EXPERIMENTAL TETHERED CORD MODEL IN GUINEA PIGS (EVALUATION OF THE RESULTS)

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ABSTRACT :

In this study, the biochemical and histopathological changes in the spinal cord after the experimental stretching were investigated.

In tethered cord formed guinea pigs, hypoxantine and lipid peroxide levels were significantly increased showing us ischemic injury ($p = 0.001$ and $p<0.005$ respectively).

In electronmicroscopic examination, besides the reversible changes like edema and destruction in gray-white matter junction, the irreversible changes like the scarcity of neurofilaments and destruction in the axons and damage in myeline sheats were observed.

Latency periods of SEP and MEP were significantly increased as their amplitude were significantly decreased. These changes showed us defective conduction in the motor and somatosensorial nerves. With 1.5 Tesla MRI the changes in the medulla spinalis could not be well visualized as the vertebral colon was too small.

We think that our animal model would be a guide for experimental tethered cord syndrome.

Key words: *Tethered cord syndrome, evoked potentials, free oxygen radicals.*

INTRODUCTION

Tethered cord is strecthing of medulla spinalis due to the localization of the conus medullaris distally. It may be congenital or acquired. Among the etiology of congenital tethered cord; short and thick filum terminale, lipoma of filum terminale, diastematomyelia, myelomeningocele and myelochisis, intradural lumbasacral lipoma, lipomeningocele and lipomyelome ningocele, neuroenteric cyst, dermoid tumors, congenital fibrovascular bands can be listed. The reasons of acquired tethered cord are the surgical interventions applied in the lumbosacral area (1, 7, 13, 14, 17).

Movements of conus medullaris is limited by factors like growth, development and daily activities, due to the mechanical stretching of the medulla spinalis. The continuous or intermittent stretching of the cord results in physiopathologic changes within seconds. Mostly, insufficiency of the microcirculation of the cord and the following hypoxia is the cause of these changes. At the beginning, damage occurs in neurons, later damage is also observed in axons and loss of spinal autoregulation occurs due to stretching of the cord. The negative effects of the neuronal ischemia is thought to be responsible for this (7, 15, 18, 19).

Our aim in this study was the observation of the biochemical and histopathological changes in the spinal cord after the experimental stretching of the spinal cord. We also tried to evaluate the neurological deficits and to decide if all mentioned changes were reversible or not.

As few experimental tethered cord studies were reported in the literature, we wished our study to be an acceptable experimental model.

MATERIAL AND METHODS

The experimental study was held in Surgical Research Laboratory. The average weight of the guinea pigs were between 480-530 g. No sex difference were made. Twelve of the guinea pigs died during the experiment, we continued our experiment with the remaining 32 guinea pigs. We used 2 guinea pigs to determine the tension force used in formation of tethered

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cord. We divided the remaining 30 guinea pigs into two groups.

1st group: 12 guinea pigs constituted the control group. The lumbosacral cord segments were extracted without tensioning the cord and 6 of them were used in biochemical studies while as the other six were used for the histopathological examination.

2nd group: In this group, there were 18 guinea pigs. After we formed tethered cord, 9 of them were separated for biochemical studies and 9 of them were examined histopathologically.

The hind limb volumes of the 18 guinea pigs in the second group were measured prior to the formation of tethered cord, also we recorded the SEP (somatosensory evoked potantials) and MEP (Motor evoked potantials) values.

The method of tethered cord formation: Intramuscular injection of ketamine 70mg/kg and Xylazine 5mg/kg was used in the anesthesia of the guinea pigs. After the anesthesia, the hind limbs of the guinea pigs above the level of knee joints were digged in to a scaled containers and in this way we were able to measure the volumes of the limbs. The toracolumbar area of the guinea pigs was prepared, which were in prone position with the legs stabilized. Following the skin incision, paraspinal muscles were dissected and four level laminectomy were done. Under the vision of microscope, dura was incised, conus medullaris and filum terminale were exposed, 6/0 silk suture was passed just at the tip of the conus and filum (Fig. 1, 2). Two guinea pigs were brought to EMG laboratory to determine how much weight would be suspended. One to seven gram weights were suspended and SEP and MEP values were recorded. When under 5 gram weight was used, no obvious change was observed in the values and traction force was determined as 5 gram. For 3 minutes 5 gram traction force applied in 18 guinea pigs and one drop of cyanoacrylate was applied to the tip of the conus and the surrounding tissues of the filum for fixation. Muscles and subcutaneous tissues were closed with 4/0 vicryl and the skin was sutured with 4/0 prolene. The animals were observed postoperatively in their cages and at the 7th postoperative day, SEP and MEP evaluations were made.

At the 10th day, the hind limb volumes were again recorded and MRI was used to image the entire vertebral colon and medulla spinalis. At the end of 12th day, lumbosacral area was again incised with a second operation and segments of lumbosacral cord was extracted. The materials taken from 9 guinea pigs were put to separate containers were frozen -70°C degree and were prepared for biochemical study. The other 9 guinea pigs' cords were put in 2.5% gluteraldehyde solution in separate containers and were sent to electron-microscopic examination.

Biochemical Studies: In normal and pathological tissues, lipid peroxide and hypoxantine levels were recorded with the use of TBARS (Thiobarbituric acid test) method. Tissues which had been frozen at -70°C were immediately homogenised in 10 volumes of ice-cold phosphate buffer (50mM, pH 7.4) using a glass-glass homogenizer. Lipid peroxidation in tissues was determined using the method of Uchiyama and Mihara. The values were calculated in terms of nmol MDA/g wet tissue (MDA: Malondialdehyde). Hypoxantine

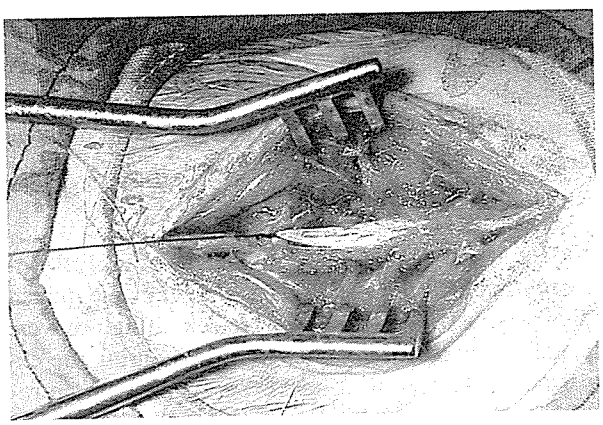

Figure 1. Photographs showing the surgical procedure

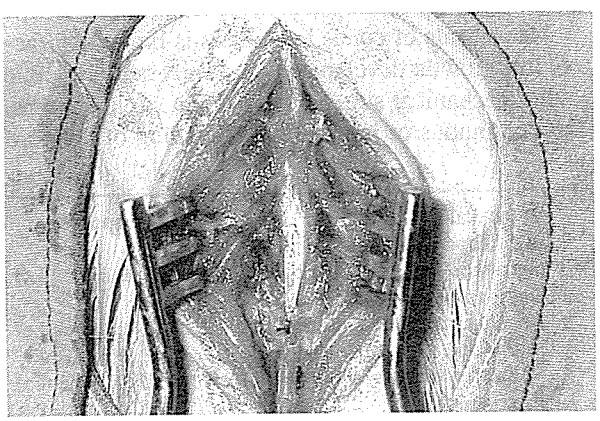

Figure 2. Photographs showing the surgical procedure

values were measured as nmol/g tissue, in normal and pathologic tissues (16).

Electronmicroscopic examination (Transmission EM): The slices taken from the normal and pathologic tissues were examined under the JEOL JEM 1200 EM.

Radiological evaluation: Entire vertebral colon is imaged with 1.5 Tesla MRI.

Record of SEP and MEP values: SEP values were recorded in terms of msn/mV prior and after the formation of tethered cord with the help of electrodes placed. For this reason, active and reference teflon needle electrodes, each 2 mm. apart, were placed to the posterior part of left thigh along the sciatic nerve. Continuous alternating current stimulus is given Medelec MS 25 device (0.2 msn. duration and approximately 6 mA amplitude). The results were recorded with the help of reference electrodes placed at the nasion and active electrodes placed at the durametter in neighborhood of the burr-hole made on the guinea pig skull corresponding to the sensorymotor cortex. The ground electrode was placed on the upper thoracic region (Fig. 3).

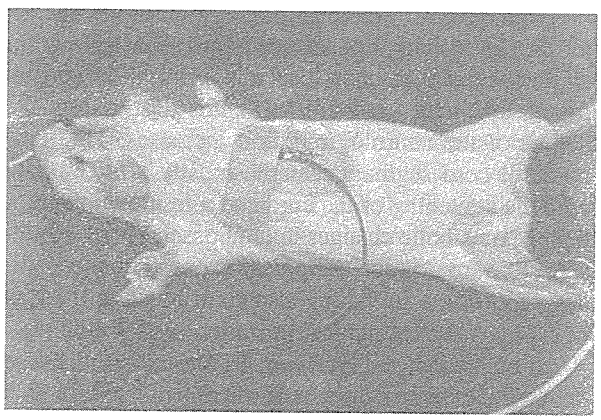

Figure 3. Photographs showing the record of the SEP and MEP values with electrodes in the guinea pig.

MEP values were recorded prior and after the tethered cord formation, with the help of electrodes placed. Also with three electrodes placed to the areas mentioned below, a stimulus 5 mA amplitude and 0.2 msec. duration was given. The response were recorded from the left thigh with the electrodes placed 2 cm. apart. The ground electrode was placed to upper thoracic region.

Statistical evaluation: In the comparison of the lipid peroxide and hypoxantine levels Mann-Whitney U test and for comparing MEP and SEP values Wilcoxon matchedpairs signed-ranks test was used. For significance $p<0.05$ was accepted (SPSS, 5.0, 1992).

RESULTS

Prior and after the formation of tethered cord (at the 10 th postoperative day) hind limb volumes were compared and it was found that there was an average of 1.1±0.2 cc. reduction.

Biochemical findings: In the tethered cord group, the measured lipid peroxide and hypoxantine values were significantly increased compared to the control group. In normal guinea pigs' cord segment lipid peroxide level was measured per gram wet weight of tissue vlue of 64±5.7 nmol was calculated, in guinea pigs with tethered cord lipid peroxide level was found as 84±4.7 nmol/g. Hypoxantine levels, in normal groups were measured as 478.8±68.0, however in tethered cord group it was found 651.2±71.5 nmol/g. This was accepted statistically significant (for the lipid peroxide group $p=0.001$ and for hypoxantine group $p<0.01$) (Table 1).

Table 1. Biochemical Results (Mean ± SD)

Group	Lipid Proxide (nmol/g)	Hypoxantine (nmol/g)
Normal	64 ± 5.7	478.8 ± 68.0
Tethered group	84 ± 4.7	651.2 ± 71.5

$p<0.05$ comparison of the tethered cord cases with the normal groups

Histopathological findings: The slices taken from the control group and tethered cord group were evaluated with EM, the samples taken from tethered cord cases showed that axonal degeneration, damage in myelin sheats and scarce appearance of the neural filaments were present (Fig. 4). Also, the nerve cell count was decreased compared to the control group.

Radiological evaluation: 1.5 Tesla MRI device was useful in the evaluation of the laminectomy area and vertebral canal width but the morphological changes at the conus level and lumbosacral cord segment could not be evaluated (Fig. 5).

Results of SEP and MEP: The guinea pigs with tethered cord were compared in terms of the results. Latency periods were significantly increased, as their amplitudes were significantly decreased. In SEP and

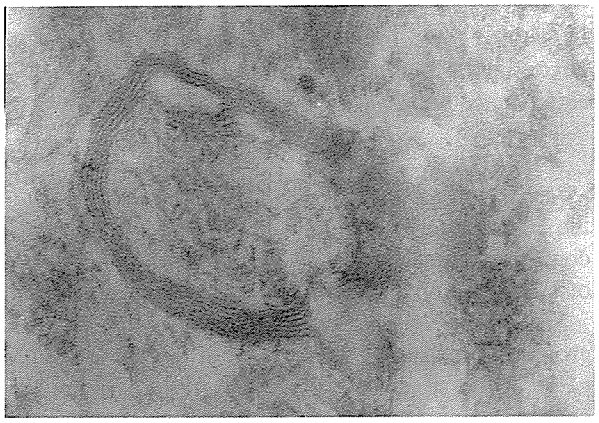

Figure 4. In the electron microscops (X 30000), the scarce neural filaments and damage in the myelinization and axonal degeneration was observed.

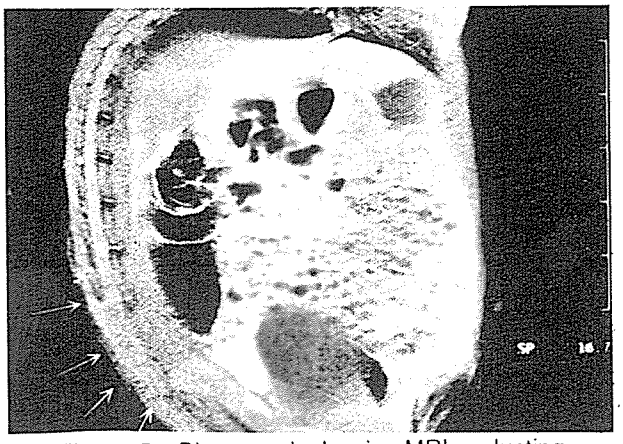

Figure 5. Photograph showing MRI evaluating.

MEP measurements, latencies were recorded in terms of msec and amplitudies were recorded in terms of mV. Prior to tethering SEP latency was found to have a mean value of 11.27±0.88 msec, however after the formed tethered cord it was lenghtened to value of 14.15±0.51 msec. Amplitude was, 1.25±0.11 mV before the tethering of the cord, however it was reduced to 0.91±0.10 mV we formed tethered cord. Likewise MEP latency was lenghtened to 12.50±1.71 msec from 10.46±1.18 msec, its amplitude was reduced from 3.05±0.26 mV to 0.75±0.05 mV. These were statistically significant (For SEP $p<0.0005$ and for MEP $p<0.05$ was calculated), (Fig. 6), (Table II).

DISCUSSION

The tethered cord syndrome is a clinical syndrome characterized by motor and sensory loss, associated with pain, scoliosis and sphincter disorders.

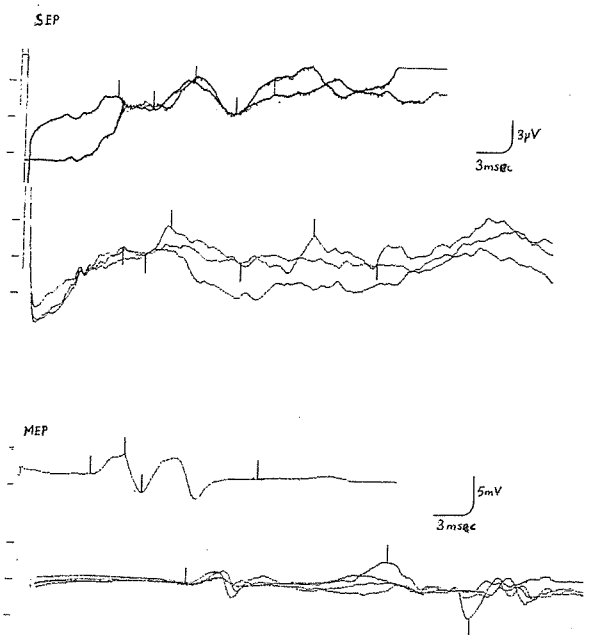

Figure 6. Graphies showing SEP and MEP values recorded prior and after the formation of tethered cord.

On the basis of the pathologic findings formed after the development of tethered cord, the destruction of the oxidative metabolism of lumbosacral cord is found, and with the increase of this destruction, the degree of the neurologic deficit increases. The lumbosacral cord segment was the mostly affected area with the stretching of the cord and a result we thought that the metabolic changes in lumbosacral cord was responsible from the neurological status. The mechanical effect of the stretching also contributed to the loss of axonal integrity and myelinization (2, 3, 4, 15, 19).

In our study, the ischemia in the cord and tissue damage was shown by biochemical studies, in which measurement of lipid peroxide and hypoxantine levels were used. In ischemic tissues, hypoxantine was accumulated and increased the tissue destruction. In our study, in the tethered cord formed cases, hypoxantine levels were significantly increased in the cord. Average hypoxantine levels in normal cord segments was measured 478.8±68.0 nmol/g, however these levels were increased to 651.2±71.5 nmol/g in tethered segments. This was an indicator of the significant ischemia in the cord. In the tissue ischemia and damage, there were significant increase in the free oxygen radicals (11, 12). Lipid peroxide level was a parameter showing us the formation and increase in the levels of

Table 2. SEP and MEP Values (Mean ± SD) (msec/mv)

Group	SEP (latency / amplitude)	MEP (latency / amplitude)
prior to tethered	11.27 ± 0.88 / 1.25 ± 0.11	10.46 ± 1.18 / 3.05 ± 0.26
after tethered	14.15 ± 0.51 / 0.91 ± 0.10	12.50 ± 1.71 / 0.75 ± 0.05

$p<0.05$ for SEP and MEP prior to tethering and afterwards.

these radicals (2, 16). In our study, in the cords of the tethered cord cases, with use of TBARS method, lipid peroxides levels were significantly increased. Prior the tethered cord levels were measured as 64±5.7 nmol/g, after the formed tethered cord these levels were increased to a value 84±4.7 nmol/g. This showed us the damage in the cord.

With the electron microscopic examination, besides the reversible changes like edema and destruction in gray-white matter junction, the irreversible changes like the scarcity of neurofilaments and destruction in the axons and damage in myelin sheaths were observed.

In our study, prior and after the formation of tethered cord, we recorded SEP and MEP values and we found that there were significant differences among those. SEP, was a parameter used in the evaluation of the physiological integrity of the IA group fibers, transmitting the vibration and proprioceptive senses along the posterior tract of the medulla spinalis (4, 8-11).

In one study, the two most important factors in the deterioration of SEP and MEP values were shown to be degree of injury of medulla spinalis and decrease in medulla spinalis blood flow (3). With the decrease of blood flow increase in the latency periods of SEP and MEP, the decrease in the amplitudes and loss of configuration occured. According to the degree of stretching, the damage in the cord varied and the neurological deficits ranged from loss of sensation in lower extremities to complete paraplegia. In our study, besides showing the significant changes in SEP and MEP values, at the 10th postoperative day we measured the volumes of the hind limbs of the guinea pigs again and observed significant degree of atrophy development. This was an indicator of decreased motor nerve transmission.

Nowadays MRI is the most important device used in the diagnosis of the tethered cord syndrome and in the evaluation of the cord. The localization of the conus, ischemia of the cord and desctruction in the cord was shown by MRI (6). We used 1.5 Tesla MRI device in the evaluation of the guinea pigs with tethered cord syndrome. The width of vertebral canal and laminectomy area were well shown, however, the localization of conus and the changes in medulla spinalis could not be well visualized. We decided that MRI evaluation could not be used in guinea pigs or in other small experimental animals (As vertebral colon is too small).

CONCLUSION

In the tethered cord syndrome, the degree of stretching of the cord and the degree of the damage in lumbosacral segment of medulla spinalis were the determining factors in the etiology of such pathological changes. If cord stretching resulted irreversible changes like destruction and damage in myelinization, though untethering was done and the stretching was relased, some of the neurological deficits could not recover, however we expect that metabolic dysfunctions could recover because of the increase in the blood flow of the cord postoperatively (5). Operation should be done immediately if we made the diagnoses just after the birth or during the childhood and also if there was minor or no neurologic deficits. In unoperated tethered cord cases, progression with time or irreversibility of the neurologic deficits could be seen. In the child or adult tethered cord cases, in which significant neurologic deficits were seen, if surgical treatment was selected to release the stretching of the cord, neurological deficits could recover in some of them. In a lot of cases, postoperatively pain sensation was reduced or completely recover, however sphincter tonus loss changed minimally. In the postoperative neurological recovery, the stretching degree of the cord had the primary role. In our study, when the tension was applied to the cord with the weights under 5 gram, we observed no significant changes in the SEP and MEP values, however with 5 gram or above weights these values significantly deteriorated and histopathological

examination revealed irreversible structural changes. We believe, our experimental model, would be a guide for later experimental studies searching the changes formed release of the tethered cord in the animal models with tethered cord syndrome.

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