

# INTERVERTEBRAL SPACE INFECTION : AN EXPERIMENTAL MODEL

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## ABSTRACT :

*Intervertebral space infections are rarely seen complications of lumbar discal hernia operations. However they are important because their definite diagnosis and treatment are difficult, their treatment is expensive and they cause loss of work. The treatment is medical except some special cases and it's very hard to grow the pathogen microorganism from the cultures of both blood and intervertebral space specimens. Because of these reasons sufficient knowledge about the bacteriological and histopathological changes couldn't have been obtained.*

*In this experimental study, a intervertebral space infection model was made in rats under laboratory conditions. The groups to which *S.aureus*, *K.pneumoniae*, *P.aeruginosa* and physiological saline had been inoculated, were examined microbiologically and histopathologically after three weeks. Among the inoculated microorganisms, *S. aureus* continued its activity and caused disestructive changes after this period of time. Also vacuolar myelopathia was detected in the spinal cord and spinal roots of all three pathogen inoculated groups.*

**Key Words:** *Intervertebral space infection, experimental model.*

## INTRODUCTION

The etiopathogenesis of intervertebral space infections which are among the main problems of intervertebral discal hernia operations has not been determined and because they are usually treated medically, the histopathological changes occurring within the intervertebral space has not been observed clearly. So experimental models need to be done to light this subject and to determine the histopathological changes.

It has not been decided whether or not the pathology of intervertebral space infections are a result of an active, acute infection because the pathogen can only be isolated in 50% of the cases and the clinical symptoms occur 3 weeks later than the operation. Although *S.aureus* has been responsible for most of these infections, many interspace infections caused by various pathogens which resulted in different pathological changes has been reported.

Under the view of this information, an easily performed intervertebral space infection model has been designed by using albino rats. Various pathogens had been inoculated and the existence of active infection & the histopathological changes occurred in the tissues after three weeks which is the time for the appearance of clinical symptoms, has been investigated.

## MATERIAL AND METHODS

In this study, albino rats having similar properties which were raised in the laboratories of Dokuz Eylül University Faculty of Medicine, Department of Experimental Surgery have been used. The rats were from the race of *Rattus norvegicus* having 87.5% homogeneity and weighing  $250 \pm 15$  grams in average. A total of 28 rats, four groups consisting of 7 rats each, have been used for the study.

Following microorganisms according to the frequency that they cause nosocomial infections were inoculated to the intervertebral disc space of the rats: *Staphylococcus aureus* to the first group, *Klebsiella pneumoniae* to the second, *Pseudomonas aeruginosa* to the third, and sodium chloride 0.85% to the control group. The microorganisms have been prepared in the Department of Microbiology and inoculated within minutes. The bacteria were cultured in brain heart infusion broth and incubated at 30°C for 18 hours. The broth cultures were centrifugated at 4500 rpm for 30 minutes and the supernatants were thrown. The sediments had been washed three times with sodium chloride 0.85% and suspended in sodium chloride 0.85% to contain  $1-2 \times 10^8$  bacteria/ml. 0.1 ml of these cultures were also inoculated to blood agar plates and checked the number of bacteria.

The manipulations were done in department of Experimental Surgery operation room, under sterile conditions by using microscope. The rats were anesthe-

sized by Ketalar [di 2- (0-clorophenil) - 2 - (methyl amino) cyclohecsanon hydrochloride 8 mg/100 gr] intraperitoneally. Then the rats were fixed in prone position. The skin of the thoracolumbar junction was shaved and the skin and subcutaneous tissue were opened under sterile conditions. The thoracolumbar fascia was cut from 0.5 cm left side of the median line and the paravertebral muscles were dissected bluntly. The intervertebral disc was seen laterally and the microorganisms were inoculated to this region by PPD syringe. Following the inoculation fascia, subcutaneous and cutaneous tissues were closed by using 4/0 silk suture.

After the inoculations, the rats were observed till they were in total sleep and they were transferred to their cages where they were kept at  $20\pm 2^{\circ}\text{C}$  in 12 hours of light and 12 hours of dark conditions for three weeks. After this period, the rats were decapitated with ether and regions of 3 cm's of vertebral columns having the inoculation point at the center with their paravertebral tissues were removed under sterile conditions.

The pieces of the specimens containing bone and soft tissues from the inoculation point were put into sterile tubes to be processed bacteriologically. The tissue specimens were weighed and homogenised in 1 ml of brain heart infusion broth. Then, 50 mls of the suspensions were cultured to blood agar plates and incubated at  $37^{\circ}\text{C}$  for 18 hours. The biochemical properties and the antibiotic sensitivity patterns of the grown bacteria were compared with the inoculated microorganisms. The colonies were counted and expressed as units/ml (colony forming unit/ml). The growth rates of the control and the inoculated groups were compared statistically by student's t-test (21).

The main parts of the specimens were fixed in formalin and processed in the Pathology Department. The specimens were kept in bone acid for 12 hours and washed with water. Then the prerations prepared from these tissues were dyed with Hemotoxylene & Eosine and observed with light microscope by using 4, 10, 20 and 40 magnifications.

## RESULTS

Neurological deficits and the symptoms of the systematic disease didn't develop during the three weeks of observation period in rats which were healthy before the process. Post operative care was not re-

quired after the inoculation and the cutaneous sutures were eaten by rats but a problem was not observed at these regions.

When the rats to which *S. aureus* were inoculated were opened, soft tissue abscess were seen macroscopically in all of them. This macroscopic change was not seen in other groups. The bacteria were grown from all the rats to which *S. aureus* were inoculated but no growth was detected from the rats to which 0.85% sodium chloride and *P. aeruginosa* had been inoculated. The results were shown in Table I. The colonies of *S. aureus* grown from rats had been counted and the CFU/ml were  $10^5$  for all of them. The colony count for the *K. pneumoniae* grown rats were  $0.6-8.8 \times 10^2$  CFU/ml.

**Table 1:** The growth rates of inoculated and control groups.

	Growth rates (%)	p values (compered with control group)
<i>S. aureus</i>	7/7 (%100.0)	$p < 0.05$
<i>K. pneumoniae</i>	3/7 (%42.8)	$p > 0.05$
<i>P. aeruginosa</i>	0/7 (%0.0)	-
Control group	0/7 (%0.0)	-

The pathological examination of the specimens showed that the microorganism had been directly inoculated into the disc. The observed histopathological changes had shown difference according to the pathogen; the preparations of the specimens of the rats to which *S. aureus* were inoculated showed polymorphonuclear and mononuclear cell infiltration in the muscle and connective tissues and abscess formation within the fibrous connective tissue in some regions. While destruction was detected in the paravertebral muscles, bone and cartilages; the dura and medulla spinalis in the vertebral column was protected from inflammation. On the other side, vacuolar myelopathy had been observed in medulla spinalis and spinal nerve roots. Intervertebral disc space had again been protected in rats to which *K. pneumoniae* and *P. aeruginosa* were inoculated but no pathological change was detected except the post operative changes in the paravertebral tissues. A more diffuse vacuolar myelopathy was detected in the medulla spinalis of the rats which were infected by *K. pneumoniae*; however a more distributed but mild lacunar vacuolization was

detected in the rats which were infected by *P. aeruginosa*.

In the control group to which 0.85% sodium chloride had been inoculated, postoperative changes such as degeneration of the muscles, hemorrhagic areas etc. were detected but no sign of infection has been observed.

## DISCUSSION

Intervertebral disc space infections are approximately seen in 0.2-5% of intervertebral disc hernia operations (1) and can also complicate trauma, lumbar puncture, spinal anesthesia and chemonucleolysis (2, 3, 5, 8, 12, 18, 19, 24). Their long and expensive treatment and loss of work make these infections important. It has been suggested that the careful sterilization and prophylactic antibiotic use were necessary to decrease the rate of post-operative infections (23). At the same time it's known that as the length of the operation increases, the infection rate directly increases (4, 15). During the recent years, the use of microscope especially for the operations of failed back surgery syndrome has been emphasized after the wide use of microscope in Neurosurgery (4,13,15). However the use of microscope during the first operation in disc surgery will probably increase the succes of surgery and cause a decrease in the rate of infection.

Because the infection occurs within a long time, there are difficulties about the isolation of the etiological agent, and because the infections can be treated medically and the radiological imaging methods can not show the changes; detailed information about the activation of the microorganisms and the histopathological changes has not been obtained (22). Studies about this subject are being done with laboratory animals such as dogs, sheeps and rabbits (6, 7, 16, 20). These animal has the advantage of being big and can be easily examined radiologically, however they share the disadvantage of having a high cost and they are difficult to care. In this study, a model was done by using rats which are cheap, easily obtained and cared.

In the rat model, bacteriological and histopathological examinations were done. A period of three weeks which was the time when 90% of the infection was completed was taken as the reference period and infection was produced by inoculating *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* which were the most frequently isolated microorganisms.

During the recent years, the isolation of the pathogen from the cultures of blood and disc puncture specimens and the treatment according to the antibiotic susceptibility patterns have been emphasized (2, 9, 17, 18). For this reason, use of percutaneous automatic nucleotomy has been suggested which could help the debridement therapy and diagnosis (17). In contrary to all the efforts, the isolation could only be done in maximum 50% of the cases (2, 3, 5, 8, 10, 12, 14, 18, 22, 24).

The bacteria isolated most commonly from the blood and biopsy cultures was *S. aureus* and *S. epidermidis* followed this microorganism. While *P. aeruginosa* has been frequently isolated from intravenous drug users, various kinds of agents have reported to be the pathogens of postoperative spinal infections (1, 2, 5, 11, 17, 18, 24). In this study, the inoculated microorganism has been isolated after three weeks in 100% and 42.8% of the *S. aureus* and *K. pneumoniae* inoculated rats, respectively. The inoculated agent couldn't be isolated in *P. aeruginosa* inoculated rats. These results pointed out that the agents have showed different activities. The difficulty of isolation could be the result of these different activities.

The infection of the intervertebral disc tissue which was typical for the classical interspace infection has not been detected in our rat model. Although it has been observed that the inoculation was done directly to the disc space during the inoculation process and in histopathological frame, discitis didn't develop. Any information explaining this situation couldn't be found in literature. As a result of this study, it has been concluded that the thickness of the disc process (average 1.5 mm) and the high haemopoietic activity observed in the vertebral end-plate had been the preventing factors for the infection agents to localize and cause infection.

## CONCLUSION:

When the microbiological and histopathological results were evaluated, the following points were concluded:

- \* An easily performed model had been done because of the surgical anatomic similarities between humans and *R. norvegicus* rats.

- \* These rats were resistant to different environmental conditions and they were easy to feed so the loss during the study could be ignored and this lowered the expense of the study.

\* Because *S. aureus* were grown in 100% of the rats 3 weeks after inoculation, it has been concluded that this activity was responsible for the pathological changes occurred during the *S. aureus* infections. Especially the paravertebral abscesses; bone, muscle and cartilage tissue destruction which were not observed in other groups were considered to be the signs of this result.

\* Because the growth of the pathogen was not statistically significant and no growth was detected in the groups infected with *K. pneumoniae* and *P. aeruginosa* and because the histopathological changes except vacuolar changes observed in the *S. aureus* inoculated group were not detected in the other groups, it has been concluded that these agents have lost their activities after three weeks.

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