Zagazig University Medical Journal
www.zumj.journals.ekb.eg

https://doi.org/10.21608/zumj.2024.326262.3619 Volume 31, Issue 1, January. 2025

Manuscript id: ZUMJ-2410-3619
Doi: 10.21608/ZUMJ.2024.326262.3619

ORIGINAL ARTICLE

End-to-End Sciatic Nerve Repair at Different Angles of Attachment: in Albino Rat Experimental Model

Mohammad Reda Ahmad[1], Mohamed Hassan Mohamed Abd El-Aal[1], Mostafa Abdo Ahmed El-Sayed [2*], Mahmoud Abdel-Nabi Saeed[1]

[1] Plastic and Reconstructive Surgery Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

[2] Plastics Surgery Department, Hehia Hospital, Zagazig, Sharkia, Egypt

***Corresponding author:**
Mostafa Abdo Ahmed El-Sayed

Email:
dr.sasa7007@gmail.com

Submit Date: 07-10-2024
Revised Date: 16-10-2024
Accepted Date: 20-10-2024

ABSTRACT

Background: Peripheral nerve injuries pose a significant challenge in clinical practice, often leading to severe functional impairments and a reduced quality of life for affected individuals. The ability to promote nerve regeneration is critical for optimizing recovery outcomes. This study aimed to evaluate the effect of two surgical repair techniques (90-degree and 45-degree) on neuronal count and regenerative indices in the sciatic nerve of albino rats.

Methods: Eight albino rats were used and divided into two groups according to the surgical technique applied. In group I (right sciatic nerve), the sciatic nerve was transected and repaired at a 90/90-degree angle, and in group II (left sciatic nerve), it was transected and repaired at a 45/135-degree angle. After the nerve was repaired, neuronal counts were evaluated at distal and proximal segments. Regenerative indices were calculated to compare the regenerative potential of each surgical approach.

Results: The right sciatic nerve (90-degree repair) at the distal segment had a mean neuronal count of 140.38 neurons/hpf. The left sciatic nerve (45-degree repair) demonstrated a significantly higher mean neuronal count of 291.63 neurons/hpf. At the proximal site, there was a mean of 223.13 neurons per HPF in right sciatic nerves versus 336.38 neurons per HPF in left sciatic nerves. The regenerative index was also significantly higher in the 45-degree repair group, with mean values of 19.63 and 28.63, both statistically significant compared to the distal (15.25) and proximal (22.88) segment of the nerves in the 90-degree group.

Conclusions: The 45-degree oblique repair technique demonstrates higher neuronal counts and regenerative indices compared to conventional 90-degree methods of sciatic nerve repair. These techniques promise to optimize surgical techniques in peripheral nerve injuries to improve recovery.

Keywords: Sciatic nerve, neuronal count, nerve repair, regenerative index, peripheral nerve injury.

INTRODUCTION

Damage to peripheral nerves, particularly the sciatic nerve, can result in severe impairments to motor and sensory functioning and is a primary cause of long-term disability. These injuries are common in trauma situations, and while microsurgical methods have advanced, finding the best strategy for nerve restoration is still difficult. Although end-to-end neurorrhaphy is the gold standard for nerve repair, several variables might affect its outcome, including the nature of the damage, the amount of strain at the repair site, and—above all—the alignment of the nerve stumps. [1, 2].

The sciatic nerve, one of the largest peripheral nerves, is vital to lower limb function, making its restoration important in experimental and therapeutic contexts. One of the most important problems in nerve restoration is ensuring that the nerve ends are correctly aligned and oriented in space during reattachment. Inadequate nerve regeneration, abnormal axonal growth, and subpar functional recovery can all be caused by improper angular orientation or misalignment. [3, 4].

Animal models, especially the albino rat, are commonly used in peripheral nerve studies because of their capacity to regenerate nerves and their physical similarity to human neural systems. These models enable the investigation of factors influencing nerve repair outcomes in well-controlled experimental environments. [5, 6].

Although several studies have been conducted on sciatic nerve repair, nothing is known regarding the impact of the attachment angle on nerve regeneration and recovery. This work aims to study the effects of end-to-end sciatic nerve attachment angle variations on nerve regeneration and functional recovery in albino rats. By investigating different restoration angles and further optimizing surgical treatments for nerve injuries, this research aims to improve clinical outcomes for persons with similar nerve injuries. [7-9].

Using an albino rat model, the study examines the differences between the conventional 90/90-degree nerve regeneration methodology and the innovative 45/135-degree method. The study aims to compare the effects of these two distinct approaches to ascertain whether one produces superior results in terms of nerve regeneration and functional recovery. This research aims to provide important new knowledge that will improve surgical methods for sciatic nerve repair and clinical care of peripheral nerve damage.

METHODS

The sample size included eight male Sprague-Dawley rats, each weighing between 250 and 350 grams. The same eight rats were allocated into two groups based on the surgical technique applied. In group I (right sciatic nerve), the sciatic nerve was transected and repaired at a 90/90-degree angle; in group II (left sciatic nerve), the sciatic nerve was transected and repaired at a 45/135-degree angle. This arrangement facilitated a comparative investigation of nerve healing outcomes at various angles of attachment. This arrangement facilitated a comparative investigation of nerve healing outcomes at various angles of attachment. The Institutional Animal Care and Use Committee of Zagazig University approved all experimental procedures (Approval number: ZU-IACUC/3/F/66/2023).

Before the experiment, all animals underwent a one-week acclimatization period under standard laboratory conditions, including a controlled 12-hour light/dark cycle, room temperature maintained at 22-24°C, and ad libitum access to water and a standard diet. This acclimatization ensures that the animals are in optimal health and stress-free before the surgical procedures.

All procedures performed on the rats in this experiment were conducted with the highest standards of ethics and compassion. Anesthesia was administered via an intraperitoneal injection of a Ketamine/Xylazine mixture (25 mg of Ketamine and 10 mg of Xylazine per mL) at 0.1 mL per 100 grams of body weight. Following anesthesia, the rats' lower limbs were shaved in preparation for the procedures.

The transected sciatic nerves were immediately repaired using end-to-end epineural repair with 9/0 polypropylene sutures, employing microsurgical techniques. In **Group 1**, the sciatic nerve on the right side was transected and repaired at a 90/90-degree angle. In **Group 2**, the sciatic nerve on the left side was transected and repaired at a 45/135-degree angle. Twelve weeks postoperatively, all rats were sacrificed. Sciatic nerves from both sides were harvested, and a 1 cm segment proximal and distal to the epineural repair site was collected for histopathological examination to evaluate nerve regeneration (Figures 1-3).

Postoperative Care and Assessment

Postoperative monitoring and care were conducted meticulously throughout the surgical and recovery phases. Each rat was housed individually in a cage equipped with food and water. During the first seven days, water with 0.8 mg of Tetracycline per 100 kg body weight per 24 hours was used for antibiotic prophylaxis. About two weeks after surgery, after the surgical wounds had completely healed and the sutures were taken out, the rats were placed in bigger group cages, where they remained for the duration of the research.

Histological Evaluation

Rat sciatic nerve specimens were obtained from the 45- and 90-degree angle repair sites and kept for 48 hours in a 20% formal-saline solution. The specimens were preserved and then processed using an automated tissue processor, following a two-step methodology of fixation and dehydration. The tissues were fixed for 48 hours in 10% buffered formalin, and any remaining fixative was removed by rinsing them in distilled water for 30 minutes. A graded sequence of ethanol solutions was used to execute the dehydration process: two cycles of 100% absolute ethanol, lasting an hour each, followed by 90% ethanol for 90 minutes and 70% ethanol for 120 minutes.

Then, the specimens were cleared in 50% alcohol and 50% xylene for one hour, then pure xylene for 90 minutes. After removal, the specimens were impregnated in molten paraffin wax, embedded, and blocked for sectioning. Thin sections of the paraffin (4–5 µm) were prepared and stained by hematoxylin, eosin, and toluidine blue using the standard protocols. Following the staining, these sections were then observed under a microscope for histopathological features, including, but not confined to, degenerative changes, necrosis, apoptosis, metaplasia, fibroplasia, inflammation, granuloma formation, and regenerative processes.

STATISTICAL ANALYSIS

The collected data were coded and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences software version 26.0 (IBM Corp., Armok, NY, USA). The quantitative data were presented as mean, standard deviations, and ranges. Also, qualitative variables were presented as numbers and percentages. The one-sample Kolmogorov-Smirnov test can test whether a variable is normally distributed. The normality of the variables was checked with the Shapiro-Wilk test. The statistical analysis was considered significant at $p < 0.05$.

RESULTS

Eight male Sprague-Dawley rats weighing between 200 and 350 grams were selected for the study. For the Neuronal Count, the right sciatic nerve, representing the 90-degree angle, had an average Neuronal Count of 223.13 and a standard deviation of 42.51. Meanwhile, the left sciatic nerve of the 45-degree angle showed a significantly higher mean Neuronal Count of 336.38 with a standard deviation of 43.67. A p-value of 0.0002 was detected, denoting a significant difference, further reinforcing evidence of better regenerative results using the 45-degree surgical approach (Table 1).

In Neuronal Count data, the right sciatic nerve or the 90-degree angle had an average Neuronal Count of 140.38 ± 19.07. On the other hand, the left sciatic nerve in the 45-degree group revealed a highly increased Neuronal Count with a mean of 291.63 ± 18.15. The p-value of <0.0001 reflects a significant difference, emphasizing the higher regenerative result based on the 45-degree repair technique. The findings suggest that the surgical-model 45-degree repair has advantages in promoting superior nerve regeneration, manifesting as the Regenerative Index and Neuronal Count (Table 2).

In the Proximal Regenerative Index, the right sciatic nerve, repaired at 90 degrees, was found to have a mean Regenerative Index of 22.88 with a standard deviation of 2.71, while its contralateral, the left sciatic nerve that had been repaired at 45 degrees, had a mean Regenerative Index of 28.63 with a standard deviation of 3.53. The p-value is 0.0045, which means that it is statistically significant. Thus, the two techniques have a substantial difference based on their regenerative capacity, and the 45-degree approach performs better (Table 1).

For the Distal Regenerative Index, the right sciatic nerve, repaired at a 90-degree angle, had a mean Regenerative Index of 15.25 ± 3.73. In contrast, the left sciatic nerve, repaired in a 45-degree fashion, had a significantly higher mean Regenerative Index. With the p-value at 0.0333, this is significant, showing more excellent regenerative capacity than that of 90-degree (Table 2).

90-Degree Nerve Repair-Right Sciatic Nerve:

The histological sections of the 90-degree repair angle were characterized by well-oriented myelinated and unmyelinated nerve fibers with distinct nuclei. Suturing material residuals and several areas containing focal regeneration of the nerve fibers were present. As calculated under HPF microscopy, the proportional repair regenerative index ranged from 10% to 23% in the distal segments and 19% to 31% in the proximal segments. Further, the fibroblastic scar tissue also recorded mild inflammatory changes with invasion at the epineurium. Counts of neurons in the distal segments ranged from 120 to 173 cells/HPF, but when measured at the proximal segments, counts ranged from 187 to 297 cells/HPF (Figures 4 and 5).

45-Degree Nerve Repair (Left Sciatic Nerve):

Sections from the repairs with a 45-degree angle demonstrated a relatively normal appearance, with myelinated and unmyelinated nerve fibers with striking capillarization, particularly within the proximal segments. The neurons appeared healthy and had prominent nuclei; several areas of nerve fiber regeneration were observed focally. The proportional repair regenerative index in the distal segments ranged between 15% and 28%, while that of the proximal segments ranged between 23% and 37% at HPF. Inflammatory changes and epineural fibroblastic scar invasion were minimal. Similarly, neuronal counts were higher for the distal segment of the groups, with a range between 260 to 317 cells/HPF, while in proximal segments, counts ranged from 293 to 408 cells/HPF. It would appear that the oblique 45-degree nerve repair would provide a larger regenerative surface of contact, with increased neuronal regeneration and capillarization, while inducing less inflammatory and scar tissue changes than the standard 90-degree repair (Figures 4 and 5).

Table 1: Comparative analysis of the Proximal measurements of the right and left sciatic nerves in terms of the Regenerative Index and Neuronal Count.

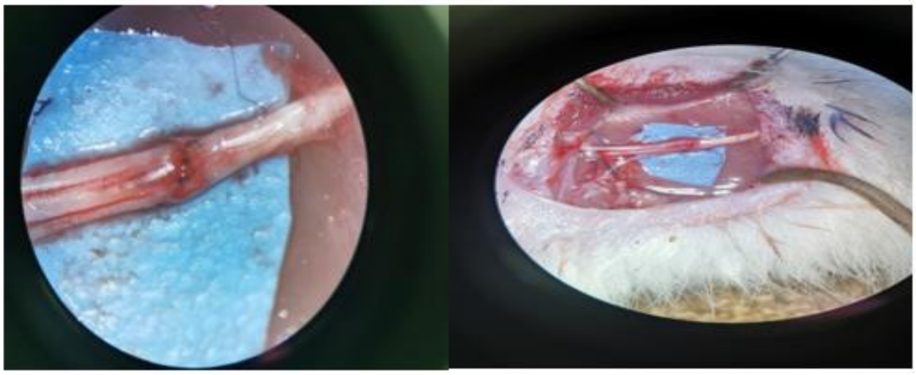
	Group I Right sciatic nerve (90 degree)	Group II Left sciatic nerve (45 degree)	P value	Statistically significant
	N = 8	N = 8		
Proximal				
Regenerative Index	22.88±2.71	28.63±3.53	0.0045	Sig.
Neuronal Count	223.13±42.51	336.38±43.67	0.0002	Sig.
Statistical test used: Tow sample T-test				
p-value≤0.05 considered statistically significant (95% confidence interval).				

Table 2: Comparative analysis of the distal measurements of the right and left sciatic nerves in terms of the Regenerative Index and Neuronal Count.

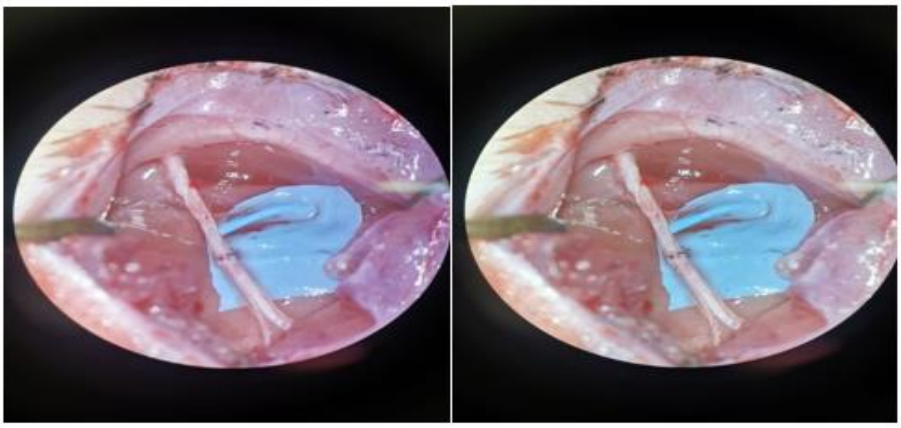
	Group I Right sciatic nerve (90 degree)	Group II Left sciatic nerve (45 degree)	P value	Statistically significant
	N = 8	N = 8		
Distal				
Regenerative Index	15.25±3.73	19.63±3.16	0.0333	Sig.
Neuronal Count	140.38±19.07	291.63±18.15	<0.0001	Sig.
Statistical test used: Tow sample T-test				
p-value≤0.05 considered statistically significant (95% confidence interval).				

Figure 1: (A) Shaving, fixation, and positioning (prone), (B) Markin

Figure 2: The animal Surgical Preparation

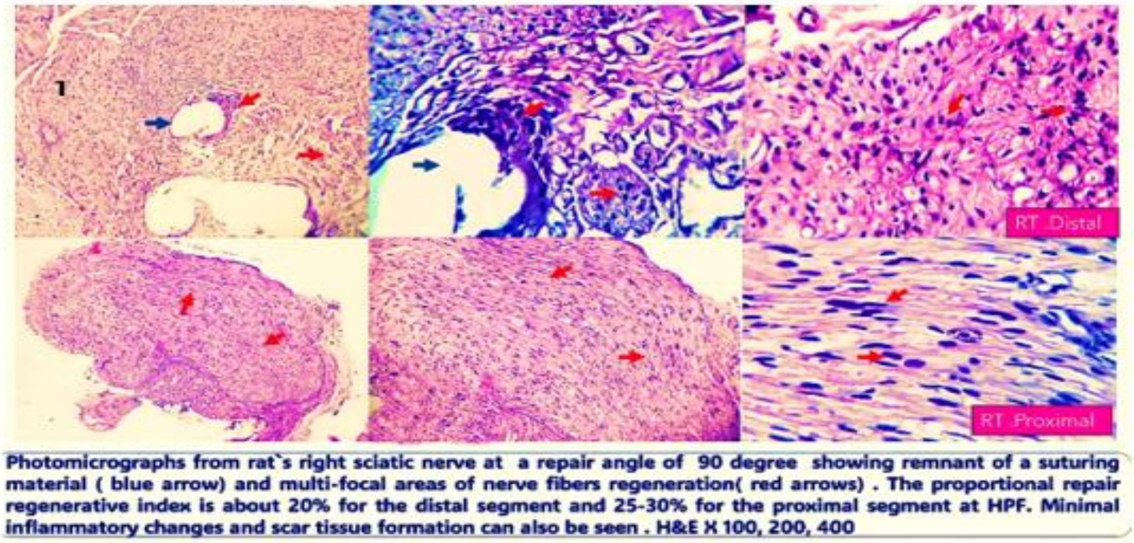


A

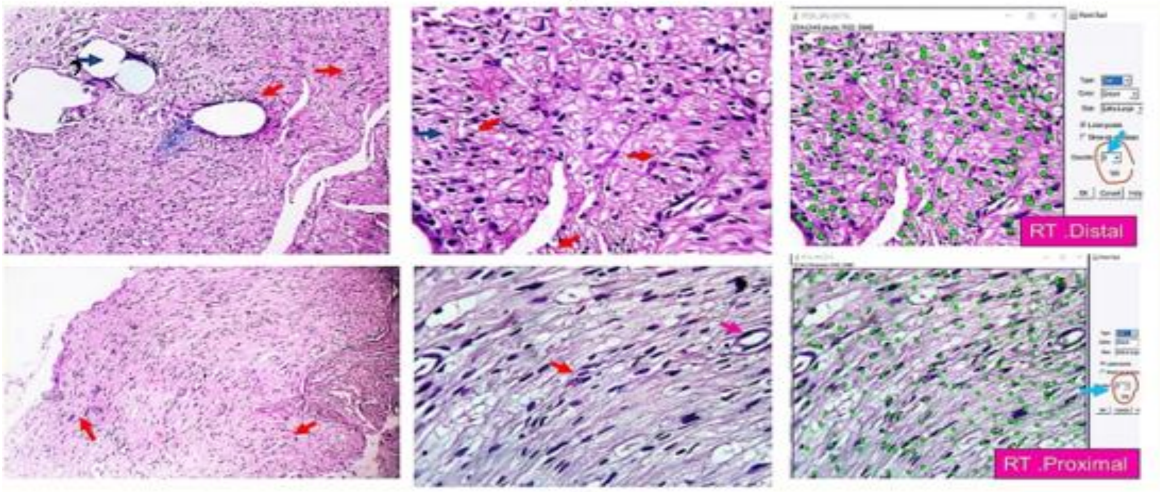


B

Figure 3: (A) Left side, (B) Right-side sciatic nerve after good repair



A



B

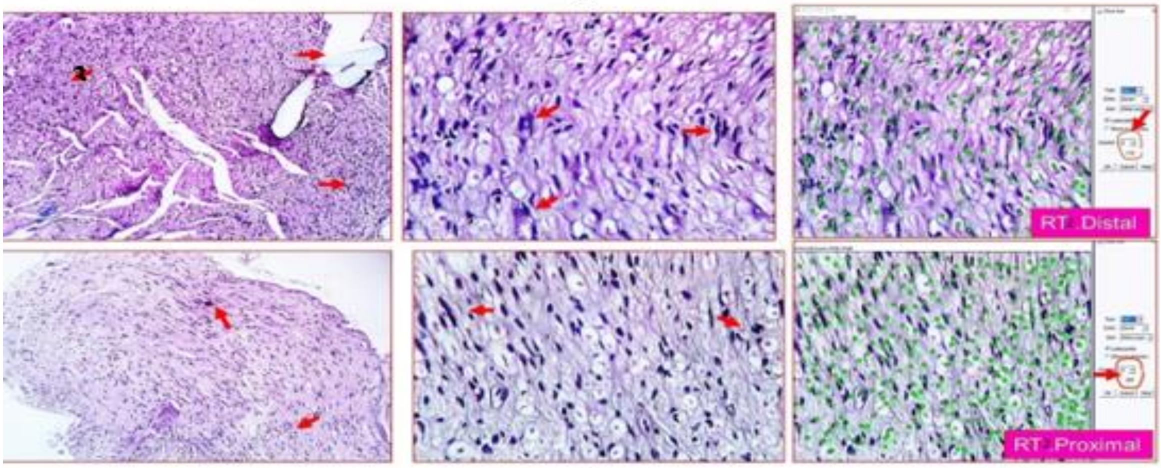
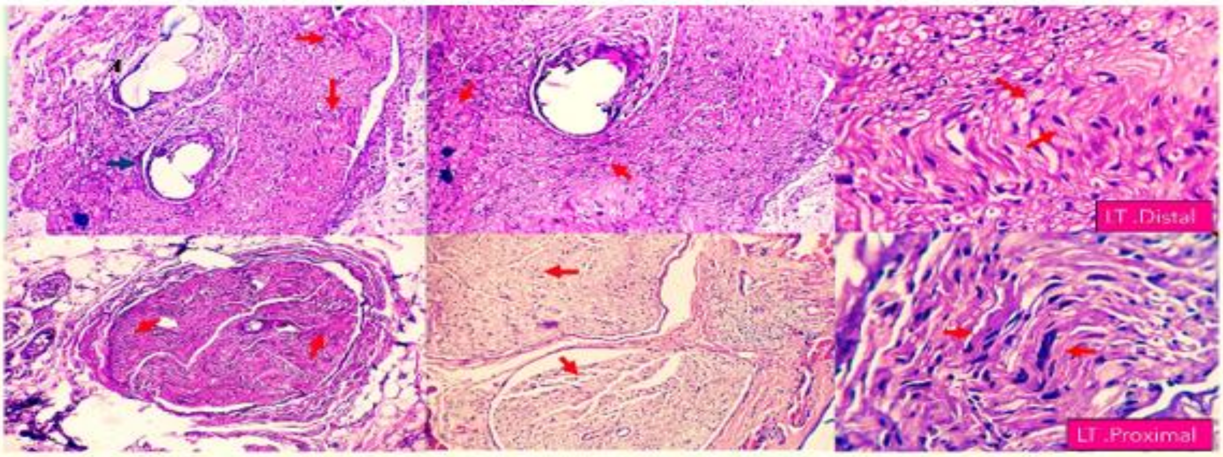


Figure 4: Photomicrographs from a rat`s right sciatic nerve at a repair angle of 90 degrees demonstrating histopathologic changes and the computably estimated neuronal numbers in both the proximal and distal segments.



Photomicrographs from rat`s left sciatic nerve at a repair angle of 145/45 degree showing remnant of a suturing material (blue arrow) and multi-focal areas of nerve fibers regeneration(red arrows) . The proportional repair regenerative index is about 25-28% for the distal segment and 30-33% for the proximal segment at HPF . Mild inflammatory changes and scar tissue formation can also be seen H&E X 100, 200, 400

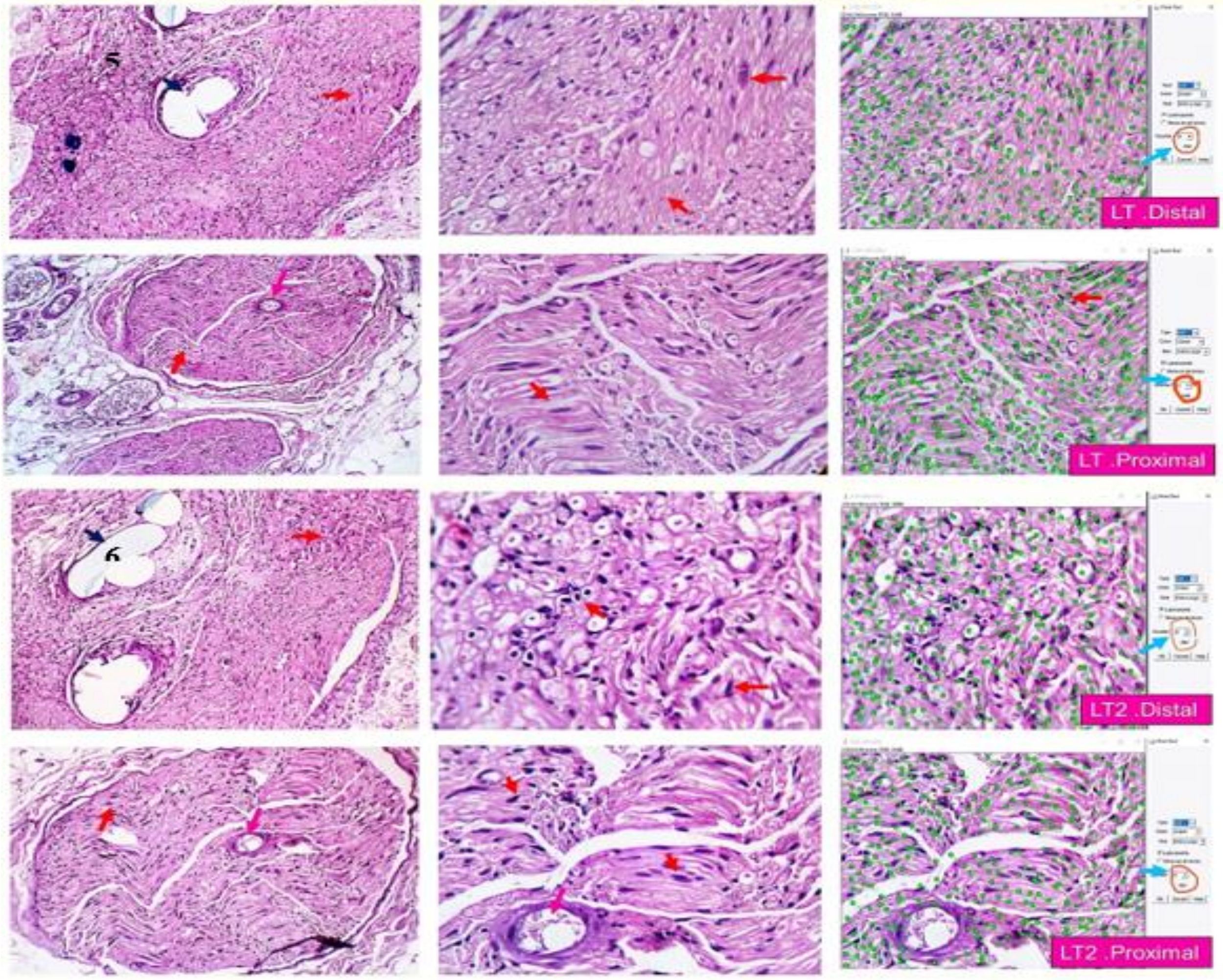


Figure 5: Photomicrographs from a rat`s left sciatic nerve at a repair angle of 145/45 degrees demonstrating histopathologic changes and the computably estimated neuronal numbers in both the proximal and distal segments

DISCUSSION

Sciatic nerve injuries represent one of the most important clinical problems because of their impact on motor and sensory function. In general, peripheral nerve injury and damage to the sciatic nerve remain one of the functional therapeutic challenges in developing microsurgical techniques. Many studies during the past years have detailed various methodologies for the improvement of the outcomes of nerve repair by nerve grafting, neurotization, and nerve conduits. However, functional recovery was still far from ideal, especially when the nerve ends were aligned in a non-straight fashion. [10]

The present study compares the outcome of end-to-end repair of the sciatic nerve at two angles of attachment, 45° and 90°, in an albino rat experimental model. Our findings reflect significant disparities in regenerative potential between the two repair techniques, with 45° oblique repairs showing better results for neuronal count and both proximal and distal regenerative indices. The finding supports our hypothesis that the greater the obliquity of the angle of repair, the lesser the hindrance to axonal regeneration and, thus, the better nerve recovery.

Our results demonstrated that the difference in the regenerative index significantly differed between 90-degree repair, with a mean of 15.25, and 45-degree repair, with a mean of 19.63. It would, therefore, follow that 45-degree oblique repair allows for better nerve regeneration in its distal segment compared to conventional 90-degree repair. The results have shown that the mean regenerative index for the 45-degree repair was significantly higher in the proximal segment of the sciatic nerve, with a value of 28.63 compared to the 90-degree repair, at 22.88. These findings are believed to advocate the increased obliquity of the repair angle being advantageous for nerve regeneration.

Several studies in this domain come to the same conclusion. For instance, Xiao-Chu et al. [11] Note that lower angles of repair, only between 30 and 45 degrees, are better for nerve regeneration than higher angles, which range from 60 to 90 degrees. Their study ascertained that the lower-angle repairs indeed had faster and fuller axonal regeneration that would provide a better general result in nerve function.

Again, in 2002, Yan et al. [12] demonstrated that oblique nerve repairs resulted in significantly more nerve fiber sprouting and muscle recovery than the latter or transverse repairs. Their results reflected that oblique repair is associated with increased muscle force, weight, and regenerated nerve fibers.

In support of this view, Lian et al. [13] succeeded in identifying, in 2020, that nerve coaptation at an angle of 30 degrees secured significantly better nerve recovery compared to 60- and 90-degree repairs. Their findings also justify that making oblique sections creates a better environment for nerve regeneration.

In our result analysis of neuronal counts in the distal and proximal segments of the sciatic nerve, there were significant differences between the two surgical techniques employed. Considering an average neuron count per HPF, the right sciatic nerve with 90-degree end-to-end repair had 140.38 neurons per HPF. The left sciatic nerve with the 30-degree oblique repair recorded an enormously higher neuronal count, at 291.63 neurons per HPF. The marked difference thus states that the regenerating response was more favorable in the nerve that had undergone the oblique repair technique.

Consequently, in the proximal segment, the right sciatic nerve had a neuronal count of 223.13 neurons per HPF, while the left sciatic nerve had an increased neuronal count of 336.38 neurons per HPF. These observations again suggest that an angle of repair at 30 degrees, compared with the conventional repair at 90 degrees, contributed to the best outcomes after nerve repair.

Kosins et al. [14] reported that immunological demyelination significantly increased neuronal count, axon density, and nerve fiber diameter following acute injury of the sciatic nerve and favored enhanced nerve regeneration. Kuyucu et al. [15] documented that treatment with Exenatide could significantly increase axonal number in the injured sciatic nerve after injury, thus improving nerve regeneration.

CONCLUSION

It can be concluded that the angle of repair significantly influences the quality of the regeneration of the sciatic nerve and postoperative complications. The general approach of end-to-end repair at a right angle results in poor neuronal regeneration, increased scar tissue formation, and escalation of the inflammatory response. In turn, the repair angle was slanted at 45 degrees, which showed a larger repair surface area, resulting in enhanced neuronal regeneration, neovascularization, and less inflammation or fibroblastic scar invasion. The histological features indicated that the 45-degree repair angle provided higher regenerative indices and neuron counts, favoring the proximal segments for a better functional recovery.

These findings support revising current techniques in nerve repair to include oblique angles, perhaps allowing for better structural and functional recovery. More research is needed to clinically confirm these findings and examine the long-term effects involved with different angles in nerve repair. Evidence does support an oblique nerve repair technique as one that may prove most beneficial in comparison to traditional transverse approaches in influencing nerve regeneration.

Funding

This research did not receive a specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

REFERENCE:

1. Bhandari L, Fleissig Y, Hoey R, Beare JE, Yarberry C, Yoshida S, et al. Comparison of End-to-End Technique, Helicoid Technique, and Modified Helicoid Weave Repair Technique in a Rat Sciatic Nerve Model: A Pilot Study. Cureus. 2020;12(7):e9196.
2. Bingham JR, Kniery KR, Jorstad NL, Horkayne-Szakaly I, Hoffer ZS, Salgar SK. "Stem cell therapy to promote limb function recovery in peripheral nerve damage in a rat model" - Experimental research. Ann Med Surg (Lond). 2019;41:20-8.
3. Casal D, Mota-Silva E, Iria I, Pais D, Farinho A, Alves S,et al. Functional and Physiological Methods of Evaluating Median Nerve Regeneration in the Rat. J Vis Exp. 2020;(158):10.3791/59767.
4. Eltahir M.I.M, Altun G, Abdik Z, Mead A.O, Kocaman A, Alsharif M.H.K, et al., "Possible effect of ginger oil and sesame oil on peripheral nerve regeneration after sciatic nerve crush: A stereological and histological study in a rat model," 2021.
5. Lemke A, Ferguson J, Gross K, Penzenstadler C, Bradl M, Mayer RL, et al. Transplantation of human amnion prevents recurring adhesions and ameliorates fibrosis in a rat model of sciatic nerve scarring. Acta Biomater. 2018;66:335-49.
6. Ma Y, Gao H, Wang H, Cao X. Engineering topography: effects on nerve cell behaviors and applications in peripheral nerve repair. J Mater Chem B. 2021;9(32):6310–25.
7. Mohamadi F, Ebrahimi-Barough S, Nourani MR, Ahmadi A, Ai J. Use new poly (ε-caprolactone/collagen/NBG) nerve conduits along with NGF for promoting peripheral (sciatic) nerve regeneration in a rat. Artif Cells Nanomed Biotechnol. 2018;46(sup2):34-45.
8. Semmler L, Naghilou A, Millesi F, Wolf S, Mann A, Stadlmayr S, et al. Silk-in-Silk Nerve Guidance Conduits Enhance Regeneration in a Rat Sciatic Nerve Injury Model. Adv Healthc Mater. 2023;12(11):e2203237.
9. Wei Z, Hong FF, Cao Z, Zhao SY, Chen L. In Situ Fabrication of Nerve Growth Factor Encapsulated Chitosan Nanoparticles in Oxidized Bacterial Nanocellulose for Rat Sciatic Nerve Regeneration. Biomacromolecules. 2021;22(12):4988-99.
10. Raza C, Riaz HA, Anjum R, Shakeel NUA. Repair strategies for injured peripheral nerve: Review. Life Sci. 2020;243:117308.
11. Chu X, Ruan H, Liu J, Fan C. Histological study on sciatic nerve repair at different angles in rats. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. CJPRS, 2011;25(5):587-90.
12. Yan YH, Yan JG, Sanger JR, Zhang LL, Riley DA, Matloub HS. Nerve repair at different angles of attachment: an experiment in rats. J Reconstr Microsurg. 2002;18(8):703-8.
13. Ouyang L, Zhang F, Liu J, Cui S, Chen K, Wang J,et al. Application of oblique nerve coaptation in autologous nerve transplantation. Ann Transl Med. 2020;8(20):1300.
14. Kuyucu E, Gümüs B, Erbas O, Oltulu F, Bora A. Exenatide promotes regeneration of injured rat sciatic nerve. Neural Regen Res. 2017;12(4):637–43.
15. Kosins AM, Scholz T, Mendoza C, Lin P, Shepard B, Evans GRD, et al. Improvement of peripheral nerve regeneration following immunological demyelination in vivo. Plast Reconstr Surg. 2011;127(5):1813-9.

Citation

Ahmad, M., Abd El-Aal, M., El-Sayed, M., Saeed, M. End to End Sciatic Nerve Repair at Different Angles of Attachment: in Albino Rat Experimental Model. *Zagazig University Medical Journal*, 2024; (294-302): -. doi: 10.21608/zumj.2024.326262.3619