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Survey of the results of acute sciatic nerve repair comparing epineural and perineurial techniques in the lower extremities of rat

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ABSTRACT

Objective: To study the result of nerve repair in the two mentioned techniques in rats to find the proper answer to the existing disagreement. **Methods:** Twenty adult male rats were included in treatment group. Acutely disconnected sciatic nerve was repaired by Epineural technique in half of the rats; in the other half perineurial technique was applied. After 80 d, the number of grown axons of distal on the repair site was calculated through the use of an optical microscope. Additionally by studying the foot print of the rats the return of neural motor activity was evaluated. **Results:** In epineural group, SFI index was: (56.33±32.30) and in perineurial group: (55.71±30.31); P value=0.930 with their being no difference between these two techniques of surgery. However, in comparing epineural and perineurial groups in the groups themselves, statistical tests showed a significant difference showing functional improvement in comparison with the day before surgery *P* value=0.0001. Statistical tests showed that the average of axons´ number distal to anastomosis site in the epineural group was (349±80) and in the perineurial group was (405±174). These groups have no significant difference regarding the number of axons (*P* value=0.36). **Conclusion:** The results of epineural and perineurial surgery techniques show no difference in nerve repair, SFI index, or axon counting in distal part.

1. Introduction

Acute peripheral nerve injuries are among the most frequently occurring injuries caused by trauma. The treatment of injuries is important because of high incidence of this kind of injury and the disabilities that they cause[1]. Currently, therapeutic strategies are categorized based on type of injury. When the nerve is completely disconnected, surgery and reconnecting two head stumps is the only therapeutic strategy[2]. Major drawbacks of this method include: difficulty in repairing nerves with a small radius; the possibility of causing further injury by suturing; and also potentially causing inflammation around the suture points[3,4]. This is why scholars continue to search for better methods and alternative solutions to aid both patients and surgeons in reducing cost and improving the process.

After nerve disruption, the distal region will be affected by Walerian degeneration leaving only the Schwann Sheath. To restore it, if one repairs two heads of disconnected nerve, the growth of nerve axons will be happen from proximal region towards distal part inside the Schwann Sheath. There are some draws back about the

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epineural and perineurial treatment techniques. And in the past years there were barely any prospective studies in this field with quantitative measurement of axons that healed and transverse the repair site. These numbers of axons can explain the functional result which was seen after 80 d. So, we decided to study and compare the result of nerve repair in two fore mentioned methods in RAT to find proper answer.

2. Materials and methods

This study was conducted in the Animal Laboratory of Clinical Research Training Center of our Hospital on 2-4 month male rats weighted 250-300 g and approved by research and ethics committee.

In this study, 20 male healthy rats aged between 2-4 month average weights of 250-300 g were selected and all were placed in numbered individual cages in a room set for 21 ℃ temperature. They had daylight and night darkness cycle through windows and had sufficient food and water. The rats were randomly divided into two A, B groups of 10, then they received anesthesia by taking Ketamine (75 mg/kg) and Xylazine (10 mg/kg) via Peritoneal and underwent surgery. First 10 ones were selected as A group and other 10 were selected as B group. Before surgery all rats were foot printed , and then they underwent surgery with an incision of about 4 cm in right Gluteal region, after opening the skin and fascia lata and splitting Gluteal muscles, sciatic nerve was exposed and, 1.5 cm before nerve terminal branches, were cut off. In group A Rats (Epineural) disconnected nerve was repaired by two 10-0 Prolene sutures in the epineural layer. In the B group, the rats (Perineural) fascicules existing in the nerve's body were repaired by one 10-0 Prolene suture for each fascicule .All sutures were from the same material therefore it did not affect the results. Then for 7 d Co-amoxiclav syrup (1 mg/kg) was administered to them through drinking water.

10 d and 50 d after surgery all Rats were foot printed. 80 d later (2.5 months) again they were foot printed and on the same day they underwent a surgery. In it, part of the nerve which was repaired was separated and put in a special container containing Glutaraldehyde solution at 4 ℃. Related containers were numbered from numbers 20 to 1 randomly. In conventional time all the samples were transferred to Pathology Laboratory of School of Medicine for further study and counting the axons. Axon counting is from nerve section and the results are recorded in tables in order to be evaluated and compared. Neuron cuts from each section were tabulated using dissector counting techniques[5]. In this technique, counting axons of related neurons in a prepared section is based on a type of sampling technique. In order to determine neuron density we select a cut sample from each sciatic nerve. From each sample, 10 cuts prepared and then the collection of cuts were analyzed. Sampling procedure was in a way that by placing a graded square behind the microscope eyepiece, a specified scale for measuring microscopic fields was designed. The numbers of each neuron's axons in one field out of four fields, in a zigzag way, were counted and recorded.

On the other hand in order to study Sciatic Functional Index, foot print technique, a standard technique among common research techniques, was used and their results were recorded in other tables and compared. The following formulas were used for this purpose:

$$SFI = -38.3(PLF) + 109.5\ (TSF) + 13.3\ (ITSF) - 8.8$$

PLF=EPL − NPL/NPL
TSF=ETS − NTS/NTS
ITSF=EITS − NITS/NITS
E = Experimental
N= Normal
PL = Print length
TS = Toe spread
ITS = Intermediate toe spread
PLF = print length factor
TSF = Toe spread factor
ITSF = Intermediate toe spread factor

The formulas are obtained based on a study from of Bain, Machinnon, Hunter[6]. SFI index varies between zero (Normal Nerve) and minus 100 (Complete Nerve Disconnection). The electrophysiological study were not done, as SFI can completely shows the functional result of the repair .Also counting the axons in the distal part can show the existence of repaired axons in the distal part anatomically ,so it means that functional result is the direct effect of axonal re-growth. In other word, anatomical and functional results can easily show the quality of repair in both groups.

Information collected from data collection forms were entered into Virasat 18 statistical software and SPSS

statistical software. Charts and Tables were used to describe data. In order to compare results, in case of normal distribution, Test Independent Sample is used. In case of not normally distributed, Non Parametrical tests such as Man whitney-U is used. This study is conducted under the verification of the Research Council of University and laws related to animal use were followed in this study.

3. Results

The conclusion was made on comparing the SFI index based on foot print and based on axon counting.

3.1. Examining SFI index function based on foot print

Average SFI index in the day before the surgery in Epineural group was −10/94±13/88 and in the tenth day it was −75/9±15/6 and in the fiftieth day it was −74/2± 22/47 and in the eightieth day after surgery it was −64/18± 21/52. In the Perineural group SFI index before surgery was −10/61±20/58 and in the tenth day it was −76/47±10/69 and in the fiftieth day it was −73/4±8/1 and in the eightieth day after surgery it was −62/30±15/53.

In comparing these two groups, epineural and perineural, their average difference was not significant. General SFI index in the Epineural group was −56/33+32/3 and in the perineural group it was −55/71+30/31 and PV= 0/930 and there was no difference between these two surgical techniques. But in comparing epineural and perineural groups compared to the day before surgery, statistical tests showed significant difference which defined performance improve compared to the day before surgery PV=0/0001.

SFI index of the day before surgery was compared to day 10, 50 and 80 in Epineural technique. SFI index in the days 10, 50 and 80 was significantly different from the day before surgery (*P*=0/0001).

Also, SFI index in perineural technique in the day before surgery was compared to the day 10, 50 and 80. SFI index in perineural technique in the day before surgery was significantly different from the days 10, 50 and 80 (P=0/0001). So, sciatic nerve function improvement in each of the techniques was compared and evaluated to the day before surgery. In comparing SFI index in Perineural technique with Epineural technique, according to (*P*=0/93), there is no significant difference between these two techniques regarding SFI index improvement (Figure 1). Average number of axons in epineural group was 349±80 and in the perineural group it was 405±174, and these two groups don't have any significant difference with each other in regard of the number of axons (PV=0/36) (Figure 2). The comparison of studied indexes; PLE, TSE and ITSE in epineural and perineural techniques is presented in Figure 3.

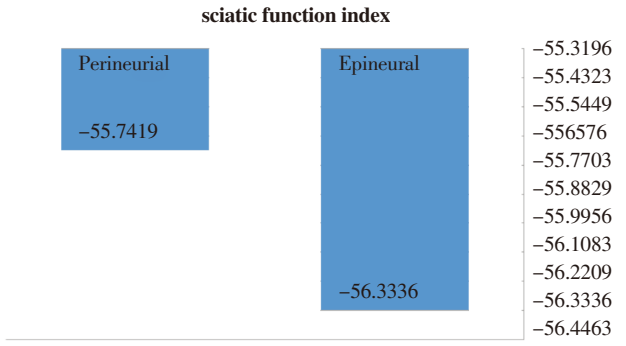

Figure 1. SFI index comparison diagram in rats that have undergone epineural and perineural techniques.

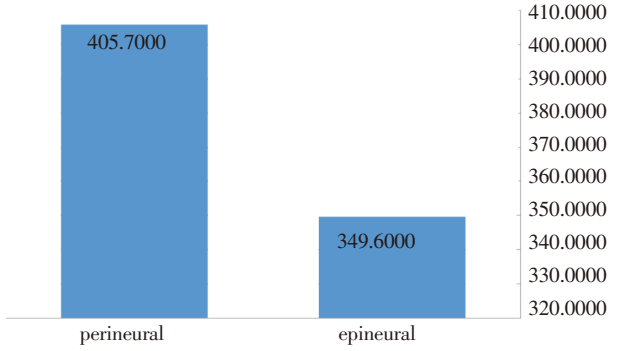

Figure 2. Studying the amplitude of the number of axons in epineural surgery technique versus perineural surgery technique.

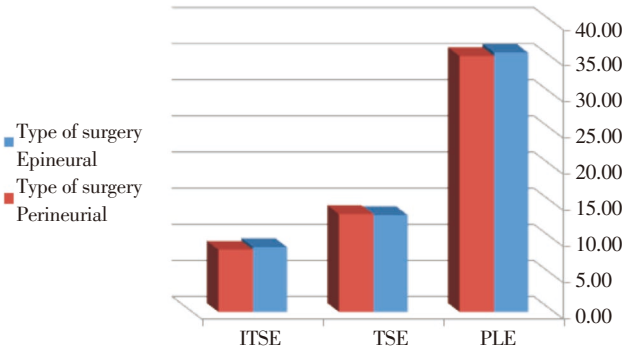

Figure 3. The comparison diagram of studied indexes in epineural surgery technique versus perineural surgery technique. PLE= Print length experimental; TSE=Toe spread experimental; ITSE=Intermediate toe spread experimental.

4. Discussion

Peripheral Nerves' injuries can impose high costs and

negatively impact the function of the area affected. This also leads to collateral reverse consequences for families and society. Varying treatments of these injuries may lead to less complications as well as maintenance and survival of neurons. This issue is an important and valuable discussion in studies. One of the research methods in this field relates to the use of Axotomy model or complete nerve disconnection[7]. By using this method sensory and motor neurons' death will result[7-9]. Which is the common method in adult rats studies[10-11]? Between 7 to 50 percent of sensory neurons die after peripheral nerve injury[7].

In ours study Epineural technique was compared to perineural technique for repairing peripheral nerves. In the presented study, there was no significant difference in average of motor indexes, SFI motion index. Previous researches such as the Young[12] and Tupper[13] studies were in agreement with our results and no difference between these two techniques was observed.

For further discussion and in order to consider a deeper evaluation, we should consider a few subjects.

First, how was our evaluation method of nerve repair? It is hard to compare the results from different studies as they used different methods. However, SFI index is introduced as a valid criterion for evaluation of repairing nerve in many studies[6].

We have to consider the fact that the repaired nerve may affect the targeted muscle or organ but sometimes because of joint stiffness, no motion occurs. If we accompany this index with tenacity measurement index and joint tonicity and stiffness, we may find adjustment in its quantitative perspective. To overcome this potential problem we also used axon counting index, to evaluate the number of axons that traverse the anastomosis site.

Hart and et al[8] have found that in this technique the necrotic cells also attain some coloring so we shouldn't merely rely on it. Therefore this index has to be used in accordance with other measurements.

There are studies that have reported the extent of neuronal death, as a consequence of peripheral nerve repair, by using neuronal counting[10,14]. In the present study, to create a more accurate situation, we used studies of animal behavior with SFI index and axon counting in pathology view simultaneously. No significant difference was found in either of methods. In other words SFI index shows functional recovery of repaired nerves that shows no differences in the two groups. And Axonal counting shows numbers of axons that traverse the anastomosis site, Anatomical results of the repair which again shows no differences.

The counting of axons was done after 80 d, so all of the nerves that transverse the repair site was easily counted. All of the axons colored by special color, so merely the counting of axons were reliable .And it shows how much of the axons healed after 80 d. Also The SFI can completely show the functional result of the repair after 80 d. In other words counting the axons in the distal part can show the existence of repaired axons in the distal part anatomically, and SFI can show functional effect of repair and they go with each other. Therefore it means that functional result is the direct effect of axonal re-growth .In other word, anatomical and functional results can easily show the quality of repair in both groups.

It seems that there is no difference between these two repairing methods, therefore Epineural surgery technique may be a better choice for a surgeon as it is a simpler process with less surgical time. Moreover, this method can cause less inflammation and scarring to the anastomosis site with less interference in process of nerve repair.

So, being virtually no difference between the two methods, what is actually important in nerve repair? With an overview of the new articles, it is been said that neuronal death is related to factors such as age, animal's breed, Trophic factor deprivation and/or synthesis of neurotoxic agents caused by disconnection[15,16] therefore anti-inflammatory actions (agents) for preventing reactions leading to the destruction of neurons immediately after injury seems to be very important. It is assumed that the distal segment has a significant role in providing Trophic factors retrogradely to the ends of injured nerves[17]. Glover blames loss of axoplasm in the cell death[18] while Johnson blames decrease in growth factors freed from target tissue and Schwann cells for cell death[29].

Ma and et al in the repair of nerves by Epineural technique have found a decrease in sensory neurons' death and through this prevented motor neurons' death[20]. Our results match with these findings due to the improvement rate in SFI index in both epineural and perineural surgery techniques. In comparison with the initial days, post surgery results were significant (P<0/05) and significantly increasing. The SFI index in epineural group went from -75 to -56 and in perineural group from -76 to -55, so our results support the classic method of nerve repair[21].

Growth rate in peripheral nerves, reported to be 1 to 3

millimeter/day[22], and it is obvious that before an injured nerve cell nucleus has a chance to turn into retrograde degenerative changes (as a result of deprivation of target organ's trophic factors and/or injured neuron), Connection performed either by epineural technique or by perineural technique would allow injured nerve cells nucleus to be repaired, and preparing the field for nerve survival.

It seems that retrograde trophic agents' densities in these two techniques have shown no difference as it is found in previous studies[17, 20-22, 29].

Prospective research on recognizing these materials and their effective concentration and using them exogenously may cause functional improvement in reconstructive surgeries, before cell death and the cell apoptosis process occur.

Epineural and perineural reconstructive techniques in repairing nerves have no difference in SFI index (P>0/05). Applying epineural and perineural reconstructive techniques in repairing nerves makes no difference in counting regenerative axons in distal part after 80 d (P>0/05).

Conflict of interest statement

All authors declare that there are no competing interests to declare.

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