td>
</tr>
</tbody>
</table>
Graph 1: Showing Mucosal findings.

Graph 2: Showing Submucosal findings.

Graph 3: Showing Inner circular muscle layer hypertrophy.

Graph 4: Showing Inner circular muscle layer thinning.

Graph 5: Showing Outer longitudinal muscle layer hypertrophy.

Graph 6: Showing Outer longitudinal muscle layer thinning.

Graph 7: Showing % of Presence of myenteric plexus.

Graph 8: Showing Presence of myenteric plexus.
(Proximal Vs Distal Segment)
DISCUSSION

Anorectal malformations are rare birth defects concerning anus and rectum and approximately 1 in 2,500 to 1 in 5,000 new born babies are affected by anorectal malformations. Various degrees of severity were distinguished, ranging from mild anal stenosis over anal atresia with or without fistula to persistent cloaca or even cloacal exstrophy. Furthermore, anorectal malformations frequently manifested with other malformations, approximately 64% of all anorectal malformations patients have one or more additional extra-anal anomalies. Previous studies have shown that associated malformations are more frequent in “high” defects that are complex and difficult to manage with a poor functional prognosis than in “low” defects that are less complex and easily treated with an excellent functional prognosis. Associated malformations mainly include the genitourinary system (21-61% and more), spine and spinal cord (10-25%), rest of the gastrointestinal tract (9-20%). Anorectal malformations with high lesion presents with a fistula that communicate rectum to the bladder, urethra or vagina [16]. The management of the anorectal malformations is principally surgical. Although presently several new techniques improved the outcome, but the management still remains to be unsatisfactory because constipation, incontinence and soiling are reported to be common sequelae after surgical repair of anorectal malformations by available techniques [17, 18]. These complications are resulted due to impairment of the structure and function of smooth muscles of rectum, further studies on histological aspect of anorectal malformations may provide such details that may be effective and beneficial in surgical management of the condition.

The present study has given us a vast number of histopathological findings which corresponds with studies of other workers while some other observations were contrary to the findings noted by these workers. A number of new observations noted by us through detailed evaluation of proximal and distal segments of anorectal malformations are also mentioned that have not been published in the literature so far. In this discussion we will compare the earlier findings noted by other workers with observed findings of this study and also elaborate some new observations (tables 1-4).

CONCLUSION

To conclude The present study emphasizes that the malformed segments in anorectal malformations were found structurally abnormal with severe impairment in distal segment. This study supports the excision of distal segment that will be the definitive surgery for better functional outcome. However, further detailed studies on histopathology, contractile function, electrophysiology, immunohistochemistry and biochemical assays involving more number of cases are required for better understanding and management of these problems.

Conflicts of Interests: None

REFERENCES

Amrish Tiwari et al., HISTOLOGICAL STUDY OF NEONATAL BOWEL IN ANORECTAL MALFORMATIONS.