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Termino Lateral Neurorrhaphy (TLN): A New Approach for Nerve Repair

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ABSTRACT

For the past decade, Termino-lateral neurorrhaphy (TLN) has been the subject of great deal of clinical and scientific debate. The efficiency of TLN, its ability to preserve donor nerve function, the necessity of disrupting donor nerve connective tissue layers during the procedure. The mechanism by which the TLN affords re-innervations and even the definition of the procedure have been questioned. For this reason this experimental study was conducted to outline the pattern of axonal sprouting in TLN. The study was conducted on 30 rats, using the tibioperoneal model. They were divided into 3 groups, group I that represents the control group, group II where a window is done in the tibial nerve and group III where TLN has been done. The results were evaluated using histological examination (light microscopy, electron microscopy) and morphometry. The results in group I revealed no signs of degeneration or regeneration which indicates reliability of animal model, in group II no axonal sprouting was detected indicating that the epineurial window alone was not sufficient for allowing collateral sprouting and subsequent nerve regeneration and repair. In group III TLN resulted in collateral sprouting with the same axonal number and myelin thickness as the control group indicating excellent nerve regeneration and repair. This study concluded that TLN is a suitable and reliable technique for nerve regeneration and repair without affection of the donor nerve.

INTRODUCTION

When possible, transected peripheral nerves are repaired in a tension-free, end-to-end fashion. In cases where the proximal nerve stump is not available for end-to-end repair, surgeons must seek alternative repair techniques. One of such techniques is end-to-side cooptation known as terminolateral neurorrhaphy (TLN) **[1]**. It involves suturing the distal stump of a transected nerve to the side of intact, adjacent nerve. In recent years, there has been a resurgence of interest in termino lateral neurorrhaphy (TLN) with numerous articles reporting the success of end to side nerve repair technique in the promotion of axonal regeneration in laboratory animals **[2,3]**. Several questions were raised regarding the efficacy of TLN, its ability to preserve donor nerve function, the necessity of disrupting donor nerve connective tissue layers during the procedure, the mechanism by which the TLN provides reinnervation and even the definition of the procedure **[4]**. Despite these doubts, recently published reviews of literature on TLN conclude that end-to-side neurorrhaphy, without deliberate disruption of donor nerve axons, is a promising clinical technique **[4,5]**.

The aim of this study is to investigate, experimentally, the technique of end to side neurorrhaphy in peripheral nerve repair for its possible applications as an alternative technique in the repair of peripheral nerve injury.

MATERIAL AND METHODS

Thirty male white rats (300-350 gm) were used in this study, the animals were housed in a suitable environment in the micro vascular lab of the plastic surgery department, Ain Shams University. The animals were maintained in a 12 hour light dark cycle with food and water available.

Anesthesia: The rats were anaesthetized with intraperitoneal Sodium Phenobarbital (25 mg/kg i.p.). Anesthesia was maintained with supplements as needed.

Surgical procedure: A "T" shaped incision was made on the skin of the dorsum of the left thigh measuring 3x3 cm, a similar incision is done in the underlying muscle thus exposing the entire sciatic nerve. The peroneal nerve at its branching point was exposed and dissected from the underlying fascia (Fig. 1a & b). This branching point is usually 1.2 to 1.5 cm from the sciatic notch. Subsequent steps were done according to the group.

Group I (control): In this group, closure of the wound in layers is done. 5/0 vicryl suture was used for the muscle and 4/0 silk suture for the skin.

Group II: An epineurial window elliptical in shape about 1.5 to 1.7 cm from the sciatic notch was done in the tibial nerve where the epineurium only was removed making sure that there was no disturbance in the underlying perineurium. The peroneal nerve is kept in its place. The wound was closed in layers in similar fashion to group I.

Group III: The same steps, up to doing an epineurial window were also done, added to it the peroneal nerve was severed. The proximal severance site was ligated with 8/0 suture, rotated 100 degree and implanted into the adductor muscle group with 8/0 suture (Fig. 2a & b). The distal segment of the severed nerve was sutured to the epineurial window in an end to side fashion using 10/0 nylon suture (Fig. 3a & b). The wound was closed in layers as in the other groups.

RESULTS

All animals survived after surgery and there were no major functional impairment or infection. After 7 weeks, all specimens were harvested and processed for histological examination using light microscopy analysis and selected area was examined using electron microscopy.

A- *Light microscope:*

Group I:

i- *Proximal part of tibial nerve:*

Showed normal shape and regular myelinated axons.

ii- *Distal part of tibial nerve:*

Showed no evidence of degeneration or regeneration. However, there were few sections that show minimal areas of focal demyelination.

iii- *Peroneal nerve:*

Showed no evidence of degeneration or regeneration (normal axon shape, myelin). However, the axons are of smaller size than that of tibial nerve.

Group II:

i- *Proximal part of tibial nerve:*

There were no changes in axon shape or distribution, myelin shape. However, there was marked thickening of epineurium.

ii- *Distal part of tibial nerve:*

Showed thickening of epineurium without evidence of degeneration or regeneration. However, there were very few areas of focal demyelination.

iii- *Window area:*

Showed thickening of epineurium with abundant connective tissue deposition. There was no evidence of "mushrooming" which is indicative of perineurial affection. However, there were no axons found in the epineurium of window area or outside it (Fig. 4).

iv- Peroneal nerve:

Showed no evidences of regeneration or degeneration with normal distribution and shape of axons. However, there was thickening of epineurium with deposition of connective tissue (Fig. 5).

Group III:

i- *Proximal part of tibial nerve:*

Showed normal axonal shape and distribution. There was no evidence of degeneration or regeneration. However, there were focal areas of demyelination.

ii- *Distal part of tibial nerve:*

Showed no evidence of degeneration or regeneration. There were focal areas of demyelination with intact perineurium (Fig. 6).

iii- *Peroneal nerve:*

Showed thickening of the epineurium. There was well arranged myelinated and non myelinated nerve axons smaller in size than in group I. There was no evidence of degeneration. There was a sizable blood vessel (vascularised peroneal nerve).

iv- *End to side junction:*

The junction between peroneal and tibial nerve was intact with evident epineurium. The fibers near the junction took an oblique pattern while entering peroneal nerve. Both myelinated and unmyelinated fibers were present transversing the junction. There was no evidence of degeneration. However, while the axons appear smaller in size than control group (Fig. 7).

B- *Electron microscope:*

The results of electron microscopic study showed:

Group I:

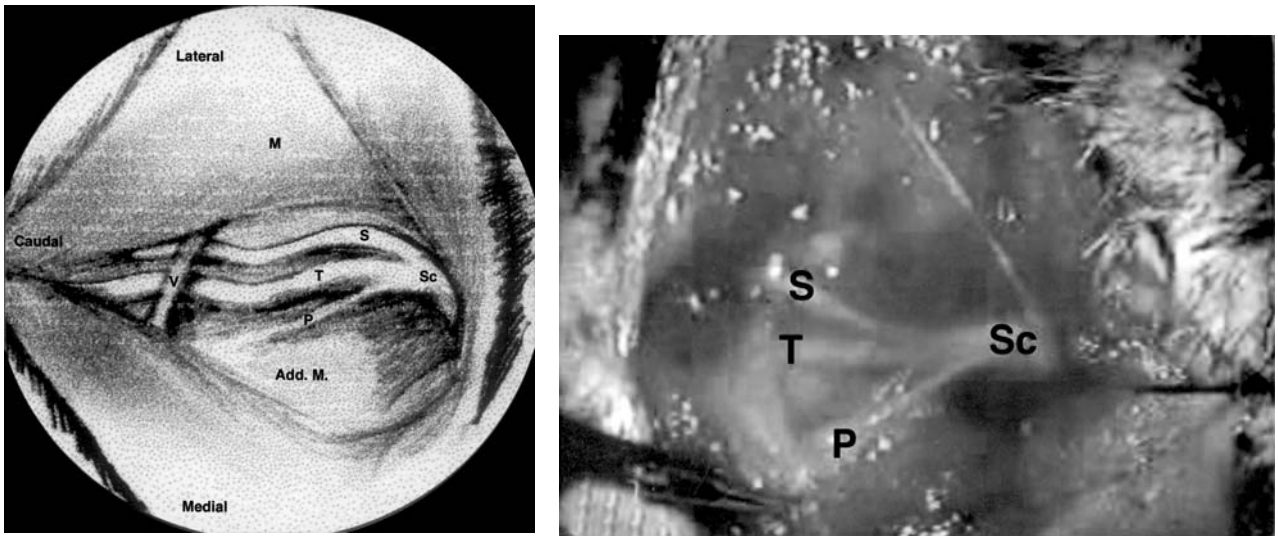
No signs of degeneration or regeneration with normal ultra structure of the nerve.

Group II:

No axonal sprouting occurred from tibial nerve, intact perineurium of tibial nerve.

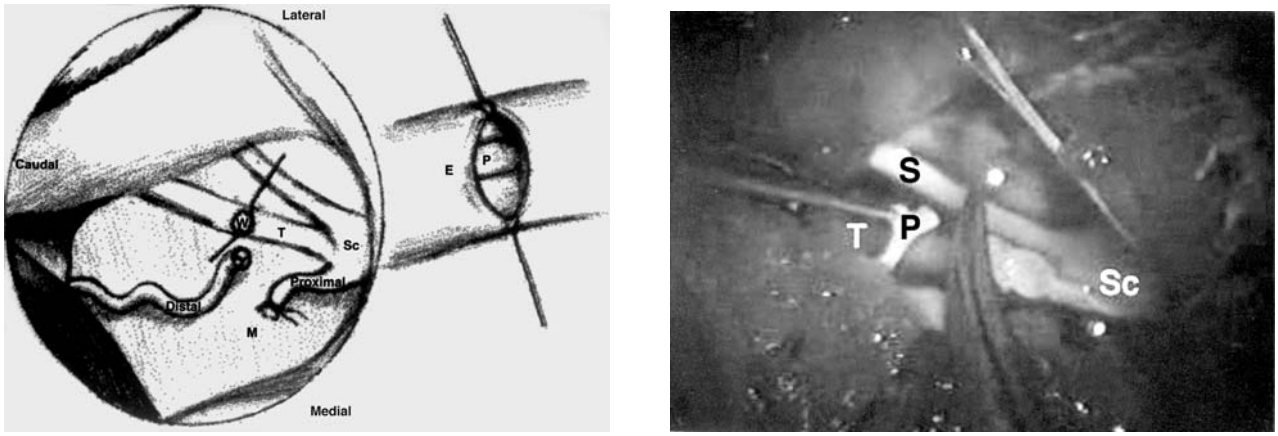
Group III:

Well formed myelinated axons were detected in peroneal nerve with near normal ultra structure of nerve fiber, no signs of degeneration or regeneration were found in distal segment of tibial nerve.



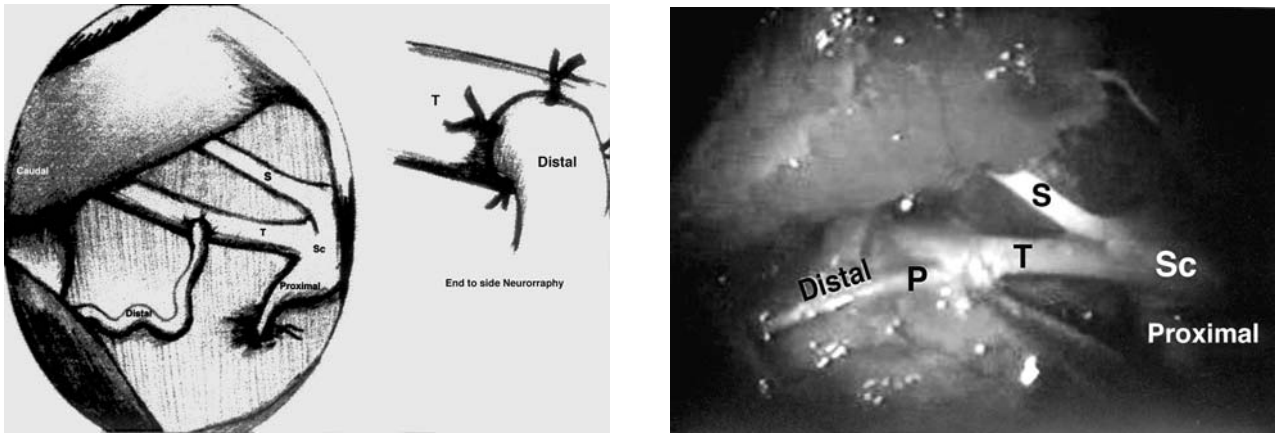
(A) (B)

Fig. (1:A-B): Normal anatomy of sciatic nerve branches in rat. (M: Muscle, Sc: Sciatic nerve, S: Sural nerve, T: Tibial nerve, P: Peroneal nerve).



(A) (B)

Fig. (2:A-B): Peroneal nerve ligated proximally, Transection of the Peroneal nerve, creation of an epineurial window in the tibial nerve. (M: Muscle, Sc: Sciatic nerve, S: Sural nerve, T: Tibial nerve, P: Peroneal nerve).



(A) (B)

Fig. (3:A-B): Distal part of peroneal nerve sutured to the tibial nerve window (TLN) and proximal part implanted into underlying muscle. (M: Muscle, Sc: Sciatic nerve, S: Sural nerve, T: Tibial nerve, P: Peroneal nerve).

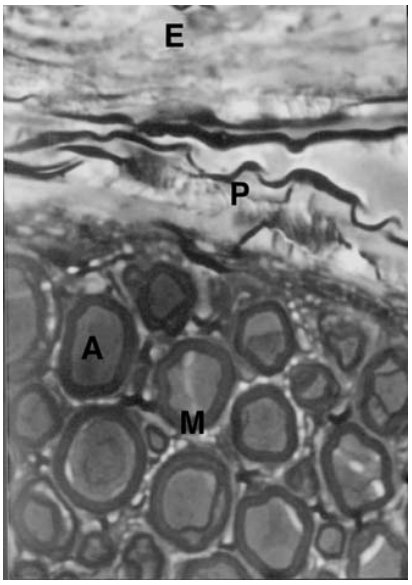


Fig. (4): Semi-thin section of window area of tibial nerve (T.S.) stained by touldine blue, there is thickening of epineurium. (E = epineurium, P = perineurium, A = axon, M = myelinx 400).

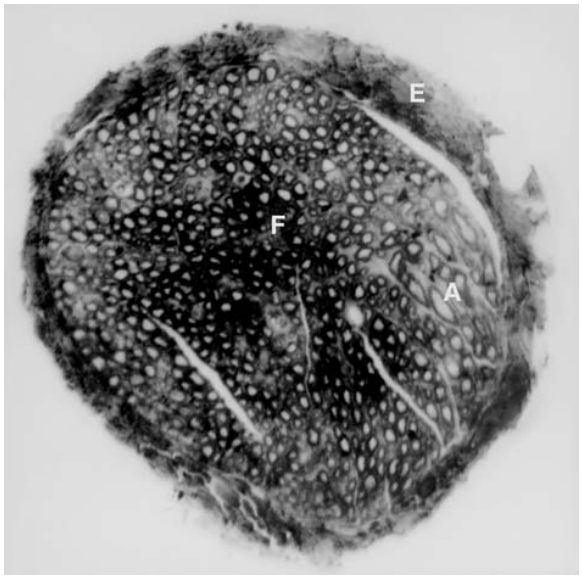


Fig. (5): Semi-thin section of peroneal nerve (T.S.) stained by touldine blue, no evidence of degeneration or regeneration, thickening of epineurium. F = fascicle, E = epineurium, A = axons, M = myelin x 250.

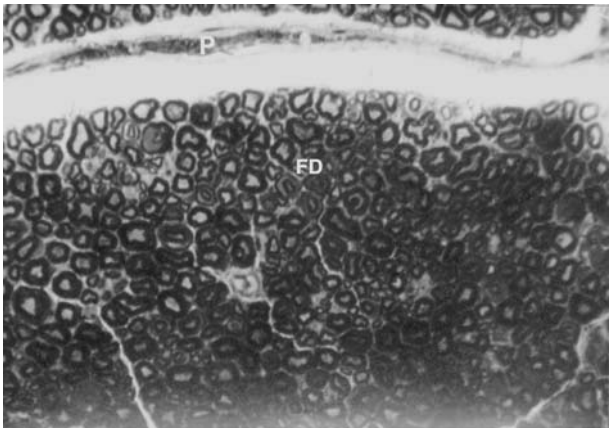


Fig. (6): Semi-thin section of distal part of tibial nerve (T.S.) stained by touldine blue, magnification x 250, no evidence of degeneration or regeneration. FD = focal areas of demyelination, P = perineurium.

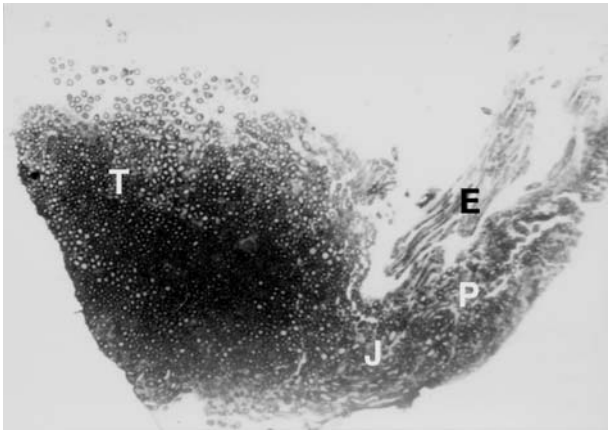


Fig. (7): Semi-thin section of junction between tibial & peroneal nerve (T.S.) stained by touldine blue, there is continuous epineurium no evidence of degeneration, there is regenerating axons of smaller size (black arrow). E = epineurium, T = tibial nerve, P = peroneal nerve, J = junction x 40.

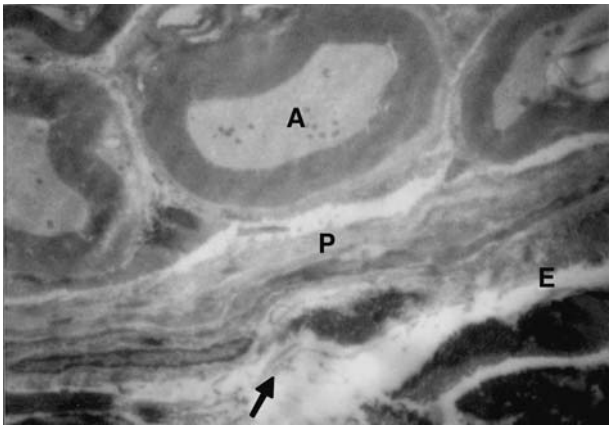


Fig. (8): An electron micrograph of tibial nerve at window area in group II, showing intact perineurium, epineurial thickening and collagen deposition, no axons are found in epineurium, site of window (white arrow) urinyl acetate and lead citrate x 5000. A = axon, P = perineurium, E = epineurium.

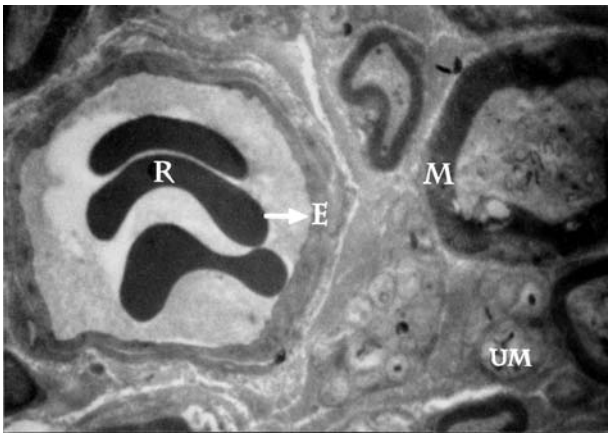


Fig. (9): An electron micrograph of peroneal nerve in group III (after end to side anastomosis) A, showing both myelinated and unmyelinated nerve axons, blood vessel showing RBCS, no evidence of degeneration. E = endothelium (white arrow), M = myelin, UM = unmyelinated axons, R = RBC urinyl acetate and lead citrate x 5000.

DISCUSSION

Following Viterbo's original report of end to side neurorraphy in 1992, there have been some concerns regarding TLN from a neuroscience point of view **[6]**. This probably affected the volume of research with a very few published articles between 1992 and 1995. Over the last years there have been several reports trying to reach for many unsolved questions about the reliability of the technique and its possible use in clinical cases **[1-5]**.

The tibio-peroneal model used in this study remains the most commonly used model in literature, it has however and the disadvantage that proximal axotomy could provide regenerating axons to the more distal graft segment. This has been termed “contamination” or “invasion” of the distal peroneal nerve.

Al-Qattan **[5]** reduced the "contamination" problem by transfixing the proximal part of tibial nerve in a distal location by cutting a segment off and then directing the stump away from the TLN. Chen **[7]** used a blocked tube to seal the proximal end of the tibial nerve to prevent the same problem. Tham **[8]** has used a silicone tube to seal the coaptation site, all of these trials succeeded in decreasing the possibility of "contamination”.

In this study measures have been taken to avoid this phenomenon by implanting the proximal part of peroneal nerve into the nearby muscles. The results showed that in the control group there were no axons outside the epineurium or within the epineurium, which proves that the surgical procedure used did not result in any injury to near by nerves, also the method of preventing contamination is reliable. These findings make us believe that both the animal model is very reliable and that it excludes false results due to surgical technique.

There are a variety of methods of assessment of TLN. Mennen **[9]** used walking track analysis as a method of evaluating movements that resulted from this type of neurorraphy and proved that TLN is effective with some change of gait of the rat.

The source of regenerating axons in the recipient nerve following TLN is a matter of debate until now. There are three possible sources of regenerating axons: (a) trauma to small branches at surgery site or during preparation or suturing of the donor nerve which will lead to axonal injury and a process of degeneration up to the first node of Ranvier with formation of numerous axonal sprouts. Some of these sprouts will pass through the end to side anastomosis and others will pass to the donor nerve. (b) contamination from the transected proximal stump and (c) true collateral (nodal) sprouting from the TLN coaptation site.

McCallister **[1]** clearly demonstrated how this contamination occurs. Axons from the proximal peroneal stump travel in the outer epineurium of the intact tibial nerve prior to entering the sutured distal segment of the peroneal nerve.

In this study the tibio-peroneal model proved to be a very reliable animal model with the results excluding the first two possible sources of TLN, so we believe that true collateral sprouting is the only way of repopulation of axons through TLN. The electron microscopic examination although was unable to clearly define the origin of the axons in the TLN, yet its finding showed, beyond doubt, that clusters of myelinated nerve axons repopulating the peroneal nerve must have come from the tibial nerve.

Zhang et al. **[11]** performed TLN between the peroneal nerve and tibial nerve in adult rats and used fluorescent microscopy in proofing that there is true collateral sprouting at the level of the node of Ranvier. The effect of this axonal sprouting on the donor nerve following TLN and whether the parent (donor) nerve is downgraded (i.e. has any loss of function) is an issue that can affect the possible use of this repair.

Zhang et al. **[11]** studied this issue and came up with the fact that there is a small degree of reduction of function which was observed early, with later improvement. He measured the conduction velocity of the nerve after few months of doing TLN and found that it have reached about 95% of the control group.

Mennen **[9]** utilizing TLN in reinnervation of median nerve through ulnar nerve found that there was a small degree of downgrading in human patients which was not of clinical significance.

In this study there were no signs of degeneration or regeneration in the tibial nerve (donor nerve) with repopulation of the peroneal nerve with myelinated axons of the same count and myelin thickness but of smaller diameter than that of the control group. This indicates that TLN can provide adequate reinnervation of denervated muscle and can do so without inducing functional deficits in muscles innervated by the donor nerve. Few sections showed focal demyelination in the donor nerve distal to TLN, yet it is an occasional finding and does not indicate degeneration.

Factors and variables affecting axonal collateral sprouting following TLN are extremely important since the final aim would ideally be to apply the TLN concept in the clinical situation with predictable results. Lundeborg et al. **[12]** have confirmed that the removal of an epineural window would allow for axonal regeneration more than if it is not removed as it stands as a barrier. Noah et al. **[4]** have documented that removal of both epineural and perineural layers would allow for better axonal regeneration which is expected as another barrier layer is removed. However, Hu **[13]** could not find a statistical difference between experimental animals having TLN with or without windows at the coaptation site.

These results with debatable findings have made us think of creating a new group where epineural layer is removed and in the same time it is not attached to a distal segment. This is to appreciate the importance of the presence of a distal segment. According to the results in-group II, where only an epineural window is created there were no signs of any axonal sprouting outside the tibial nerve or in the epineurium. This suggests that the major stimulus in inducing collateral sprouting is the denervated muscle and the attached distal segment of the nerve. The theory suggested for this is the release of nerve growth factors that actually stimulate the collateral sprouting. This concept was strongly backed up by the work of McCallister et al. **[1]** who proved that the external supplementation of growth factors to the end to side coaptation site gives a superior response and more collateral sprouting, also he mentioned the fact that Schwann cells in the donor nerve is very important in inducing such collateral sprouting. In our study since the samples were harvested after 7 weeks from operation early findings as penetration of Schwann cells was not searched for.

Matsuda et al. **[14]** demonstrated that invasion of Schwann cells from the distal segment into the epineurium of the donor nerve was a crucial step for initiation of collateral sprouting from the intact nerve. In this study, results coincided with his results proving that creation of epineural window alone without the presence of a Schwann cell source did not result in regenerating axons. Instead we demonstrated there is thickening of the epineurium at the window area. Actually the problem is that we need the maximum amount of collateral sprouting without the damage of the donor axons, it is evident that removal of the epineural layer is the maximum layer that can be removed without damage to the donor nerve axons and that technically it is impossible to remove the perineural layer without damage of donor axons.

Yen et al. **[15]** used the helicoids form of attachment where it resulted in more axonal sprouting which was explained by the fact that there was an increase in surface contact.

Yuksel et al. **[16]** demonstrated that the axonal regeneration is possible with side to side coaptation and that the results were satisfactory and even superior to the end to side technique.

In this study end to side nerve repair was done using the most common way in experimental studies where the epineurium of peroneal nerve was sutured by four stitches to epineurium of window area in tibial nerve, the results showed that there were axonal collateral sprouting after seven weeks which reached that of control group as regard number of axons and myelin thickness with decrease in the axonal diameter. These results were comparable and even better than presented by Noah et al. **[4]** as regard the numbers of axons present in peroneal nerve. The technique of doing the end to side anastomosis is important in actually reaching a good coaptation and avoiding misdirection of axons rather than the type of technique used in nerve repair.

Lundborg et al. **[12]** used nerve graft (not attached to any end organ) and he noted that the rate of axonal regeneration was higher using degenerated grafts. This was explained by the presence of more Schwann cells in these grafts. However Zhang et al. **[11]** have demonstrated that a fresh TLN is superior to a delayed end to side repair when the recipient nerve is attached to an end organ. Attachment of the distal end of the recipient nerve or conduit to an end organ may enhance neurotrophic events and will likely give a superior response using TLN.

REFERENCES

1- McCallister W.V., Tang P. and Trumble T.E.: Is end to side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. J. Reconstr. Microsurg., 15: 597-604, 1999.

2- Motsumoto M., Hirato H. and Nishiyama M.: Schwann cells can induce collateral sprouting from intact axons; Experimental study of end - to - side neuropathy using a Y chamber model. J. Reconstr. Microsurg., 15: 281-286, 1999.

3- Noah E.M., Williams A. and Jorgenson C.: End to side neurorrhaphy: A histological and morphmetric study of axonal sprouting into an end - to - side nerve graft. J. Reconstr. Microsurg., 13: 99-106, 1997.

4- Noah E.M., Williams A. and Fortes W.: A new animal Model to investigate axonal sprouting after end - to - side neurorrhaphy. J. Reconstr. Microsurg., 13: 317-325, 1997.

5- Al-Qattan M.M.: Terminolateral neurorrhaphy: Review of experimental and clinical studies. J. Recons. Microsurg., 17: 99-107, 2000.

6- Viterbo F. and Trindade J.C. and Hoshino K.: End - to-side neurorrhaphy with removal of epineurial sheath. An experimental study in rat. Plast. Reconstr. Surg., 94: 1038-1047, 1994.

7- Chen Y.G.: The effect of deinnervated muscle and schwan cells on axon collateral sprouting. J. Hand Surg., 23A: 1025-1033, 1998.

8- Tham S.K.Y.: Motor collateral sprouting through an end - to - side nerve repair. J. Hand Surg., 23A: 844-851, 1998.

9- Mennen U.: End - to - side sutures in the human patient. Hand Surg., 3: 1-7, 1998.

10- Terzis J.K., Sun D.D. and Thanos P.K.: Historical and basic science review, past, present and future of nerve repair. J. Reconstr. Microsurg., 13: 215-225, 1997.

11- Zhang Z., Soucacos P.N. and Beris A.E.: Long term evaluation of rat peripheral nerve repair with end - to - side neurorrhaphy. J. Reconstr. Microsurg., 16: 303-309, 2000.

12- Lundborg G., Rosen B. and Dahlin L.: Tubular repair of the median nerve in the human forearm: Preliminary findings. J. Hand Surg., 19B: 273-276, 1994.

13- Hu G.: Terminolateral neurorrhaphy with autogenous venous nerve conduit (AVNC): Histologic study. J. Reconstr. Microsurg., 15: 627, 1999.

14- Matsuda H., Hato Y. and Osamu I.: End - to - side nerve cooptation: Clinical applications for cross face nerve grafts. J. Reconstr. Microsurg., 13: 376-382, 1997.

15- Yan J.G., Matloub H.S. and Sunger S.R.: Nerve sprouting after terminolateral neurorrhaphy. J. Reconst. Microsurg., 15: 376-3, 1999.

16- Yuksel F., Karacaoglu E. and Gluer M.: Nerve regeneration through side to side neurorrhaphy sites in a rat model: A new concept in peripheral nerve surgery. Plast. Reconstr. Surg., 52: 404-412, 1999.