Original Research
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Comparison of nerve repair with end to end, end to side with window and end to side without window methods in lower extremity of rat

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Abstract

Background: Although, different studies on end-to-side nerve repair, results are controversial. The importance of this method in case is unavailability of proximal nerve. In this method, donor nerves also remain intact and without injury. In compare to other classic procedures, end-to-side repair is not much time consuming and needs less dissection. Overall, the previous studies in this field have just evaluated nerve recovery by non functional or functional parameters. As it has proven, the results of functional and non functional studies are not always matched. Therefore, this study was designed to evaluate this method by functional (Sciatic function index) and non functional parameters (histochemical study).

Methods: In this experimental study, forty adult male Wistar rats (200-250g) were used their left proneal nerve was cut and divided into four groups: 1- control group (n=10). In this group, nerve was exposed and cut and implanted onto adductor muscles, 2- end-to-end anastomosis (n=10). In this group, peroneal nerve cut and two segments were anastomosed end-to-end, 3- end-to-side anastomosis with window (n=10). In this group, peroneal nerve was cut and anastomosed with window to tibial nerve, 4- end-to-side anastomosis without window (n=10). In this group, peroneal nerve was cut and anastomosed without window to tibial nerve. After 1, 8 and 16 weeks, functional (Sciatic function index) and after 16 weeks non functional studies (histochemical study) were performed and the results compared.

Results: All experimental groups (group 2, 3, 4) motor recovery at 8th, 16th week were not statistically different ($p>0.05$). In histological study axons count in end -to-side with window anastomosis were higher than other experimental groups ($p<0.05$).

Conclusion: According to our findings, the authors believe that end to side neuroraphy leads to axon growth and comparable functional recovery with end to end neuroraphy in rat model. Due to the fact that the diameter of nerves and muscles which might be neurotised in human are much bigger and not comparable with rat, it is suggested to set up some set of experiments on the bigger size animals such as primates in further studies for generalization of results to human being.

Keywords: end-to-side nerve anastomosis, end-to-end nerve anastomosis, motor recovery, histologic study

Introduction

End-to-side (or terminolateral) nerve transfer was described in the early 20th century as a technique for therapy of facial nerve injury [1]. Technically, the distal end of a transected nerve is sutured to the lateral side of an uninjured nerve. End-to-side neurorrhaphy was first reported in the beginning of

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the 20th century by several authors. These early studies showed that nerve repair occurred through end-to-side method. However, due to the lack of functional results, this technique was quickly abolished. The end-to-side technique was revived in the late 1980s by Viterbo et al [2]. Again, with development of in neurobiology and experience of microsurgical techniques, this method was revived [3].

By 2000, Pietro G [2] cut the rectos femoris nerve of rabbit and anastomosed by end-to-side method to vastus medialis nerve and finally function of nerve returned. Kanje , et al [4] in 2000 and Yamauchi [5] at 2000 and also Hayashi [6] at 2004 after the nerve repair by end-to-side method in experimental studies, emphasised the collateral sprouting. In 2005 Bonitoti and colleagues used one nerve as a donor nerve for repair of two nerves and showed that even one nerve can be used for repair of two nerves by end-to-side method. In 2005 Schmid hummer [8] and co-workers, cut the median nerve of babone and anastomosed by end-to-side to motor branch of ulnar nerve, the baboons could do apposition. In 1998 Francios [9] and colleagues performed neurotization of musculocutaneus nerve form ulnar nerve by end-to-side method. In 2004 Pienaar [1] and his team repaired the brachial plexus injuries in human by end-to-side method which was not successful after months and they left this method. In 2007 Gilbert [10] and his colleagues used end-to-side method in repair of brachial plexus and also the result was not satisfactory.

The importance of end-to-side method is the fact that, it is very useful in cases that proximal nerve is not available and also in this method, donor nerves remain intact and without injury. Finally in comparison to other classic procedures, end-to-side repair needs less times and dissection. Despite of several studies, the results of this method are very controversial and the majority of studies just evaluated nerve recovery by only non functional or merely functional parameters. As it has proven, the results of functional and non functional studies are not always matched. Therefore, this study was designed to evaluate this method by Siatic Functional Index as a functional (SFI) and non functional parameters (histochemical study).

Methods

Forty male wistar rats, 200-250g were purchased from the Razi Institute animal facility. The rats were prepared for standard aseptic surgery and anesthetized using intraperitoneal ketamin and xylazine. The left sciatic nerve and its three major branches were exposed via a gluteal muscle-splitting incision, and the peroneal nerve was sectioned 1 cm down from its connection to the sciatic nerve. The rats were randomly divided into four groups.

Group 1 (Control): The proximal and distal ends of sectioned peroneal nerves were curved back at the 90 degree angle and implanted into the adductor muscles (n=10).

Group 2 (End-to End neurorraphy): The proximal and distal segments of sectioned peroneal nerves were sutured. (10-0 nylon, microscopic magnification) (n=10).

Group 3 (End-to-side neurorraphy with window): The distal ends of sectioned peroneal nerves were sutured to the tibial nerve trunk after a 1mm diameter window in the epineurium. (10-0 nylon, microscopic magnification) (n=10).

Group 4 (End-to-side neurorraphy without window): The distal ends of sectioned peroneal nerves were sutured to the tibial nerve trunk without removal of epineurium (n=10).

Motor recovery-walking track analysis: To evaluate the proneal nerve recovery, walking track analysis was carried out at 1, 8 weeks and 16 weeks after surgery [11]. To obtain the walking pattern, the hind paws of rats were pressed onto an inkpad and rats were allowed to walk up a small inclining gangway (slope 20-, length 1 m, width 14 cm), which was lined with white paper [12].

All of rats had a few pre-training runs.

The footprints parameters were measured: [1] The print length (PL, distance from the heel to the third toe), [2] the toe spread (TS, distance from the first to the fifth toe), and [3] the intermediate toe spread (ITS, distance of the second to the forth toe). All these measurements were taken from the left experimental paw (EPL, ETS, EITS) as well as from the right non-operated paw (NPL, NTS, NITS) of each rat tested. Using data, the SFI, which results from the differences between the injured and the intact contra lateral paw, was calculated by the modified formula from Bain et al as follow [11].

SFI= - 38.8 (EPL:NPL) / NPL + 109.5 (ETS:NTS)/NTS + 13.3 (EIT:NIT)/NIT: 8.8.

Histology: In this study, at 16th week after surgery tissue specimens from the distal part of the peroneal nerve 1 cm below the anastomosis sites in all of experimental groups were excised and fixed with paraformaldehide 4% and stained with tetraoxide osmyiom 1%. Then the nerves stained with toluidine blue 1% and observed under the light microscope using Olympus AX 70. Japan.

Statistical procedure: Data from the experiments were expressed as mean ±standard deviation (SD). Statistical differences were analyzed by one way ANOVA test. Bonferroni post HOC analysis was used for multiple group comparisons to determine the statistical significant differences. Moreover, a P-value < 0.05 was considered as statistically significant.

Results

Motor recovery-walking track analysis: Analyses of the rat walking pattern by recording its footprints and calculating the SFI are a well established and commonly used method for the assessment of motor nerve recovery after sciatic nerve injury [11].

Based on the statistical analysis of SFI there was no significant difference between control group in the 1thweek (50.26) and 8th (48.49±15.93) and 16th (54.76±18.25).

In the end-to-end group there was a statistical difference between 1st week (53.62) compared to 8th (13.78±8.29, P=0.00) and 16th week (11.644±9.93, P=0.00), but there was no a statistical difference between 8th and 16th week (p=.793).

In the end-to-side with window group there was a statistical difference between 1st week (45.86) compared to 8th (26.56± 8.03 P=0.00) and 16th (16.71±8.71, P=0.00) week, and also between 8th and 16th week (p=0.17). In the **e**nd-to-side without window

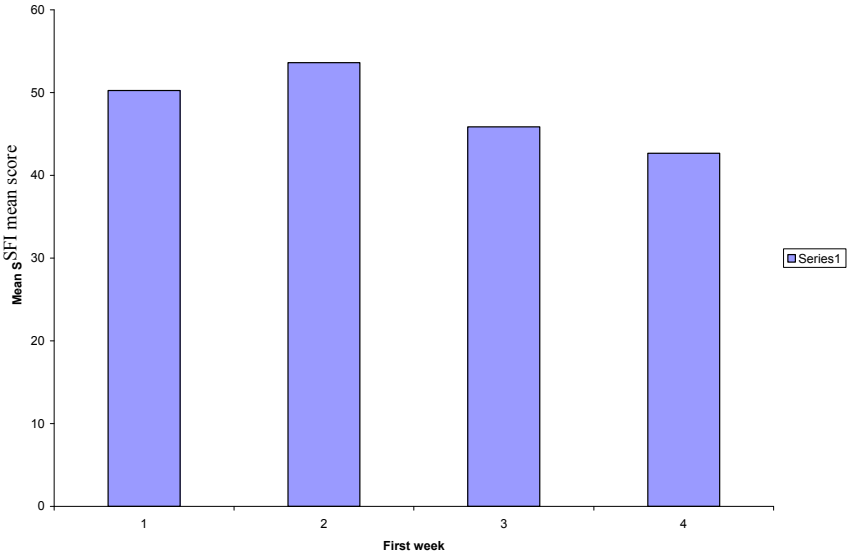

Fig 1. The SFI mean in experimental groups first week after surgery. 1=control, 2=end-to-end, 3=end-to-side with window, 4=end-to-side wtihout window.

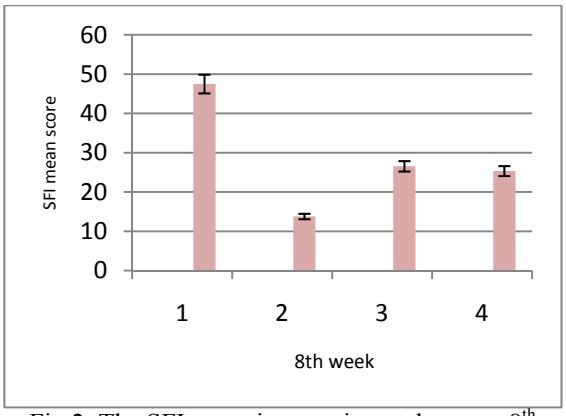

Fig 2. The SFI mean in experimental groups 8th week after surgery (p<0/05). 1=control, 2=end-to-end, 3=end-to-side with window, 4=end-to-side wtihout window.

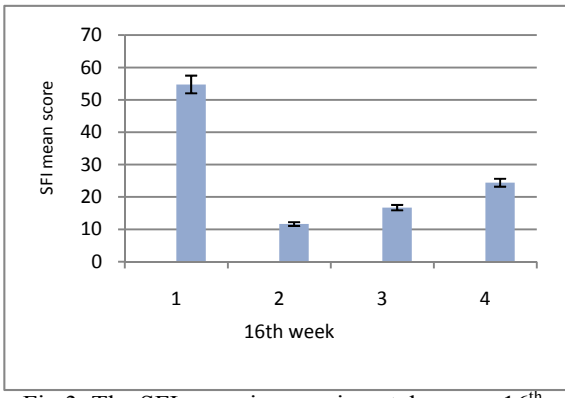

Fig 3. The SFI mean in experimental groups 16th week after surgery (p<0/05). 1=control, 2=end-to-end, 3=end-to-side with window ,4=end-to-side wtihout window.

group there was a significant difference between 1st week (42.66) compared to 8th (25.34±8.89, P=0.39) and 16th (24.40±12.60, P=0.22) week, but there was not a significant difference between 8th and 16th week (P=836). Based on the statistical analysis of SFI between all groups after 8th and 16th

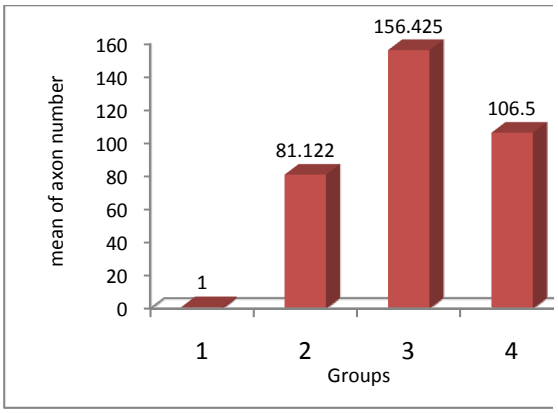

Fig. 5. The axon mean numbers after 16 weeks in all groups. 1=control, 2=end-to-end, 3=end-to-side with window, 4=end-to-side wtihout window.

week after surgery, there was a significant difference between group 1 and other groups [2-4], (p=0.02, 0.016, 0.014). There was a statistical difference between group 2 and 3 after 8th week (p=0.01) but there was not a statistical difference between them after the 16th week.

There was no significant difference between group 2 and 4 after the 8th and 16th week after the surgery (p>0.05). And also there was no significant difference between group 3 and 4 at 8th and 16th week (p>0/05). (Figs. 1, 2, 3).

Histological study

On examination of the cross sections at 16 weeks after surgery, the axons count in end - to-side with window group was significantly better than in end-to-end and end-to-side without window groups (Figs. 4, 5 and Table 1).

Discussion

It has been established that end-to-end neu-

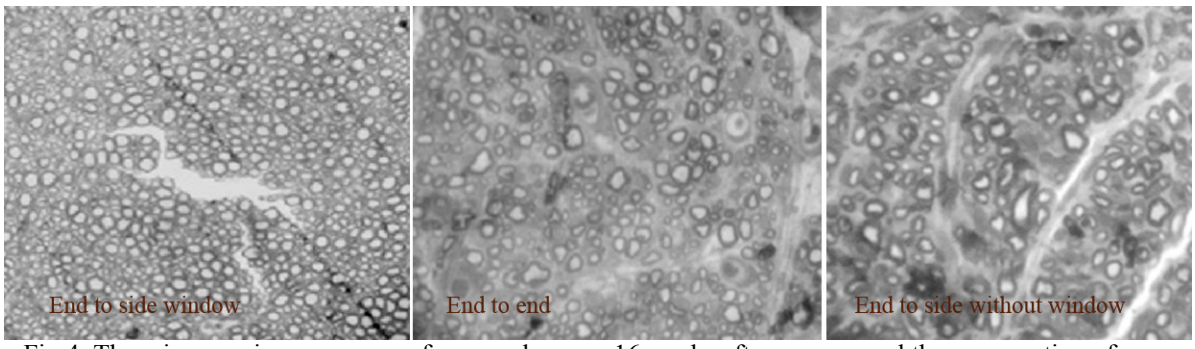

Fig 4. The microscopic appearance of peroneal nerves 16 weeks after surgery and the regeneration of axons. Scale bare ×100 (end-to-side with window), ×100(end-to-end, end-to-side without window).

Table 1. Axon numbers in experimental groups.

Groups	Axon number	
control	R*21*=0	
	R*61*=0	
	R*62*=0	
	R*63*=0	
	R*64*=0	
	R*65*=0	
	R*66*=0	
	R*67*=0	
	R*68*=0	
	R*69*=0	
end-to-end anastomosis	R*51*=106	R*56*=71
	R*52*= 68	R*57*=68
	R*53*=76	R*58*=80
	R*54*=83	R*59*=94
	R*55*=92	R*60*=69
end-to-side with window	R*22*=133	R*27*=173
	R*23*=148	R*28*=130
	R*24*=153	R*29*=226
	R*25*= 180	R*30*=135
	R*26*= 122	R*31*=162
end-to-side without window	R*32*=105	R*37*=95
	R*33*=86	R*38*=115
	R*34*=104	R*39*=81
	R*35*=145	R*60*=120
	R*36*= 115	R*61*=97

rorrhaphy is the treatment of choice when both ends are available. In peripheral nerve injuries with unavailable proximal stumps or long nerve defects, nerve repair can be potentially performed with end-to-side neurorrhaphy technique. End-to-side nerve repair has recently attracted special interest in experimental practice. As stated by Viterbo [13] recovery in end-to-side nerve repair is possible that may be explained by fiber regeneration (sprouting). Collateral axonal sprouting is believed to be essential for successful reinervation following end-to- side nerve anastomosis as emphasised by several studies [10, 4-5]. It has been well identified that stimulation of neurotrophic factors which arise from the recipient distal nerve stump, may induce nerve sprouting.

To study the functional recovery of injured nerve, we compared end-to-end, end-to-side with window and without window anastomosis in common proneal nerve.

As we showed in this study, Functional and non functional parameters are not always matched following nerve repair, the current study demonstrated that functional recovery (SFI) in experimental groups was not statistically different (Figs. 1, 2, 3) while non functional parameters showed statistical difference (Fig. 5).

Toshihiro Fujiwara showed that functional recovery of the denervated target organs was promoted by axonal augmentation after the reversed end-to-side repair [13]. Also Tunc C. ogun showed that End-to-side nerve repair can be a viable alternative to nerve grafting in patients with long gaps between the ends of the injured nerve [14]. In agreement with our study, Francisco and colleagues neurotisised musculocetaneus nerve by ulnar nerve by end-to-side method successfully [9]. There are some controversies with results obtained by other research groups and some other Authors [1,10,15] didn't recommend end-to-side nerve repair. It seems that these controversial results are caused by two factors: firstly, axonal regeneration is not selective. It means that some-

times despite of good nerve regeneration, only sensory fibers regenerate into recipient nerve and vice versa. As emphasized by Giovanoli and Schmid in rabbits, the motor nerve branch of rectos femoris cut and sutured end-to-side to the motor branch the vastus medialis after creating an epineural window and functional recovery was very good [2]. Secondly, several factors play pivotal roles in the nerve repair such as age that may be different in several studies [9].

Based on this study, result of end-to-side with window was better from point of histological study (Fig. 5]. It seems that this may be explained by collateral sprouting of donor nerve [10,4,6,1,5]. One of the most important factors that influence in regeneration of injured axon is neurotrophic factors. It seems that these factors had high influence in end-to-side with window repair compared to other groups. Difference in axon count in end-to-side with window and without window seems to arise according to some authors [1] that claim, the more invasive end-to-side technique, the grater the degree of axonal regeneration through the coaptation site, presumably as a result of unintentional donor axon transaction.

According to our findings, the authors believe that end to side neuroraphy leads to axon growth and comparable functional recovery with end to end neuroraphy in rat model. Due to the fact that the diameter of nerves and muscles which might be neurotised in human is much bigger and not comparable with rat, it is suggested to set up some set of experiments on the bigger size animals such as primates in further studies for generalization of results to human being.

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