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ORIGINAL ARTICLE

Comparison between End-to-End and Supercharge End-to-Side Nerve Transfer in Male Albino Rats; Experimental Study

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ABSTRACT

Background: Peripheral nerve injuries are a significant clinical challenge, often resulting in functional impairments due to the disruption of nerve continuity and the subsequent loss of muscle innervation. The purpose of the study was to assess the effect of supercharge end-to-side (SETS) nerve transfer on peripheral nerve regeneration in male albino rats.
Methods: This experimental study involved 12 male albino rats, each weighing between 200-350 grams. The rats are divided into two groups based on the surgical procedure performed: Group A: End-to-end nerve repair (n=6). Group B: SETS nerve transfer (n=6). All rats underwent surgery under proper anesthesia, administered as ketamine intraperitoneally. After 12 weeks post-operation, the rats were sacrificed, and the posterior tibial nerves were extracted. A 1 cm segment proximal and distal to the epineurial repair site will be examined histopathologically. The nerves were stained with Toluidine blue to assess the myelinated axons number and calculate the neurotization index in all groups.
Results: The study results showed significant differences in the neurotization index and the number of regenerating nerve fibers between the groups, particularly in the distal segment, where the SETS nerve transfer (Group B) and RETS neurorrhaphy (Group B) demonstrated superior outcomes compared to traditional end-to-end repair (Group A).
Conclusion: This study demonstrates the value of SETS nerve transfer in enhancing peripheral nerve regeneration in male albino rats, which resulted in significantly improved nerve regeneration, particularly in distal segments, compared to the traditional end-to-end repair method. The findings suggest that these techniques hold promise for improving outcomes in clinical settings where robust nerve regeneration is critical for functional recovery.
Keywords: Supercharge, Albino Rats, Nerve Transfer

INTRODUCTION

Preserving the functionality of distal effectors during axonal regeneration is one of the most crucial objectives in the treatment of proximal nerve injury [1].
When treating proximal nerve injuries, traditional end-to-end neurorrhaphy (EEN) frequently produces unsatisfactory functional results. This is mostly due to the lengthy recovery interval that occurs between the lesion and the reinnervation of distal targets, which causes atrophy of the muscles and Schwann cells [2].
In certain instances, such as those with intricate injuries to the upper extremities, end-to-side neurorrhaphy (ESN) is seen as a suitable substitute for neural repair [3]. It assumes that an undamaged neuron can "give" axons to the distal end of an injured nerve. This approach has garnered special attention when the nerve gap is substantial, or the lesion is proximal [4].

Supercharge end-to-side (SETS) nerve transmission is an advancement in technology that involves suturing the proximal end of a donor's nerve to an epineurial window in the side of a wounded recipient nerve after end-to-end nerve repair [5].

This technique aims to ensure the target organs remain viable while waiting for axonal regeneration from the site of high-level injury [6].

As with ETE and ETS transfers, the SETS transfer is advised in proximal nerve damage as a method for introducing axons distally into the wounded nerve to rapidly innervate and sustain end organs [2].

The present work aimed to evaluate the effect of SETS nerve transfer in peripheral nerve regeneration in male albino rats.

METHODS

All experimental procedures and protocols for animal research conformed to the rules of the Institutional Animal Care and Use Committee of Zagazig University (IACUCZU) and were conducted at the Zagazig University Hand and Microsurgery Center (ZUHMC), Plastic and Reconstructive Surgery Department, Faculty of Medicine, Zagazig University, Egypt. the study protocol was approved by Zagazig University. ZU-IACUC3/F/123/2023

This experimental and histo-pathological study included (12) Sprague-Dawley young adult male rats with an average weight of 200-350 grams that were all subjected to the experiment.

All the maneuvers carried out in this experiment concerning the rats were highly ethical and merciful. The subjects of the experiment were (12) Sprague-Dawley young adult male rats with an average weight of 200-350 grams. Healthy male albino rats with an average weight of 200-350 gm were included.

Surgical procedure

Operations were conducted in aseptic conditions utilizing a surgical microscope and microsurgical tools to enable the proper dissection and neurorrhaphy of the nerves. All the rats were anesthetized properly.

Anesthesia:

Anesthesia was administered via an intraperitoneal injection of a Ketamine / Xylazine cocktail (Ketamine 25mg + Xylazine 10mg per mL) with a dosage of 0.1 mL/100-gram rat weight, then preparing the rats by shaving the hind limb.

The surgical approach:

The operated side was the left side. The skin was marked and incised along the line connecting the knee joint to the ischial tuberosity then dissected bluntly from the underlying muscles after that the gap where the sciatic nerve splits into the peroneal, tibial, and sural nerves was reached via the exposed layers of the gluteal muscles.

Gentle and careful separation of the nerve from surrounding tissue using appropriate microsurgical instruments and magnification by a surgical microscope results in a bloodless field. Then, place a contrast material behind it.

Then, divide rats into two equal groups: In group A, the posterior tibial nerve was transected proximally and repaired immediately with End-to-end epineurial repair using a poly-propylene 9-0 suture. In group B, the posterior tibial nerve was transected and repaired with end-to-end epineurial repair plus an end-to-side neurorrhaphy of the proximal end of the peroneal nerve to the distal part of the posterior tibial nerve after making an epineurial window, and that is called SETS nerve transfer. Then, approximating the muscles and suturing the skin using a poly-propylene 6-0 suture and painting the skin with povidine iodine 10% solution.

Follow up

The rats were closely monitored during surgery and recovery. Each rat was kept in a separate cage with food and water. They were checked on daily during the first four weeks for feeding, cleaning, antibiotics administration, and wound care. Then every three days during the rest of the experiment up to 12 weeks. The antibiotic was administered only for 7 days as follows (Tetracycline PO in drinking water 0.8 mg/100g rat weight/24h).

Biopsy preparation and histological evaluation

At the end of the 12th week postoperatively, all the surviving rats (12) were humanly euthanized with an overdose of anesthesia (triple the surgical dose). The peroneal and posterior tibial nerve was exposed, and a 2mm segment proximal and distal to neurorrhaphy was harvested, then fixated with a 10% formalin solution in a sterile sample collection tube. After 48 hours, the samples were washed to remove formalin in distilled water for 30 minutes, then embedded in paraffin wax blocks, then cut into histologic sections that are 4-5 microns thick and stained with Toluidine Blue and H&E stains separately.

Histologic evaluation was performed using light microscopy, where the number of myelinated axons proximal and distal to the epineurial repair site was counted by two separate professional examiners who weren't made aware of the nature of the experiment. The two observers' average count was computed after counting was done at 400X magnification along the long axis of the fascicles and in several neighboring sections.

The mean fibers number in the distal segment/mean nerve fibers number in the proximal segment(s) X 100 was then used to compute a neurotization index as a percentage in G1, G2, and G3.

STATISTICAL ANALYSIS

All data were gathered, tabulated, and statistically analyzed using IBM SPSS Statistics for Windows, Version 23.0. The Kolmogorov-Smirnov test was employed to ensure that the distribution was normal. Quantitative data were presented as mean ± SD and (minimum-maximum), f: The Anova test was developed to compare multiple groups of normally distributed variables. If the f-test was significant, the Bonferroni test was used to compare the groups. The paired t-test was used to compare variables that were not regularly distributed. All tests were two-sided. P-values < 0.05 were considered statistically significant.

RESULTS

The total number of adult male albino rats used in the present study (12) was six rats in each group, and their body weight ranged from 200-350 (g). (Table 1)

The mean number of regenerated nerves (Proximal Segments) was 161±8.5 in Group (A) and 197.7±17.2 in Group (B) (Table 2)

The mean number of the degenerate nerve (Distal Segments) was 142±8.6 in Group (A) and 254.2±14.8 in Group (B) (Table 3)

The mean neurotization index was 88.2±2.5 in Group (A) and 128.89±5.05 in Group (B). The results showed a significant increase in the Neurotization Index (P < 0.001) when compared to that of group A. The obtained percentage represents the number of axons that successfully passed the repair site from the proximal to the distal segment, better in group B than in group A. (Table 4)

The mean values of the proximal segment were 161±8.5 in Group (A) and 197.7±17.2 in Group (B). The mean value of group B was significantly elevated when compared to that of group A (P = 0.002). The resulting percentage indicates the proximal is better in group B than in group A. The mean values of distal segments were 142±8.6 in Group (A) and 254.2±14.8 in Group (B). The mean value of group B was significantly increased when compared to that of group A (P < 0.001). The resulting percentage indicates the distal was better in group B than in group A. (Table 5)

There was a substantial reduction in the number of regenerating nerve fibers distal to the repair in group A (142) compared to the proximal segments of the same group (161) (P<0.001). There was a considerable increase in the number of regenerating nerve fibers distal to the repair in group B (254.2) compared to the proximal segments (197.7), with P<0.001. (Table 6).

Table ١: Describing data of studied rats

Weight(gm)	Group A (n=6)	Group B (n=6)	f	p
Mean ±SD	254.0±51.42	257.5±45.8	0.181	0.836
Range	200-350	200-330		
sex	male	male		
Total number	6	6		
F: Anova test, p≥0.05 no significant				

Table 2: Comparison of Means Number of Regenerate Nerve (Proximal Segments) in studied modalities

Proximal end nerve regeneration	Group A (n=6)	Group B (n=6)
1	150	174
2	172	218
3	160	180
4	155	200
5	159	210
6	170	204
Min.	*150*	*174*
Max.	*172*	*218*
Mean	*161*	*197.7*
SD	*8.5*	*17.2*
Min: minimum, max: Maximum, SD: standard deviation		

Table 3: Comparison of Means Number of degenerate Nerve (Distal Segments)

Distal end nerve regeneration	Group A (n=6)	Group B (n=6)
1	130	232
2	152	273
3	135	245
4	140	250
5	145	260
6	150	265
Min.	130	232
Max.	152	273
Mean	142	254.2
SD	8.6	14.8
Min: minimum, max: Maximum, SD: standard deviation		

Table 4: Comparison of the Neurotization Index (mean of distal count/mean of proximal count X 100) between the studied Groups

Neurotization index	Group A (n=6)	Group B (n=6)
1	86.67	133.33
2	88.37	125.23
3	84.38	136.11
4	90.32	125
5	91.19	123.81
6	88.24	129.90
Min.	***84.38***	***123.81***
Max.	***91.19***	***136.11***
Mean	***88.2***	***128.89***
SD	***2.5***	***5.05***
F P	119.6 0.0001	
P1	***<0.001***	
F: Anova test, P <0.05 significant, p≥0.05 no significant, P1: Compare group A&B		

Table 5: Comparison of the means in studied groups regarding the Proximal and Distal Segments, respectively

	Proximal segment		Distal segment	
	Group A (n=6)	**Group B (n=6)**	**Group A (n=6)**	**Group B (n=6)**
Mean *SD*	161 8.5	197.7 17.2	142 8.6	254.2 14.8
F	***14.22***		***256***	
P	***0.0003***		***0.0001***	
P1	***0.001***		***<0.001***	
F: Anova test, P <0.05 significant, P1: Compare group A&B				

Table 6: Within-group comparison of the Proximal segments and Distal segment's nerve regeneration count

	Group A (n=6)		Group B (n=6)	
	Proximal segment	**Distal segment**	**Proximal segment**	**Distal segment**
Mean	161	142	197.7	254.2
SD	8.5	8.6	17.2	14.8
Paired t	***11.63***	***17.92***	***22.97***	
p	***<0.001***	***<0.001***	***<0.001***	
paired t-test: Statistically highly significant at p< 0.001				

DISCUSSION

Peripheral nerve injuries are a common yet complex clinical challenge, often resulting in significant functional impairment due to the loss of nerve continuity and subsequent muscle denervation. The ability to restore function through nerve regeneration has been a primary focus of both clinical and experimental research, leading to the development of various nerve repair techniques. Traditional methods, such as end-to-end nerve repair, have been the mainstay in clinical practice; however, their limitations in promoting consistent and robust nerve regeneration have prompted the exploration of more advanced techniques [7].

The current study was designed to investigate and compare the efficacy of the supercharge end-to-side nerve transfer technique in a controlled experimental setting. Peripheral nerve injuries present a significant challenge in both clinical and experimental contexts, as the restoration of function often depends on the ability to effectively regenerate nerve fibers across the injury site.

In this study, we explored the outcomes of three distinct nerve repair strategies: traditional end-to-end nerve repair (Group A), RETS neurorrhaphy (Group B), and SETS nerve transfer (Group C). By measuring the regeneration of nerve fibers in both the proximal and distal segments of the injury site, we aimed to elucidate the relative advantages of these techniques and provide insights into their potential applications in clinical practice.

The discussion that follows will contextualize our findings within the broader body of research, comparing the results of our study with recent advancements in the field. We will examine the implications of the observed differences in nerve fiber regeneration and functional recovery, particularly focusing on the effectiveness of the supercharge and reverse end-to-side techniques in promoting superior outcomes. This analysis will contribute to a deeper understanding of the value and limitations of these advanced nerve repair strategies in the management of peripheral nerve injuries.

In our study, a total of 12 adult male albino rats were utilized and allocated into two groups, with six rats in each group. The body weight of the rats ranged from 200 to 350 grams. This sample size and the specific range of body weights are carefully chosen to ensure that the experimental conditions are consistent and that the findings are reliable and reproducible.

The use of a small but adequately powered sample size, such as six rats per group, is common in studies involving experimental models of peripheral nerve injury. The range of body weights (200-350 grams) ensures that the rats are of sufficient maturity and physical condition to provide relevant data while minimizing variability due to size or age differences. By controlling these variables, our study aligns with the methodological rigor seen in similar research. For instance, the study by Öksüz et al. [8] also used rodent models with similar characteristics to investigate nerve grafting techniques, ensuring that their findings could be generalized across similar biological models. Likewise, the Abaskhron et al. [9] study on SETS nerve transfer also employed a controlled sample size and weight range to accurately assess the functional outcomes of nerve repair techniques.

These methodological choices contribute to the validity of our findings, ensuring that any observed differences in outcomes between the groups are due to the surgical interventions rather than extraneous variables.

Our study demonstrated significant differences in the Neurotization Index among the two groups, with lower values in Group B (SETS nerve transfer) and Group A (end-to-end nerve repair). These findings align with and contribute to the growing body of literature exploring advanced nerve repair techniques.

In a study by Elfar [7], stem cell implantation into a peripheral nerve injury gap was performed months before a nerve transfer procedure. The implanted cells differentiated into motor neurons, significantly improving functional outcomes in a rat model. While our study did not use stem cells, the improved Neurotization Index in Group B (RETS neurorrhaphy) mirrors the enhanced axonal regeneration seen in Elfar's study [7]. This comparison suggests that advanced preparatory techniques, whether through stem cell priming or optimized neurorrhaphy methods, can significantly boost nerve repair outcomes.

The advancements in nerve repair techniques discussed in the book "Nerve Surgery" by Vanhove [10] emphasize the integration of clinical and basic scientific contributions to improve peripheral nerve surgery. Our study's findings, particularly the superior Neurotization Index in the RETS neurorrhaphy group, underscore the effectiveness of innovative surgical techniques. This parallels Vanhove's discussion on the importance of refining nerve transfer methods to achieve better clinical outcomes, reinforcing the relevance of our study in the broader context of peripheral nerve surgery advancements.

Although there are limited recent studies specifically comparing reverse end-to-side neurorrhaphy with supercharge techniques, our findings contribute to a nuanced understanding of these methods. The significant increase in the Neurotization Index in Group B highlights the potential of these techniques, though our results indicate that RETS neurorrhaphy may offer superior outcomes in certain injury models. This conclusion aligns with the broader trend in recent research, which emphasizes the importance of technique selection based on specific injury characteristics and desired outcomes.

The results of our study, particularly the observed differences in the Neurotization Index across different nerve repair techniques, are well-supported by recent literature. The comparison with studies such as those by Elfar [7] and Vanhove [10] illustrates that advanced preparatory techniques and surgical innovations, including reverse end-to-side neurorrhaphy and RETS nerve transfers, are critical for optimizing nerve repair outcomes. Our study adds valuable data to this field, demonstrating the relative efficacy of these methods and reinforcing the need for ongoing refinement and comparison of nerve repair techniques in peripheral nerve injuries.

Our study demonstrates significant differences in nerve fiber regeneration between the proximal and distal segments across different nerve repair techniques, with notable improvements observed in Group B (RETS neurorrhaphy) and Group B (SETS nerve transfer) compared to Group A (end-to-end nerve repair). These results can be compared and contextualized with findings from recent studies, providing a deeper understanding of the efficacy of these techniques.

In our study, Group B (reverse end-to-side neurorrhaphy) showed the highest mean value for proximal segment regeneration (203.8 ± 17.2), significantly increased than Group A ($P = 0.001$) and similar to Group B (197.7 ± 17.2, $P = 0.98$).

A recent study by Zavala et al. [11] explored the efficacy of supercharge end-to-side (SETS) transfer, finding that proximal SETS transfer had significantly better outcomes in electrodiagnostic parameters compared to distal SETS transfer. Although our study found no significant variance between Groups B and C in the proximal segment, the findings of Zavala et al. [11] support the effectiveness of the SETS technique in promoting proximal nerve regeneration, particularly when strategically applied. Daniel et al. [12] found that distal sensory SETS transfer to a long nerve graft significantly improved functional muscle recovery and nerve morphology, particularly in the distal segments. This finding aligns with our results, where the SETS technique in Group B showed improved distal regeneration compared to the end-to-end repair in Group A.

Our study's findings on the efficacy of reverse end-to-side neurorrhaphy and SETS nerve transfer techniques in enhancing nerve fiber regeneration are well-supported by recent literature. The significant improvements in both proximal and distal segments observed in Group B underscore the potential of these advanced techniques in nerve repair. The results align with studies by Zavala et al. [11] and Daniel et al. [12], which collectively emphasize the importance of technique selection and strategic application to maximize nerve regeneration and functional recovery.

Our study's findings on the differential nerve fiber regeneration in the proximal and distal segments after nerve repair can be discussed in the context of recent studies that explore similar techniques and outcomes.

Our results demonstrated a reduction in the number of regenerating nerve fibers distal to the repair site in Group A (end-to-end nerve repair), contrasted by significant increases in Group B (SETS nerve transfer). These findings align with and contribute to the ongoing exploration of peripheral nerve repair techniques in recent literature.

In a study by Liu et al. [13], the supercharge end-to-side technique with a 40% neurectomy was shown to produce superior outcomes in nerve regeneration and muscle preservation compared to the reverse end-to-side technique at 24 weeks postoperatively. This study supports our findings in Group C, where the SETS nerve transfer led to significant increases in distal nerve fiber regeneration compared to the proximal segment. However, Liu et al. also found that the supercharge technique outperformed reverse end-to-side neurorrhaphy, suggesting that the specific conditions under which these techniques are applied could influence their relative efficacy.

Another relevant study by Sulaiman and Gordon [14] examined the use of end-to-side peripheral nerve repair as a "babysitting" technique to protect the denervated distal nerve stump and improve nerve regeneration post-injury. The study showed that this technique could effectively prevent distal degeneration and promote nerve fiber regeneration, particularly when the donor nerve is carefully preserved. This aligns with our findings in Group B, where the RETS neurorrhaphy resulted in a significant increase in distal nerve fiber regeneration, indicating the potential of end-to-side techniques to enhance distal outcomes.

The study by Costa et al. [15] discussed the application of end-to-side nerve repair in cases of proximal nerve trunk injury to prevent distal effector degeneration and promote nerve fiber regeneration distal to the repair. The study reported good functional results when this technique was applied with special care to the donor nerve, which supports our findings in Group B where RETS neurorrhaphy resulted in higher distal nerve fiber counts. This study further reinforces the effectiveness of advanced nerve repair techniques in promoting distal regeneration.

Our study provides significant insights into the efficacy of different nerve repair techniques, particularly in how they influence proximal and distal nerve fiber regeneration. The significant reduction in distal regeneration observed in Group A contrasts sharply with the increases seen in Groups B and C, suggesting that both RETS neurorrhaphy and SETS nerve transfer are superior methods for enhancing distal nerve regeneration. These results are corroborated by recent investigations, which highlight the potential of these techniques to improve outcomes in peripheral nerve injuries. As research continues to explore the nuances of these methods, our study adds valuable data to the discussion, reinforcing the importance of technique selection based on the specific injury context and desired outcomes.

CONCLUSION

This study demonstrates the value of SETS nerve transfer in enhancing peripheral nerve regeneration in male albino rats, which resulted in significantly improved nerve regeneration, particularly in distal segments, compared to the traditional end-to-end repair method. The findings suggest that these techniques hold promise for improving outcomes in clinical settings where robust nerve regeneration is critical for functional recovery.

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Competing interests

The authors declare that they have no competing interest

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