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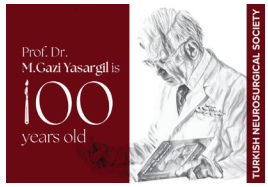

Evaluation of Inclusion Cysts and Granulation Tissue After Prenatal and Postnatal Myelomeningocele Repair

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ABSTRACT

AIM: To evaluate the long-term outcomes of nine patients who underwent myelomeningocele repair via fetoscopic surgery, open fetal surgery, and postnatal surgery.

MATERIAL and METHODS: The presence of inclusion cysts and the thickness of granulation tissues at the surgical site were analyzed using spinal magnetic resonance imaging (MRI) at a 7-year follow-up to determine their impact on clinical outcomes.

RESULTS: The spinal defect levels ranged from L2 to S2. Granulation tissue at the surgical site was thicker in the prenatal open and postnatal repair groups when compared to the fetoscopic repair group. Follow-up spinal magnetic resonance imagings (MRIs) detected an inclusion cyst in one patient from the fetoscopic repair group, whereas all patients who underwent prenatal open repair and the two who underwent postnatal myelomeningocele repair developed inclusion cysts. Clinical outcomes were more favorable in the fetoscopic repair group compared to those who underwent open repair. Patients who underwent prenatal repair exhibited varying degrees of neurogenic bladder dysfunction. Although none required urological intervention, their bladder function necessitated close monitoring, and their neurological outcomes were noticeably better than their urological outcomes.

CONCLUSION: We believe that inclusion cysts and granulation tissue affect the clinical outcome of patients after myelomeningocele repair and should be monitored during spinal follow-up.

KEYWORDS: Spina bifida, Myelomeningocele, Fetal surgery, Fetoscopic repair, Inclusion cyst, Granulation tissue

ABBREVIATIONS: MRI: Magnetic resonance imaging, **MOMS:** Management of myelomeningocele study

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INTRODUCTION

The benefits of fetal spina bifida repair over postnatal repair were established following the publication of the Management of Myelomeningocele Study (MOMS) (1). The key findings of that study included a higher rate of hindbrain herniation reversal, a reduced incidence of shunt-dependent postnatal hydrocephalus, and improved independent ambulation rates in patients who underwent fetal repair compared to those who had postnatal repair.

Spinal inclusion cysts (SICs) can develop when epidermal or dermal cells become implanted in the thecal sac, either embryologically or due to surgical intervention. The most common cause of SICs is spinal surgery, making them a recognized complication of such procedures. These cysts may necessitate reoperation due to neurological decline, radiological progression, or cyst enlargement. The reported incidence of SICs following postnatal spina bifida repair ranges from 2% to 16% (9,18,22). However, the occurrence of inclusion cysts following prenatal fetoscopic or open repair and their impact on clinical outcomes remain unclear.

This study evaluated the presence of inclusion cysts and the thickness of granulation tissue at the surgical site in patients who underwent prenatal fetoscopic, open, or postnatal myelomeningocele repair. It also examined the pathophysiology of inclusion cysts and granulation tissue formation at the surgical site and their impact on clinical outcomes in patients who underwent prenatal myelomeningocele repair.

MATERIAL and METHODS

This study retrospectively analyzed clinical and radiological data from patients followed at our center after myelomenin-gocele repair. Written informed consent was obtained from all participants. The study was conducted in accordance with the guidelines of the Declaration of Helsinki. and was conducted with the approval of the Institutional Ethics Committee (No 0618.2020).

Prenatal fetoscopic and open myelomeningocele repair procedures were performed at 26 or 27 weeks of gestation. The spinal defect levels ranged from L2 to S2. Fetoscopic repair involved reconstruction of the neural placode and dura mater closure. The procedure included mobilization of the sac, excision of epithelialized skin from the placode, incision into the arachnoid surrounding the placode, and circumferential release of the sac connection. Additionally, the rostral spinal cord was freed from the sac. Following neural placode reconstruction and dura mater closure, the defect was covered with a bovine pericardium patch, which was sutured to the skin.

In the prenatal open repair and postnatal repair groups, the surgical procedure involved sequential closure of the neural placode, dura mater, lumbar fascia, subcutaneous tissue, and skin. A bovine pericardium patch was not used in these patients.

This study included three patients from each surgical group: fetoscopic repair, prenatal open repair, and postnatal myelomeningocele repair, all treated at our institution. Spinal magnetic resonance imaging (MRI) scans, including axial and sagittal unenhanced and contrast-enhanced T1- and T2-weighted images, were analyzed 7 years postoperatively.

Neurofunctional assessment at the 7-year follow-up evaluated ambulation status, ambulation scale, and urological function. Ambulation was classified according to the Hoffer criteria (11) into four categories: community/public ambulatory, home ambulatory, nonfunctional ambulatory, or nonambulatory. Additionally, a separate category was included for patients with normal ambulation, defined as those who did not require a walking aid or wheelchair for mobility.

Public/community ambulators were defined as patients who could walk both indoors and outdoors for most activities, occasionally requiring crutches or braces. A wheelchair was only needed for long-distance travel outside their community. Household ambulators were those who could walk indoors with the aid of orthoses and could transfer in and out of a chair or bed with minimal or no assistance. However, they sometimes used a wheelchair for indoor activities at home or school and relied on a wheelchair for all community-based activities.

According to the Hoffer criteria, nonfunctional ambulators were patients who could walk during therapy sessions at home or school or in a hospital setting but depended entirely on a wheelchair for other forms of mobility. Nonambulators were those who could only move using a wheelchair but were generally able to transfer from the wheelchair to a bed independently.

The urological assessment included evaluating bladder sensation, use of clean intermittent catheterization, need for oral anticholinergic medication, bladder capacity, detrusor activity, bladder compliance, detrusor sphincter dyssynergia, maximum detrusor pressure, and post-void residual urine volume.

RESULTS

Spinal MRI at the 7-year follow-up detected an inclusion cyst in only one patient who underwent fetoscopic repair with a single-layer bovine pericardium patch. In contrast, inclusion cysts were present in all patients who underwent prenatal open repair and in two patients from the postnatal repair group (Figure 1).

Granulation tissue at surgical site was thicker in the prenatal open repair group (mean, 4.29 mm) and the postnatal repair group (mean, 3.57 mm) compared to the fetoscopic repair group (mean, 2.23 mm) (Figure 2). Although the sample size was insufficient for statistical analysis, fetoscopic repair was associated with thinner granulation tissue and fewer inclusion cysts than the other groups. The primary distinguishing factor between the groups was the use of a bovine pericardium patch in the fetoscopic repair group. Table I presents the granulation tissue thickness for each patient.

Ambulation status, ambulation scale, and constipation were assessed at the 7-year follow-up, along with a urological evaluation (including bladder sensation, use of clean intermittent catheterization, use of oral anticholinergic agents, bladder capacity, detrusor activity, bladder compliance, detrusor sphincter dyssynergia, maximum detrusor pressure, and post-void

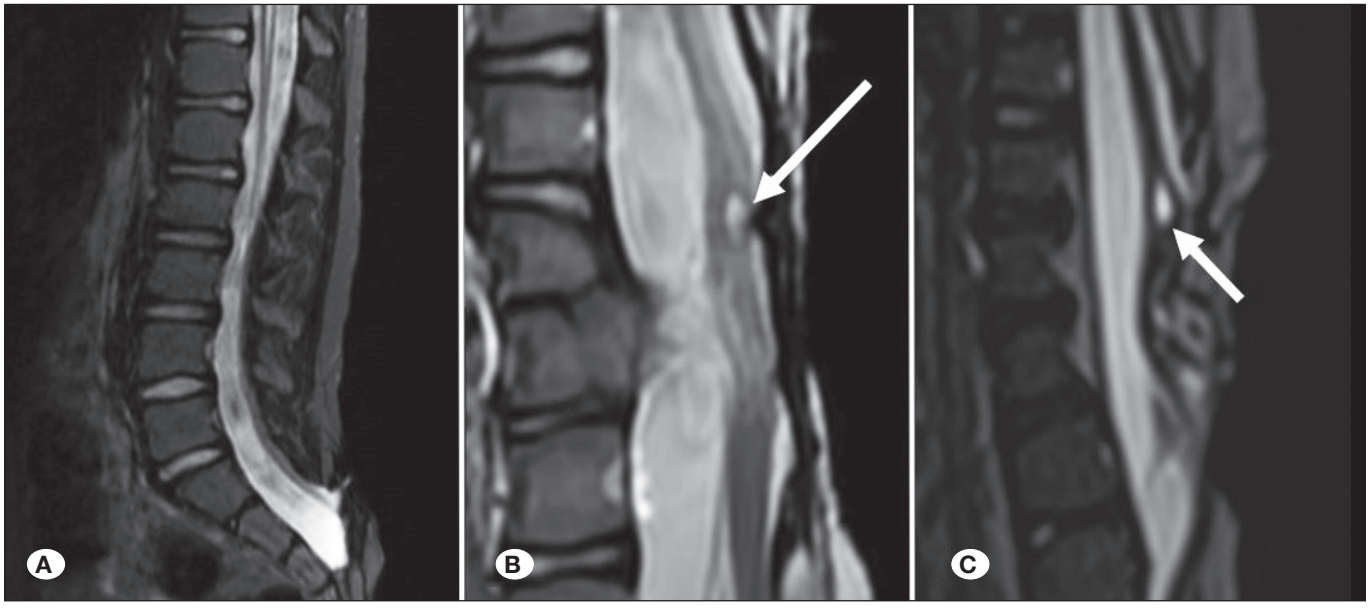

Figure 1: Sagittal spinal T2-weighted magnetic resonance imagings (MRIs) at the seven-year follow up: **A)** A patient in fetoscopic repair group, no inclusion cyst was determined. **B)** A case with inclusion cyst in open repair group. **C)** A case with inclusion cyst in postnatal repair group.

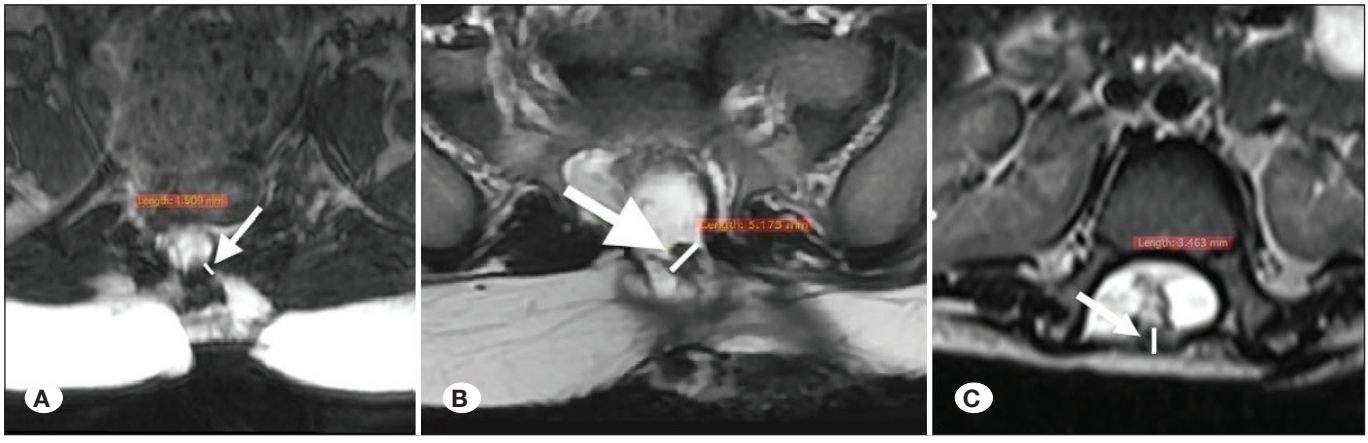

Figure 2: Axial spinal T2-weighted magnetic resonance imagings (MRIs) at the seven-year follow-up: Granulation tissue; **A)** A patient in the fetoscopic repair group, **B)** a patient in the open repair group, and **C)** a patient in the postnatal repair group.

residual urine volume). Table II presents the clinical outcomes for fetoscopic and open repair.

Ambulation was assessed using the Hoffer classification: community/public ambulatory, home ambulatory, nonfunctional ambulatory, or nonambulatory. An additional category was included for patients who were considered normal ambulators, meaning they did not require a walking aid or wheelchair for mobility.

When comparing ambulation between the fetoscopic and prenatal open repair groups, patients who had a bovine pericardium patch repair showed better clinical outcomes. Two of these patients were classified as normal ambulators, and the patient who developed an inclusion cyst on spinal MRI was able to walk indoors and outdoors for most activities, occasionally using crutches or braces for mobility.

At the 7-year urological evaluation, all patients had bladder sensation; however, one patient in the prenatal open repair group, who also had the thickest granulation tissue (5.17 mm), required clean intermittent catheterization and reported chronic constipation. None of the patients in the fetoscopic repair group had constipation.

Table III presents the urological evaluations of patients who underwent fetal surgery. The fetoscopic repair group demonstrated better bladder capacity. All patients exhibited varying degrees of neurogenic bladder dysfunction. Although none required urological intervention, their bladder profiles required close monitoring, and their neurological outcomes were clearly better than their urological outcomes.

DISCUSSION

Hutchins et al. and Meuli et al. investigated acquired spinal cord injury in fetuses with myelomeningoceles and its impact on fetal surgery (12,17). Their findings supported the idea that fetal surgery could protect the exposed, initially intact spinal cord, prevent secondary neural injuries, and preserve neural function in fetuses with spinal dysraphism. The "two-hit" theory was proposed to explain the motor deficits observed in myelomeningocele patients: congenital myelodysplasia combined with intrauterine spinal cord injury as gestational age progresses (8).

Table I: Measurement of Granulation Tissue at the Surgical Site

Case No.	Type of Surgery	Granulation Tissue Thickness-millimeters
1	Fetoscopic	1.51
2	Fetoscopic	2.97
3	Fetoscopic	2.21
4	Open hysterotomy	4.48
5	Open hysterotomy	5.17
6	Open hysterotomy	3.22
7	Postnatal	3.46
8	Postnatal	4.47
9	Postnatal	2.78

Heye et al. acknowledged the benefits of fetoscopic repair in promoting independent ambulation in spina bifida patients. However, 30% of their patients developed inclusion cysts within the first 2 years of life, and they concluded that inclusion cysts could lead to neurological function loss, which they termed a "third hit" (10).

The pathophysiology of inclusion cysts following spina bifida repair, and whether their origin is embryonic or iatrogenic, remains a topic of debate. The precise effect of inclusion cysts on clinical outcomes is still unclear.

After fetoscopic spina bifida repair, Danzer et al. identified inclusion cysts in 10 out of 54 patients at a median age of 27 months (5). Following the surgical removal of these cysts, four patients experienced loss of normal bladder function and required clean intermittent catheterization, while one patient suffered a loss of normal motor function in the lower extremities. Their study highlighted that inclusion cysts are not benign. In contrast, none of our patients experienced neurological decline or impaired urinary function. In our cohort, no patient required reoperation for an inclusion cyst.

In a study three patients who underwent open spina bifida repair, Mazzola et al. found normal motor function in the lower

Table II: Clinical Outcome After Fetoscopic and Open Repair at the Seven-Year Follow-Up Examination

Case No.	Type of surgery	Anticholinergic use	Bladder capacity (ml)	Detrusor activity	Compliance	DSD	MDP (cmH_2O)	PVR (ml)
1	Fetoscopic	(+)	104	OAB	Low	(+)	60	107
2	Fetoscopic	(+)	120	OAB	Low	(+)	180	82
3	Fetoscopic	(+)	180	OAB	Normal	(+)	70	166
4	Open	(-)	150	OAB	Normal	(-)	50	44
5	Open	(+)	100	OAB	Normal	(+)	50	25
6	Open	(+)	34	OAB	Low	(+)	170	28

OAB: *Overactive bladder,* ***DSD:*** *Detrusor sphincter dyssynergia,* ***MDP:*** *Maximum detrusor pressure,* ***PVR:*** *Postvoiding residual urine.*

Table III: Seven Years Long-Term Urological Outcome of the Patients After Fetal Surgery

Case No.	Type of surgery	Anticholinergic use	Bladder capacity (ml)	Detrusor activity	Compliance	DSD	MDP (cmH_2O)	PVR (ml)
1	Fetoscopic	(+)	104	OAB	Low	(+)	60	107
2	Fetoscopic	(+)	120	OAB	Low	(+)	180	82
3	Fetoscopic	(+)	180	OAB	Normal	(+)	70	166
4	Open	(-)	150	OAB	Normal	(-)	50	44
5	Open	(+)	100	OAB	Normal	(+)	50	25
6	Open	(+)	34	OAB	Low	(+)	170	28

OAB: *Overactive bladder,* ***DSD:*** *Detrusor sphincter dyssynergia,* ***MDP:*** *Maximum detrusor pressure,* ***PVR:*** *Postvoiding residual urine.*

extremities and no bladder dysfunction at birth (15). However, spinal MRI, conducted due to the development of motor function loss in both legs in two patients and bladder dysfunction in one, revealed inclusion cysts linked to spinal cord tethering. They concluded that patients who have undergone prenatal spina bifida repair should be closely monitored for any deterioration in motor or bladder function.

In our study, when comparing ambulation between the fetoscopic and open repair groups, we found that patients who had the bovine pericardium patch repair showed better clinical outcomes. Two patients were normal ambulators, and one patient could walk both indoors and outdoors for most activities, rarely needing crutches or braces for mobility.

Mazzone et al. examined the impact of fetal spina bifida repair on urological outcomes and suggested that fetal spina bifida repair might improve lower urinary tract function. They reported that 32% of their patients had normal urodynamic studies during follow-up (15,16). Pastuszka et al. concluded that prenatal spina bifida repair led to significant improvements in social urinary continence, thus reducing the risk of urinary tract infections and constipation (20). Gerber *et al*. found a lower incidence of high-risk bladders in patients who underwent fetoscopic myelomeningocele repair, with a trend toward clinically significant improvements compared to those who had open repair, in all evaluated parameters (6). While the long-term urological outcomes of patients after fetal myelomeningocele repair remain debated, recent studies have emphasized the limited contribution of fetal surgery to improving lower urinary tract function. The MOMS investigators evaluated bladder function in patients at 30 months and at school age. Although fetal repair showed some positive outcomes, such as reduced need for catheterization, decreased bladder trabeculation, and increased volitional voiding, the urodynamic results were comparable to those of patients who underwent postnatal repair (2,3). Macedo et al. and Parizi et al. prospectively followed their patients and found that antenatal surgery contributed minimally to bladder function (14,19). Our findings align with these contemporary studies. All our patients exhibited neurogenic bladder dysfunction to varying extents. While none required urological intervention, their bladder profiles required close monitoring, and their neurological outcomes were clearly better than their urological outcomes.

In their milestone study comparing prenatal and postnatal spina bifida repair, Adzick et al. found that prenatal surgery reduced the need for shunting and improved motor outcomes at 30 months (1). They concluded that ongoing follow-up is essential to determine whether these early benefits are sustained and to evaluate the impact of fetal surgery on bladder and bowel continence.

In our study, constipation was not observed in the fetoscopic group but was present in one patient from the open repair group. Follow-up urological evaluation showed that all patients had bladder sensation, though one patient in the open repair group required clean intermittent catheterization.

To the best of our knowledge, no previous human studies have measured granulation tissue at the surgical site after myelomeningocele repair. In an animal model studying the local host response to commercial dura patches used for fetal repair of spina bifida aperta, Kunpalin et al. demonstrated that covering the rabbit spinal cord with dura patches and skin closure preserved motor neuron density and reduced the inflammatory response (13). In our study, we measured the granulation tissue at the surgical site and found it to be thicker in the open (mean, 4.29 mm) and postnatal (mean, 3.57 mm) groups compared to the fetoscopic repair group (mean, 2.23 mm). These results suggest that the use of a dural patch results in less tissue reaction.

The surgical approach for spina bifida repair involves a multilayer closure of the neural placode, dura mater, lumbar fascia, subcutaneous tissue, and skin. While the closure of the neural placode and dura mater has remained consistent over the years, different methods for soft tissue closure are still debated in the medical literature. Materials used as scaffolds or defect coverings in animal models include collagen- and gelatin-based scaffolds, small intestine submucosa, and polymers such as silicone, high-density polyethylene, and polypropylene (23). Gurer et al. successfully used bovine pericardium patches combined with fibrin sealant at the fascial level between the dural sac and skin in the postnatal repair of open spina bifida (7).

Another study demonstrated successful fetoscopic repair using a biocellulose-based patch (Bionext®. Bionext, São Paulo, SP, Brazil) over the neural placode, resulting in a good neurological outcome (4).

In our study, the fetoscopic repair group, which used a bovine pericardium patch, had thinner granulation tissue compared to the other groups. In the open and postnatal repair groups, a primary multilayer closure of the neural placode, dura mater, lumbar fascia, subcutaneous tissue, and skin was performed.

Our results suggest that the increased granulation tissue in open fetal surgery is likely due to greater compression of the underlying tissue after the closure of the paravertebral fascia, subcutaneous tissue, and skin. The bovine pericardium patch used in fetoscopic surgery may help protect the neural tissue, leading to relatively less compression of the neural placode, fewer inclusion cysts, and consequently, less granulation tissue. A study investigating the impact of allograft patch closure on the incidence of inclusion cyst formation following open fetal spinal dysraphism (myelomeningocele) repair found that the use of a dural allograft patch appears to be positively associated with the formation of inclusion cysts (21). The same study indicated that patients undergoing open fetal myelomeningocele repair were more likely to develop inclusion cysts and related symptoms. Based on the existing studies, we believe that larger sample size studies with long-term follow-up are necessary to better understand the exact pathophysiology of inclusion cysts, as well as the effect of both the cysts and increased granulation tissue at the surgical site on the clinical outcomes of patients.

CONCLUSION

We believe that inclusion cysts and granulation tissue affect the clinical outcome of patients after myelomeningocele repair and should be monitored during spinal follow-up.

The use of a bovine pericardium patch in spinal repair appears to protect the neural tissue by reducing the compression of the placode, leading to less granulation tissue and fewer inclusion cysts. While the initial findings are promising, larger studies are needed to evaluate the impact of patch use in fetal myelomeningocele repair.

Declarations

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Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author by reasonable request.

Disclosure: The authors declare no competing interests.

AUTHORSHIP CONTRIBUTION

Study conception and design: IA, SKO, BO, LAA, HC, RC, OT, DU

Data collection: IA, SKO, BO, LAA, HC, RC, OT, DU

Analysis and interpretation of results: IA, SKO, BO, LAA, HC, RC, OT, DU

Draft manuscript preparation: IA, SKO, BO, LAA, HC, RC, OT, DU, GBS

Critical revision of the article: IA, SKO, BO, LAA, HC, RC, OT, DU, GBS

All authors (IA, SKO, BO, LAA, HC, RC, OT, DU, GBS) reviewed the results and approved the final version of the manuscript.

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