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Sacral Anomaly and Pelvic Floor Muscle in Imperforate Anus:  
A Clinical and Experimental Study

YUKIHIRO INOMATA, KOICHI TANAKA, KAZUE OZAWA

Second Department of Surgery, Kyoto University School of Medicine, Kyoto.
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Summary

Influences of sacral anomalies on the postoperative continence of imperforate anus were evaluated clinically and experimentally. Postoperative continence was clinically assessed by the quantitative scoring system for 77 patients (44 of high-type anomalies and 33 of low-type anomalies). Incidence of combined sacral anomalies was 28.6% (38.6% in high type anomalies and 15.2% in low type anomalies). Combined sacral anomalies had harmful effects on the postoperative defecational function, especially on the parameters of rectal sensation and soiling. Patients with more than four segments of sacral vertebrae had relatively satisfying results on the postoperative assessment. For a teratological study to obtain a model of sacral anomalies, Donryu rats were used. Trypan blue was injected as the teratogen intraperitoneally on the ninth day of gestation. Of the 198 live fetuses, 5 rats had tail defect and sacral anomalies. Two of the 5 anomalous rats also had imperforate anus. Four of 5 rats had vertebrae intact above the first sacral segment. In these 4 rats the pelvic floor muscles had developed to some degree. All the vertebrae were agenetic below the thoracic segments in the other rat fetus. This rat had no pelvic floor muscles. These studies showed that the severity of sacral anomalies had a relationship to postoperative continence. Intact sacral vertebrae above the 4th in humans and the 1st in rats correlated with fair development of pelvic floor muscles and satisfying postoperative continence.

Introduction

In the surgical treatment of imperforate anus, maximal salvage of the pelvic floor muscles is the most effective step for the development of postoperative continence. STEPHENS is a pioneer in this procedure. Recently PENA has developed a new radical procedure for this congenital anomaly, but his philosophy is in more complete usage of the muscles. The radical operation for imperforate anus is almost completely successful, but some children still suffer from long-standing incontinence, and others cannot be free from the use of laxatives. Such difficult cases occasionally have some complicated handicaps, one of the most important of which is sacral anomaly. The mechanism by which the sacral anomaly effects the postoperative anorectal

Key words: Sacrum, Imperforate anus, Pelvis, Trypan blue, Rats.

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function is not clear in detail. This paper presents the results of clinical evaluation of the imperforate anus with sacral anomalies and the experimental study of teratogen-induced sacral anomaly in rats.

Materials

1. Clinical evaluation

One hundred fifty nine patients have been treated for imperforate anus in our institute from 1959 to 1985. Seventy seven of them were followed through clinical evaluation of postoperative function and X-ray examination of vertebral anomalies. Forty four cases had high type anomalies and 33 had low type anomalies (Table 1). To exclude the effect of aging, patients older than 4 years were chosen for this study.

2. Experimental animal

Mature male and female Donryu rats weighing 200-300 g were acquired from Nippon Rat Co. Ltd. They were housed in an air-conditioned room (23 ± 2°C) and kept under an alternating 12-hr light/dark schedule. A solid diet (Nippon CLEA Co. Ltd., Tokyo, Japan) and tap water were made available ad libitum.

Methods

1. Clinical evaluation

Sacral anomalies were examined in all the X-ray films of each patient taken from the neonatal period to the time of evaluation. Those included plain films with A-P and lateral views, urethograms, and barium enemas. If possible, the patients were asked to come to the outpatient clinic to be X-rayed. Postoperative continence were evaluated by means of a questionnaire sent to the patients or the parents. The results were scored in 4 parameters: rectal sensation, constipation, incontinence and soiling. Scores for rectal sensation and soiling were classified into 3 grades (0, 1, 2). Score for constipation was classified into 4 grades (1, 2, 3, 4) and score for incontinence into 5 grades (0, 1, 2, 3, 4). The smaller score in the parameters of constipation or incontinence was added to scores for rectal sensation and soiling to evaluate the general function. This scoring system was proposed by The Japan Study Group of Anorectal Anomalies. Statistical analysis was performed by chi-square analysis and Student’s t test.

<table>
<thead>
<tr>
<th>Type of Anomalies</th>
<th>High (n=44)</th>
<th>Low (n=33)</th>
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<tbody>
<tr>
<td>High Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectourethral fistula</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Rectovesical fistula</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Anorectal stenosis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Low Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectovesical fistula</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Rectovestibular fistula</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Rectocloacal fistula</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Anterior Perineal anus</td>
<td></td>
<td>1</td>
</tr>
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Table 1: Type of anorectal malformations (Kyoto University Hospital 1959-1985)
2. Experimental study

Male and female rats were allowed to cohabit overnight, and upon finding sperm in a vaginal smear at 10:00 AM the following morning, the day was regarded as day 0 of pregnancy. On the 9th day of pregnancy, trypan blue (Merck Co., Darmstadt, West Germany) was injected into the peritoneal cavity. The dose of trypan blue was 40 mg/kg body weight of animals. Just before each usage, trypan blue was dissolved in saline solution and made to a concentration of 20 mg/ml. On the 20th day of pregnancy, the pregnant rats were put to death by cervical dislocation, and the fetuses were obtained by hysterotomy. The external appearance of the living fetuses was examined for malformations. Vertebral anomalies were checked by soft X-ray examination of lateral and A-P views. As a control, fetuses were taken from a non-treated female rat in her 20th day of pregnancy. Fetuses with external anomalies were fixed in 10% formaldehyde solution. They were examined by horizontal or sagittal serial sections of the pelvic region and were examined histologically for the development of pelvic floor muscles.

Results

1. Clinical evaluation

In the evaluation of postoperative continence, patients with high-type anomalies had poorer results than those with low-type anomalies for the parameters of incontinence, soiling and summated scores (Table 2). Sacral anomalies were complicated in 17 cases of the high-type group

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Score of clinical evaluation for postoperative continence</th>
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<tbody>
<tr>
<td></td>
<td>high (n=44)</td>
</tr>
<tr>
<td>rectal sensation</td>
<td>1.72 ± 0.62</td>
</tr>
<tr>
<td>constipation</td>
<td>3.16 ± 0.83</td>
</tr>
<tr>
<td>incontinence*</td>
<td>2.95 ± 1.28</td>
</tr>
<tr>
<td>soiling*</td>
<td>1.14 ± 0.67</td>
</tr>
<tr>
<td>general*</td>
<td>5.36 ± 2.06</td>
</tr>
</tbody>
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(*: statistically significant p<0.01) (mean ± S.D.)

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<th>Table 3</th>
<th>Incidence of combined sacral anomalies</th>
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<tr>
<td></td>
<td>high (n=44)</td>
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<tr>
<td>male</td>
<td>rectourethral f.</td>
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<td></td>
<td>rectovesical f.</td>
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<td></td>
<td>anorectal stenosis</td>
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<tr>
<td>female</td>
<td>rectocloacal f.</td>
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<td></td>
<td>rectovestibular f.</td>
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<td>rectocloacal f.</td>
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17/44 (38.6%) 5/33 (15.2%) (f.; fistula)
Table 4  Score of clinical evaluation and sacral anomalies combined with high-type imperforate anus

<table>
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<tr>
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<th>without sacral anomaly (n=27)</th>
<th>with sacral anomaly (n=17)</th>
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<tr>
<td>rectal sensation*</td>
<td>1.96 ± 0.19</td>
<td>1.35 ± 0.86</td>
</tr>
<tr>
<td>constipation</td>
<td>3.26 ± 0.76</td>
<td>3.00 ± 0.94</td>
</tr>
<tr>
<td>incontinence</td>
<td>3.26 ± 0.90</td>
<td>2.47 ± 1.62</td>
</tr>
<tr>
<td>soiling*</td>
<td>1.33 ± 0.62</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>general**</td>
<td>6.07 ± 1.27</td>
<td>4.18 ± 2.53</td>
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(*: p<0.05, **: p<0.01)

and 5 cases of the low-type group (Table 3). The combined rate of sacral anomaly was significantly higher (p<0.01) in the high-type group than in the low-type group. In the high-type group, patients with sacral anomalies had poorer results than those without combined sacral anomalies, in the parameters of rectal sensation and soiling. Summated scores were also better for the group without sacral anomalies (Table 4). The grade of sacral anomalies ranged from minute dysplasia to agenesis. No definite relationship was found between the type of anomaly and the clinical score. However, patients with dysplasia and no defects of vertebrae had a tendency to show better results than patients with defective vertebrae. Fourteen of sixteen patients

Table 5  Sacral anomalies and scores of clinical evaluation

<table>
<thead>
<tr>
<th>Types of sacral anomalies</th>
<th>Scores of clinical evaluation</th>
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<tbody>
<tr>
<td></td>
<td>high (n=17)</td>
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who had at least 4 segments of sacral vertebrae had scores better than 6 points (Table 5).

2. Teratological study

In 198 living fetuses from 17 pregnant rats, five had partial or complete absence of the tail. In all of these 5 rats, vertebral anomalies were found. Its overall incidence was 2.5%. Imperforate anus was a complication in two of these externally anomalous animals (Table 6). Findings for these 5 cases are as follows.

1. Findings of external malformation and X-ray examination

(a) #4-7 (Fig. 1): female, body weight 4.0 g. Tail was short and fine. No other external anomaly was found. On X-ray examination, the vertebral column above the second sacral vertebra was normal. The third sacral vertebra had a defect of the bilateral transverse process, and below the 4th sacral segment vertebral segments could not be seen.

(b) #9-2 (Fig. 2): male, body weight 4.3 g. External anomaly; defect of tail and club foot of right side. It had imperforate anus. The vertebral column above the first sacral segment was intact. The 2nd and 3rd segments had no transverse processes and below the 4th, segments were defective.

(c) #14-1 (Fig. 3): male, body weight 4.1 g. It had a short tail; half the sizes of the controls. It had 4 normal segments of sacrum and 3 segments of coccyces.

(d) #14-12 (Fig 4): female, body weight 4.2 g. It was a sibling of #14-1. The tail was rudimentary, and was accompanied by imperforate anus. In the 2nd and 3rd sacral segments, bodies of vertebrae without processes were seen, and below the 4th segment the column was defective.

(e) #16-13 (Fig. 5): male, body weight 3.2 g. External anomaly; defect of tail and abdominal distension. The vertebral column was completely agenetic below the thoracic segment. This was the most serious vertebral anomaly of the 5 cases.

2. Findings of microscopic examination of pelvic floor

In the control animals, some muscular fascicles surrounded the rectum and urogenital tract in the section through the pubic symphysis and iliac bone (Fig. 6). These had connected the internal surface of the iliopubic bone and the presacral and precoccygeal region. These were named as "Musculus iliopubocaudalis". The abnormal configuration of pelvic floor muscles was as follows.

(a) (Fig. 7): Pelvic floor muscles were well developed. Caudal muscle was not seen. The external anal sphincter existed but was composed of very few muscle fibers.
Fig. 1 Fetus (a), 54-7. Female.
Perineum (left) and radiogram of skeleton (right)

Fig. 2 Fetus (b), 29 2. Male.
Perineum (left) and radiogram of skeleton (right)
Fig. 3 Fetus (c), z14-1. Male.
Perineum (left) and radiogram of skeleton (right)

Fig. 4 Fetus (d), z14-12. Female.
Perineum (left) and radiogram of skeleton (right)
Fig. 5 Fetus (c), 16-13. Male.
Perineum (left) and radiogram of skeleton (right)

Fig. 6 Fetus serving as a control. Female. Pelvic cavity. Horizontal section, x20.
Fig. 7 Fetus (a). Pelvic cavity. Left slice is more caudal than the right. Horizontal section, ×50.

Fig. 8 Fetus (b). Pelvic cavity. Sagittal section. ×20.
(b) (Fig. 8): It demonstrated imperforate anus and rectourethral fistula. The blind pouch of the rectum was surrounded by iliopubocaudal muscle. The caudal margin of the muscle was above the urethral bulb. This funnel-shaped muscular canal was shallower in the rat with sacral anomalies than in the control animal with an intact tail. The external anal sphincter was not seen as a muscular bundle.

(c) This rat had only a partial defect of the tail unaccompanied by defects of the pelvic floor muscles. Caudal muscle was also well developed.

(d) (Fig. 9): It had imperforate anus and rectoceleal fistula. The iliopubocaudal muscle surrounded the rectum and urogenital tract above the rectal blind pouch. Poorly developed muscular fibers were seen on the dorsal side of the cloacal canal. The external sphincter was not recognizable as a complete muscular bundle. Only scattered fiber was seen below the rectal pouch and this was supposed to be the rudimentary external anal sphincter.

(e) (Fig. 10): It had no pelvic floor muscles. The pelvic cavity was very narrow and the rectal canal went through loose connective tissue to the anus. In such a serious anomaly, the external anal sphincter was barely discernable.
The damaging effects of combined sacral anomaly on the postoperative defecational function of the imperforate anus have been reported by several authors. The mechanism of this effect had been supposed to originate from defective or incomplete development of the pelvic floor muscles and/or dysfunction of an anomalous and defective sacral nerve. Analysis of the mechanism and development of a reasonable treatment regimen will benefit patients with poor continence. In this study, using the scoring system for clinical evaluation of postoperative continence, we ascertained the poor results of patients with sacral anomalies. This system is becoming the standard in Japan and is available as a objective and quantitative scale for comparison of postoperative results. The significant difference was in the parameters of rectal sensation and soiling. For continence, the mean score was low in cases with sacral anomaly, but was statistically not significant. Williams pointed out that postoperative constipation of patients with sacral anomaly could be attributed to a poor sense of rectal distension. In our study, sacral anomalies had no significant effects on the score for constipation.

Severity of sacral anomaly had various effects on the defecational function. Patients with
dysplasia but no agenesis had better results, even if the dysplastic vertebra was a high sacral segment. However, strict statistical analysis was impossible due to the shortage of cases. STEPIJE17 reported that if the sacrum had more than 4 segments, the levator muscle would be normal, and if lesser than 3, the anomaly produced a dysfunction. Our results confirmed that the presence of more than 4 segments promised good postoperative function. SAeki14 also stated that cases with more than 2 defective segments of high-type anorectal malformation had poor function. YAMADA22 also pointed out the tendency toward poor results in cases with sacral anomaly, but he made no statistical conclusions. Pelvic floor muscle is controlled by somatic pudendal nerves, the origin of which is located in S2-41. The cell group located in the anterior horn of the S1-3 is called ONUF’s nucleus10 and is strongly suspected to be engaged in anorectovesical coordination19. Such neuronal architecture has a potential for injury in sacral anomalies so we cannot conclude that the poor defecational function in cases with sacral anomaly can be attributed only to poor muscular development. The effects of the nervous system can not be excluded. We conducted an experimental study of poor muscular development associated with sacral anomaly to compare with the results of our clinical study.

Trypan blue was first reported by GILLMAN5 in 1948 as a teratogen which produced many anomalies, mainly in the central nervous system. OHSHIMA12 et al. produced sacral anomalies in experiments using trypan blue and Donryu rats. However, the combination with imperforate anus in this system was only found in one rat fetus of GILLMAN’s report5. We produced two fetuses combining imperforate anus and sacral anomaly. Teratogens that cause vertebral anomaly are not rare and some agents, for example, ethylenthiourea8, retinoic acid23 and salicylate20, are known to cause a high rate of vertebral anomalies in combination with imperforate anus. Our model is unique, however, in that the vertebral anomalies are localized in the sacral region and are without any severe cranio-myoeloschisis. Clinically encountered cases are usually mild anomalies, so this combination of the Donryu rat and trypan blue will be useful as a clinical model. Teratogenicity of trypan blue is supposed to be related to the hematoma made around the distal tip of the spinal cord. The embryogenesis of the combination of imperforate anus and sacral anomalies could not be traced in this study due to the small number of anomalous cases.

In considering the results of this animal experiment, differences between the rat and human in the anatomy of the pelvic floor muscles should be taken into account. We must ascertain that the name “levator ani muscle” is used to represent the same anatomical structure in rats and humans. As the levator ani muscle, GREENE6 picked out the muscle surrounding the rectum. The origin of the muscle was on the dorsal surface of the bulbocavernous muscle. This had been thought to be the same muscle as the levator muscle in humans, and VENABLE19 also reported the muscle in the male rat. However, some disagreed and stressed that GREENE’s levator muscle was only present in the male rat17. When the pelvic floor muscles were classified into two groups1, those contributed to the pudendal nerve and those to the sacral plexus, the muscle identified by Greene was categorized as a pudendal nerve contribution. This indicated that Greene’s muscle originated from cloacal membrane and should be listed as a
muscle related to the genital organs. The iliopubocaudal muscle in rats is considered by this study, to be the same anatomical structure as the levator muscle in humans, as determined from the analysis of the rectum, bones and muscles in serial section. This result is supported by the reports of NAKANISHI and HAYES. The iliopubocaudal muscle is contributed by the iliopubocaudal nerve, which is derived from pudendal nerve plexus and is controlled by the 5th and 6th lumbar and the 1st sacral segments of the spinal cord. In our experiment, all anomalous fetuses but one had sacral defects below the 2nd sacral vertebra and had an intact or slightly maldeveloped iliopubocaudal muscle. One fetus with complete defect of the vertebral column below the thoracic segments had no striated muscle in the pelvic floor. These results indicate that the level of sacral anomaly and the development of pelvic floor muscle have a relationship. If the vertebrae above the 1st sacral one are intact, the iliopubocaudal muscle will be developed in some degree, even if the rat has combined imperforate anus. Origin of the pudendal plexus of rat are moved 3 segments cranially from those that of human beings, so S1 in rats refers to S4 in humans. From our animal experiment, if the sacrum above S4 is clinically intact, the levator muscle in humans will be well developed. This confirms our clinical evaluations and those of STEPHENS. Our model will be useful in future research of the nervous system which is beyond the scope of this study.

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和文抄録

鎖肛における仙椎奇形と骨盤底筋群の関係に関する
臨床的実験的研究

京都大学医学部第二外科
猪股裕紀洋 田中 紳一 小澤 和恵

鎖肛における合併仙椎奇形か、術後排便機能に与える影響を、臨床的な評価と、実験的に作成された仙椎
奇形合併ラットを用いて検討した。臨床的には、77例（高位型44例、低位型33例）の4才以上の患者を対象
に検討した。全例の仙椎奇形合併率は28.6％で、高位型では38.6％と低位型15.2％に比し、明らかに高率を
呈した。直腸肛門奇形研究会の評価法によって検討すると、便意と汚染の項目で、仙椎奇形合併鎖肛患者の
機能が不良であった。仙椎奇形合併例でも4椎骨節以上を有する症例では機能は相対的に良好であった。実
験モデルとして、妊娠第9日目のDONRYUラット

に trypan blue の単回腹腔内投与を行ない、198生仔
中、5例の尾欠損、仙椎奇形ラットを得た。うち2例
は鎖肛を合併しており、1例は直腸尿道瘻、1例は直
腸内挿瘻を併せていた。例中4例は、第一仙椎以上は正
常で、これらのラットでは、やや不良ながら骨盤底
筋群の形成がみられた。他の1例は胸椎以下全欠損で、
骨盤底筋群は無形成であった。これらの結果より、鎖
肛に合併した仙椎奇形は、骨盤底筋群の発育を介して
術後排便機能に悪影響を与え、その効果は仙椎奇形の
重症度にも左右される事が明らかとなった。