

Experimental Research

## Grading of epidural fibrosis in a new rabbit experimental model for epidural adhesive scar tissue formation

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### ARTICLE INFO

### ABSTRACT

#### Article History

Received 15 / 09 / 2009

Accepted 06 / 04 / 2010

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#### Key Words :

Peridural Fibrosis  
Experimental Model  
Hemilaminotomy  
Grading Of Epidural Fibrosis  
Rabbit  
Spine

This experimental study was planned to evaluate the success of rabbit hemilaminotomy procedure and proposing of a new grading system in the evaluation of epidural fibrosis. Fourteen New Zealand white rabbits were used in this experiment. Hemilaminotomy was performed in one level and 4 weeks later after operation rabbits were sacrificed. The lumbar spines were removed and immersed in 10% neutral buffered formalin for approximately 24 hours. Then each specimen was decalcified in 5% formic acid for approximately 3 weeks. Specimens were cut coronally for gross inspection. We proposed a new grading system in the evaluation of peridural fibrosis. In seven (50%) of the rabbits of hemilaminotomy sites, fibrous tissue penetrated into the spinal canal through the bone defect and produced neural compression with reaching to the posterior longitudinal ligament (Grade IV). Four hemilaminotomy sites were in Grade III fibrosis. Remaining 3 rabbits had Grade II fibrosis. In this experimental study, we modified the rabbit total laminectomy model by using of hemilaminotomy procedure in the studying of peridural scar formation. In the same time we proposed a new grading system in the evaluation of epidural scar tissue formation.

*J. Exp. Clin. Med., 2009; 26:107-111*

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## 1. Introduction

Despite intensive experimental and clinical researches, excessive scar tissue formation is a cause of failed back surgery syndrome after spinal operations (Abitbol et al., 1994; Gabriel, 1996; Çokluk and Aydın, 2005). Although many researches focusing on the prevention of fibrotic scar tissue formation around the dura mater and spinal roots, this pathological process still is a cause of unsuccessful lumbar surgery (Nussbaum et al., 1990; Minamide et al., 1999).

Many experimental studies have been used the laminectomy model for studying of excessive scar tissue formation in rabbits (Robertson et al., 1993; Dogulu et al., 2003; Çokluk and Aydın, 2005). But the laminectomy model is not completely reflecting the clinical counterparts. In clinical practice, hemilaminotomy is commonly used in the operations for lumbar disc herniations. Hemilaminotomy and laminectomy is practically different surgical interventions.

We previously developed a new experimental

model in the evaluation of epidural scar tissue formation in rabbits. We used this model in the rabbit lumbar spinal column and compared it with the laminectomy procedure in an animal experiment. In this experimental study it was evaluated the success of this model in the aspect of the amount of newly synthesized scar tissue, the degree of compression, the severity of adhesions, the advancement and the invasion of scar tissue to the potential space and grading of the scar formation.

## 2. Material and Methods

Fourteen New Zealand white rabbits weighing 250-300 grams were used in this experiment. The animals were housed in the same room but separate individual cages at the Ondokuz Mayıs University Animal Laboratory for Surgical Research. The animals were allowed free access to food and water in their cages with a 12 hour light/dark cycle and a temperature of 22°C. All the rabbits were fed a standard rabbit diet and given 3-4 days for acclimatization to the new surroundings at the animal laboratory in order

to reduce the stress which caused by transportation and changing of the environment.

The rabbits were anesthetized with Ketamine (35 mg/kg) and Xylazine (5 mg/kg). The lower half of the back was shaved with an electric clipper. A preoperative dose of intramuscular penicillin (30 000 U/kg) was given. Each of the rabbits was positioned prone on the operating table above a heating blanket with slight lumbar flexion produced by a small towel placed beneath its abdomen. A mixture of oxygen with nitrous oxide and 1% to 2% halothane was administered via a face mask to provide anesthesia and analgesia.

The lumbosacral area was cleaned with providone iodine (Betadine) soap and solution. The surgical area was draped with an aseptic transparent surgical drape

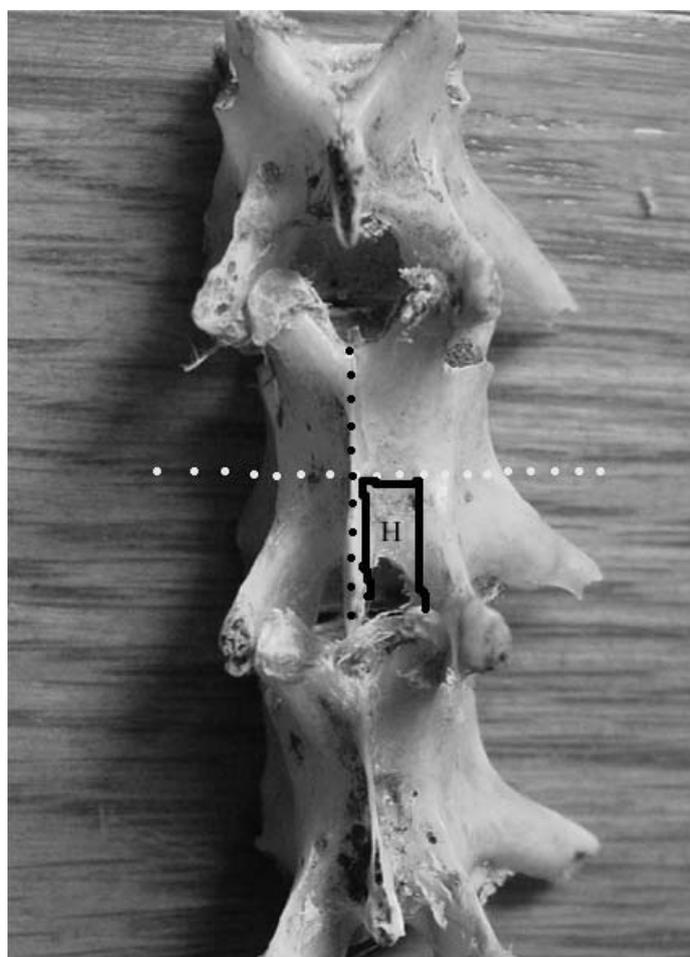
to maintain aseptic condition. Around the surgical area was covered with a sterile cotton towels. Surgery was performed under the operating microscope (Zeiss Opmi).

A midline skin incision was made from L1 to L5. The lumbosacral fascia was cut bilaterally on the lateral edge of the spinous processes. The paraspinous muscles were subperiosteally dissected from the spinous process and laminae of L2 to L4. Self retaining retractor was used for retraction of the paraspinous muscles.

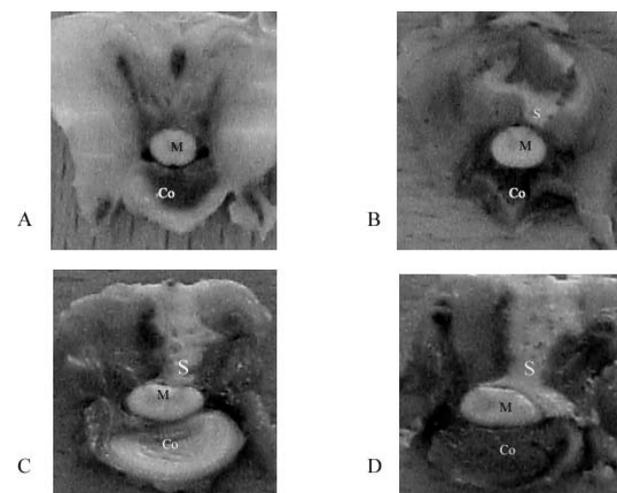
Hemilaminotomy was performed at L4 level on the right side (Fig. 1). Hemilaminotomy defect, 2 mm in wide and 5 mm in long was made in left lamina with G4-325D midas-rex diamond drill tip. Ligamentum flavum was removed with micro-scissor. Hemilaminotomy defect was also irrigated with sterile saline solution. 4-0 Vicryl sutures were used for suturing of the lumbosacral fascia. The skin was closed with 3-0 silk sutures.

The rabbits were transferred to its cages for recovery from anesthesia. One additional intramuscular injection of Penicillin (30 000 U/kg) was given approximately 24 hours postoperatively.

Animals were sacrificed at 4 weeks by a lethal dose of pentobarbital (60 mg/kg) administered via an ear vein.



**Fig. 1 :** Rabbit cadaver spinal bones in postero-anterior view. Continuing line shows the area of hemilaminotomy defect in rabbit cadaver spinal bone (white dots shows axial line, black dots shows sagittal line, H: hemilaminotomy area).



**Fig. 2 :** A. Gross macroscopic appearance of normal un-operated lumbar spinal column of a rabbit (M: Medulla spinalis, Co: Corpus of the vertebra). B. The appearance of Grade II scar tissue formation after hemilaminotomy procedure (M: Medulla spinalis, Co: Corpus of the vertebra, S: Scar formation). C. The appearance of Grade III scar tissue formation after hemilaminotomy procedure (M: Medulla spinalis, Co: Corpus of the vertebra, S: Scar formation). D. The appearance of Grade IV scar tissue formation after hemilaminotomy procedure (M: Medulla spinalis, Co: Corpus of the vertebra, S: Scar formation).

**Table 1.** Grading of epidural scar tissue in according to gross inspection findings

Grades	Gross inspection findings from coronally sectioned specimens
Grade 0	There is no visible scar tissue formation
Grade I	Scar tissue is located only in the paravertebral muscles
Grade II	Hemilaminotomy or laminectomy defect was full-filled by scar tissue without neuronal compression
Grade III	Hemilaminotomy or laminectomy defect was full-filled by scar tissue with neuronal compression
Grade IV	Scar tissue reaches to the posterior longitudinal ligament with neuronal compression

**Table 2.** Results of hemilaminotomy procedures.

Evaluation Parameters	Hemilaminotomy	
	n	%
Grade 0	-	-
Grade I	-	-
Grade II	3	21.4
Grade III	4	28.6
Grade IV	7	50
<b>Mean score</b>	<b>3.28 ± 0.82</b>	

They were perfused with 10% neutral buffered formalin solution.

The lumbar spines were removed en-bloc from L1 through L5. The cranial portion was marked with a metallic marker. The specimen was immersed in 10% neutral buffered formalin for approximately 24 hours. Each specimen was then decalcified in 5% formic acid for approximately 3 weeks. Specimens were cut coronally for gross inspection.

Peridural scar tissue on gross inspection in coronally sectioned specimens was graded into five grades as showed in Table 1. According to our epidural fibrosis grading system, Grade 0 is used for the description of the absence of epidural fibrotic tissue formation (Fig. 2A). In Grade I rabbits, scar tissue is present only in the paravertebral muscles. In Grade II epidural scar tissue formation, hemilaminotomy defect was full-filled by scar tissue without neuronal compression. In the other hand, in the rabbits of Grade III epidural scar tissue formation group, hemilaminotomy defect was full-filled by scar tissue with neuronal compression. The last grade is Grade IV. In this grade, scar tissue reaches to the posterior longitudinal ligament with neuronal compression. In our grading system, Grade II and Grade III is separated each other with the presence or absence of neuronal compression created by newly synthesized scar tissue.

In the scoring of peridural fibrosis, we used a simple technique with giving a corresponding numerical number to their grades. Grade 0 was scored with the number zero. Grade I epidural scar tissue was scored with 1 point, Grade II with 2 point, Grade III with 3 point, and Grade IV with 4 point.

### 3. Results

All animals were healthy without any neurological deficit. There were no instances of superficial infection. Three were no died rabbits after the operation.

In seven (50%) of the rabbits of hemilaminotomy sites, fibrous tissue penetrated into the spinal canal through the bone defect and produced neural compression with reaching to the posterior longitudinal ligament (Grade IV) (Fig. 2D).

Four (28.6%) hemilaminotomy sites were in Grade III fibrosis (Fig. 2C). In these sites, fibrotic scar tissue penetrated into the hemilaminotomy defect and compressed the neural structures to the opposite direction. Remaining 3 (21.4%) rabbits had Grade II fibrosis (Fig. 2B). In these levels, scar tissue penetrated into the hemilaminotomy site, adherent to the dural sac without neural compression.

The total score of hemilaminotomy procedure was found as 46 point according to our scoring system. The mean score was found as  $3.28 \pm 0.82$  in this procedure (Table 2).

### 4. Discussion:

Recurrence or persistency of low back pain and/or syatalgia after lumbar spinal surgery affects 5-40% of patients. Many of these patients require reoperation or other medical complex treatment modalities. Reoperations or other treatment modalities are not as much as successful than those of primary surgical intervention. At the end of all efforts, patients may undergo failed back surgery syndrome after his/her spinal surgical procedure despite his/her high expectancy of free from pain. This situation of a patient is not better than those of his/her first situation in terms of before the decision of the first surgery. The name of this new condition is failed back surgery syndrome. Numerous factors may be involved in failed back surgery including instability, radicular trapping that prevents the root from following its foraminal route, and inadequate or faulty surgical technique. The association of peridural fibrosis with low back pain and/or syatalgia is very clear from the results of clinical studies and personal experiences. Clinical symptoms are related to the biomechanical disability of neural, ligamentous, muscular and bone structures after the surgical intervention (Abitbol et al., 1994). Pain is related with the compression, adhesions, and entrapment to the neural structures caused by newly synthesized fibrotic tissue (Abitbol et al., 1994; Gabriel, 1996). Epidural fibrosis not only results with pain but also it results with sensory and/or motor deficiency in related dermatomes. The factors which affect the clinical findings are the amount of newly synthesized scar tissue, the degree of neuronal compression, the severity of adhesions, advancement and invasion of scar tissue into the potential spaces. Because of this efforts should be made to prevent epidural scar tissue formation and its invasion.

Although many clinical and experimental researches have been done, the exact solution for the prevention of epidural fibrosis can not be found. Despite many experimental studies focusing on the prevention of fibrotic scar tissue development around the dura mater in spinal space, this pathological processing is continuing as a main cause of unsuccessful lumbar spinal surgery. Multifactorial causes may affect the formation of excessive scar tissue (Gabriel and Friedman, 1996). Many factors related with the surgical instruments and surgical techniques can play a role in the progressing of this devastating pathological condition (Abitbol et al., 1994;

Gabriel and Fredman, 1996; Dogulu et al., 2003; Cokluk and Aydin, 2005)

Laminar bone of the lumbar vertebrae and the ligamentum flavum are the main mechanical bio-barriers between the dura mater and paravertebral soft tissues such as muscles and ligaments. In a surgery for lumbar disc herniation, a surgical corridor should be done by removing of some amount of laminar bone and the ligamentum flavum for excision and removing of the herniated disc materials. After finishing the surgical procedure, with the creation of a corridor or an open door (a piece of laminar bone and ligamentum flavum), neural structures of the spinal space lost their bio-barriers which separate them from the paravertebral soft tissues. Fibrotic tissue or scar formation begins in the paravertebral soft tissue with the promoting of surgical trauma. After the operation, newly synthesized scar tissue may easily pass through the hemilaminotomy opening.

Paravertebral soft tissues are not the only responsible origin in the production of adhesive excessive scar formation. Soft tissues involving the fibroblastic cells located in the peridural space may be another responsible origin in the development of scar formation. Briefly the origin is not only outside from the spinal space but also inside and located around the dura mater. But mainly the origin of scar formation is the paravertebral soft tissues.

At this point, we should take into consideration the function of epidural fat tissue. Its location, composition, and function are not clear enough. Its preventive effect in the developing of excessive scar tissue formation is also not clear. It may have many function in terms of biomechanical properties of the spinal dura mater and related structures. Blood supply, elasticity, to become a soft cousin for neural structures are other possible function attributed epidural fat tissue in the spinal space. The progression of scar tissue is multidirectional into the spinal canal around the neural structures (Dogulu et al., 2003).

During the surgical intervention, muscular, ligamentous and bone corridor are created for exposing and removing of disc material. This corridor can be created small or large in size according to the type of surgical intervention. A hemilaminotomy procedure with removing of a piece of laminar bone and ligamentum flavum is generally used for reach down to the affected disc level. After the operation, newly synthesized fibrotic tissue can produce new neurological symptoms and lead to the failed back surgery even in small hemilaminotomy openings (Abitbol et al., 1994; Gabriel and Friedman, 1996).

Previous experimental studies used laminectomy procedure in the rabbit peridural fibrosis model. The advantage of total laminectomy is that it is easy to do it in a small rabbit back. But this is not a counterpart model with the human hemilaminotomy procedure. We modified this model with the modification of hemilaminotomy instead of laminectomy in the rabbit lumbar spine. In clinical practice, many cases with peridural excessive scar have been operated by hemilaminotomy procedure.

Experimental models in spinal surgery should be similar with those of clinical counterparts in main procedural aspect. Our experimental model is similar to those of hemilaminotomy in clinical practice. Because of this, this modified model is superior to laminectomy model in the evaluation of peridural scar formation. Practically it is easy and simple to perform by using a microsurgical drill and instruments under the microscope.

In this experimental study, we proposed a useful grading scale for the gross evaluation of epidural scar tissue formation. According to this grading system, the amount of newly synthesized scar tissue based on the progression to the spinal spaces was divided into five grades. The first grade is the zero grades. The meaning of zero grades is the absence of scar tissue around the dura mater and paravertebral space except the common changes attributed to a surgical intervention. The following grade is Grade I. This grade is the minimum grade containing scar formation. The location of the newly synthesized scar formation is only located in the paravertebral muscles. There is no scar around the dura mater and into the hemilaminotomy opening. In this study, there is no Grade 0 and Grade I rabbits.

Grade II, III, and IV lumbar spine has newly synthesized scar formation in the paravertebral muscles, hemilaminotomy opening and epidural spaces. In the absence of neuronal compression, we graded this situation as Grade II. In this grade scar tissue is full-filled to the hemilaminotomy opening, and reached to the dura mater and/or epidural spaces, but there is no the gross findings of neuronal compression. Grade III and Grade IV have some amount of neuronal compression. In grade IV newly synthesized scar tissue formation reached to the posterior longitudinal ligament or posterior surface of the vertebral body. In Grade III, scar tissue compress to the neuronal structures to the opposite site, but it can not reach to the posterior longitudinal ligament or anterior surface of the dura mater.

For scoring of the scar formation, we proposed to use the corresponding numerical numbers such as 0, 1, 2, 3, 4. For Grade 0, it can be used 0 for scoring of this grade of scar formation. In the Grade I, it can be numerated with 1 as the scoring of pathology. Similarly Grade II can be scored with 2, grade III with 3 and Grade IV with 4. Mean scoring, standard deviation and statistical analysis can be done with these numerical values.

## 5. Conclusion

In this experimental study, we proposed a modified rabbit experimental model in the evaluation of peridural fibrosis. The compressive effect of newly synthesized scar tissue, adhesions to the dural surface, advancement of the fibrotic tissue into the biomechanically important potential space of the vertebral column may be evaluated in this modified model with gross and histopathological observation. On the other hand, the similarity of this model to the clinical counterpart (human hemilaminotomy procedure) is its

main advantage. Our suggestion is to use this model and evaluation of peridural fibrosis. grading system in the experimental studies focused on the

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