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Histological and Immunohistochemical Evaluation on Neurorrhaphy of Sciatic Nerve Using Proline Suture Versus Fibrin Glue in Albino Rats

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Abstract: The brain and spinal cord are connected to all body's organs and to the outside world by the peripheral nervous system that has a restricted capability to regenerate. Fibrin glue (FG) can be used as an alternative to suture repair to reconnect peripheral nerves. Aim: This study aims to compare the use of proline suture and FG in nerve repair through histological examination and immunohistochemical detection of PCNA. Materials and Methods: 24 albino rats divided into 3 groups GI: subjected to 3 hours of compression with a non-traumatic vascular clamp on left sciatic nerve then clamps were removed, GII: subjected to immediate repair of the nerve stumps by direct proline suture while GIII: subjected to repair with FG adhesion. After eight weeks, the nerves were removed and prepared for histological and immunohistochemical studies. Results: GI showed multiple destructive histopathological results while in GII and GIII the neural tissue was almost restored to normal reported with significant increase in PCNA positive cells. Conclusion: regeneration of nerve injury was reported for both treated groups with some privilege to FG in repairing transected sciatic nerve.

Keywords:*Neurorrhaphy, sciatic nerve, proline suture, fibrin glue, PCNA, albino rats*

Introduction

The body's complex network of peripheral nerves (PNs) connects different body's organs to the central nervous system (Carriel, Alaminos et al. 2014). Histologically, the nerve tissue, parenchyma and three specialized connective tissue layers as well as stroma are the main component of PNs (Geuna, Raimondo et al. 2009). The parenchyma is organized into peripheral nerve fibers (PNFs) as conductive units which consist of neuronal axons surrounded by Schwann cells and a thin external basal lamina. PNFs can be either unmyelinated or myelinated. The myelinated PNF in which case Schwann cells form a multilayered, lipid-rich myelin sheath. (Carriel, Campos et al. 2017).

Regarding the stroma, the epineurium, a layer of connective tissue rich in collagen and vascularized which covers of PNs externally. The parenchyma is arrange in separated fascicles, each one lined by the perineurium while a loose connective tissue is surround each PNF called endoneurium (Topp and Boyd 2006).

Traumatic injuries, tumors, and a number of clinical diseases can all have an impact on both function and structure of PNs (Moore, Kasukurthi et al. 2009).

After receiving structural injury, PNs' ability to regenerate the affected structures and restore their sensory, motor next to the vegetative functions is limited. The evacuation of a neoplasm and traumatic injuries may have a significant impact on the structure and function of the PNs, which could lower patient quality of life overall. Transection of PNs which may be complete or incomplete are passively repaired by neurorrhaphy and re-establishment of the nerve trunk and reconstruction of fascicles with a respectable functional recovery (Dahlin 2008).

When there is tissue damage and direct restoration is not possible, autologous nerve grafts are frequently regarded as the gold standard treatment (McDonald and Bell 2010). In spite of, some limitations facing nerve grafting , such as the consequent greater morbidity, the need to perform two surgical procedures at different sites, and the shortage of nerve donor sites, in addition to the loss of sensory in the area where the donor nerve was eradicated (Mafi, Hindocha et al. 2012).

One of the surgical repair is tubulization as the sectioned nerve stumps are inserted and secured inside a tubular prosthesis with the goal of directing nerve growth, preventing tissue scar invasion, and preventing the formation of neuromas. (Tos, Battiston et al. 2012). Pro-regenerative factors are added into the tube such as neurotrophic factors or stem cells (Barmpitsioti, Konofaos et al. 2011). Utilizing both biological and nonbiological tubular nerve guides, various conduits have been employed to bridge nerve gaps; the biodegradable polymers provide the benefit of not requiring an additional surgery to remove them and of maintaining a healthy nerve (Lohmeyer, Siemers et al. 2009).

In addition to that technique, there is the traditional process to reconnect a transected nerve through suture strand. However, many disadvantage associated with this technique including the suture material itself that may have an impact on the precise arrangement of fascicles and development of a neuroma in the anastomosis region as a result of the granulomatous as a response to the suture strand (Nakamura, Inada et al. 2004). Additionally, the quantity of suture strands traversing the repair site may affect the outcome of the repair (Torres, Graça et al. 2003).

Fibrin glue can be used as an alternative method to the suture in some cases. Various surgical specialties, including general surgery (Goldberg, Jobin et al. 2007), maxillofacial surgery (Sentovich 2003), cardiothoracic surgery (Giannini, Mauro et al. 2004), ophthalmology (Kjaergard and Fairbrother 1996, Sharma, Kaur et al. 2003), and neurosurgery (Yeh, Bushley et al. 2006), have been using glues and fibrin adhesives.

The administration of fibrin glue is rapid and simple, cutting down on operating time and facilitating tissue adhesion with no need for suturing and with a lower risk of infection (Alibai and Bakhtazad 1999). It has been proven that fibrin glue works successfully to restore injured peripheral nerves (Tredwell, Jackson et al. 2006). Furthermore, there is no association between the usage of fibrin and tissue necrosis or inflammation.

No fulfilled comparison between the influence of fibrin glue and suture in the nerve repair by stump-to-stump neurorrhaphy and tubulization. Therefore, more studies seek to improve the prognosis of nerve repair after complete or incomplete neural injury.

Aim of the study:

This study aims to compare between the use of proline suture and fibrin glue in nerve repair through histological examination and immunohistochemical detection of PCNA.

Materials and methods

Prolene suture and readymade fibrin glue that were used in the present study were purchased from Dr. Mohamed Shabrawichy Hospital – Doki- Egypt as shown in table1 and fig.1.

fig.1: Double syringe of fibrin glue.

Material name	Composition	Manufactures
Beriplast ® P	Mixture of fibrinogen and coagulation factor XIII	Aventis Behring - Germany
PROLENE	Isotactic crystalline stereoisomer of polypropylene	EthiconIncorporation,-New Jersey, USA

Table 1: The material brand name, type, composition and manufactures of the material used in this study

Sample size calculation

Sample size calculation was performed using G*Power version 3.1.9.2, (Faul, Erdfelder et al. 2007). The effect size *f* was 0.91 (large) according to the previous studies with alpha (α) level of 0.05 and Beta (β) level of 0.05, i.e., power = 95%; the estimated sample size (n) was 24 samples and were divided equally into 3 groups (8 samples/ group).

Experiment Animals

The present study was commenced after the approval of the Research Ethics Committee of the faculty of Dentistry of Suez Canal University (612/2023) and carried out on 24 mature male albino rats (average weight 220 to 300g). All the experimental rats were housed separately in clean metal cages under standard condition, controlled lightening and environmental temperature (25℃) receiving a standard laboratory diet and water. All rats in this study were injected with preanesthetic agent (Xylazine hydrochloride) by intraperitoneal injection 20mg/Kg, then anesthetic agent (Ketamine)***50***mg/Kg by intraperitoneal injection. In every rat, one of the thighs was shaved and sterilized with betadine, then the left sciatic nerve was exposed between the vastus lateralis and the biceps femoris by longitudinal posterolateral incision. The nerve was then dissected from the surrounding tissue with a surgical loups, then cut (divided) by lancet.

The rats were randomly divided into three groups (8 rats each): Group I: consisted of 8 rats were subjected to three hours of compression with a non-traumatic vascular clamp for left sciatic nerves and then clamps were removed, group II: consisted of 8 rats with left sciatic nerve transection and were subjected to immediate repair of the nerve stumps by direct proline suture (two stitches on each side), group III: consisted of 8 rats with left sciatic nerve transection and were subjected to immediate repair of the nerve stumps with fibrin glue adhesion, one small drop (about 50 μL) in the coaptation site. The rats were allowed to survive for eight

weeks. After eight weeks, the animals were anesthetized again, and then perfused transcranial with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The regenerated left sciatic nerves were removed and prepared for histological and immunohistochemical studies.

Histopathologic examination

Sciatic nerve tissue samples were removed and fixed in 10% NBF solution, embedded in paraffin blocks, were cut at 4 μm thickness, stained with hematoxylin and eosin (H&E) and were examined under light microscope (Olympus BX51, Tokyo, Japan).

Immunohistochemical examination:

Immunohistochemical localization was performed using proliferating cell nuclear antigen (PCNA) were examined. The slides will be dewaxed and submitted to antigenic recovery with 0.1 M citrate solution Ph 6.0. Endogenous peroxidase activity was blocked for 30 min with 0.3% hydrogen peroxide followed by 1% protein blocking for 30 min. The slides will be incubated overnight with the anti- PCNA antibodies and examined by computer image analyser system, Leica Qwin 500 software (Leica Microsystems LTD, CH9435 Meerbrugg Type: DFC295 (12730469), Input: 12v/170 MA, Serial number: 0557060916, Switzerland) was used to measure the area percent for the number of immune positive cells.

Statistical analysis

All data were collected, calculated, tabulated and statistically analyzed using the following statistical tests. A normality test (Shapiro-Wilk) was done to check the normal distribution of the samples. Descriptive statistics were calculated in the form of Mean ± Standard deviation (SD). One-way ANOVAs was used to compare between three groups. Bonferroni post hoc tests were used for pairwise comparisons. P value ≤ 0.05 is considered statistically significant. All analysis was done using the computer program SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels 0.05 (P- Value ≤0.05).

Results

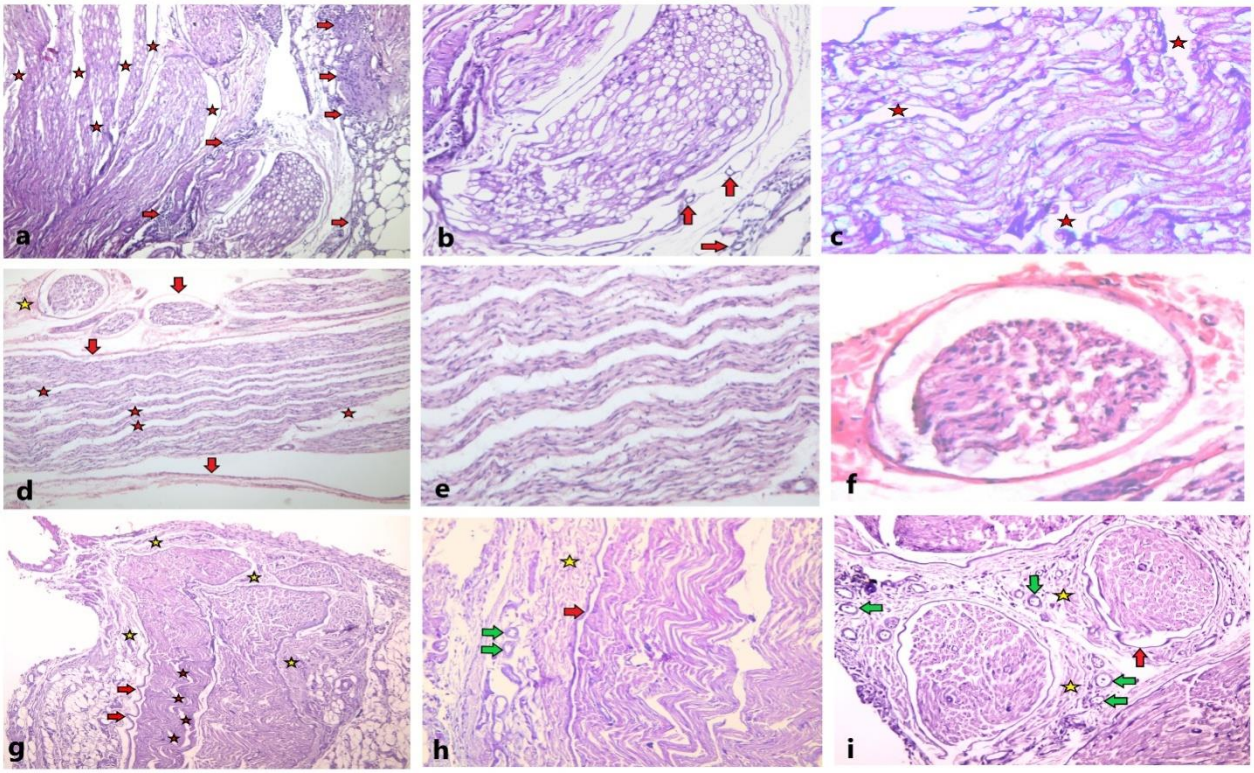
Histopathologic results

By examining group I, the compressed sciatic nerve tissues showed multiple histopathological results as follows, severe spongious appearance as a result of remarkable increase in axonal cytoplasmic vacuolization, loss of myelin sheath, axons and endoneurial tubes distortion, with discontinuity for the perineurium. Cellular infiltrations for inflammatory cells at epineurium were detected as well as constricted blood vessels. The histological appearance of the neural tissue sections was nearly restored to normal in both groups where the spongious appearance almost disappeared except in few segments. However, few vacuolization can be seen in sutured group II that showed regular nerve fibers with different diameters, regeneration of Schwann cell's nuclei peripheral to the myelin sheath and blood capillaries in endoneurium. In group III where fibrin glue was applied, the proliferated and regenerated Schwann cells is significant even though few segmental demyelinated nerves can be detected. Well organized fascicles with marked reduction in cytoplasmic vacuolization in addition to continuity of perineural and regeneration of epineural connective tissue with almost no inflammatory cells and dilated blood vessels were observed fig.2. Immunohistochemical staining showed significant increase in PCNA positive cells numbers in both treated groups than those of the sciatica groups ($p<0.05$). However, the number of PCNA positive cells were higher in group III with adhesive fibrin glue than the sutured group II fig.3.

Statistical results

The results showed that there are clearly significant differences between groups for PCNA localization. Using one-way ANOVAs ($F=14.19$, $P=0.0016$) at a significant level $P< 0.05$. The pairwise comparison showed a significant difference between group II and group III with group I while there is no significant difference between them. Group III recorded the high mean value (15.67±3.18) followed by group II (13.65±3.23) while

group I was the lowest (5.83±1.51). Mean and Standard deviation (SD), minimum and maximum values for



PCNA for different groups were presented in table 2 and fig.4

Fig.2 shows the histopathological examination of sciatic nerve tissues of all groups, H&E x100, x200, x400 respectively as (a,b,c) for group I, (d,e,f) for group II and (g,h,i) for group III.(a) showed disorientation for neural fascicles, loss of some neural fibers which replaced with empty space (red stars) with discontinuity of perineurium and several scattered inflammatory cells within epineurium (red arrows). (b,c) showed sever disintegration for myelin sheath and Schwann cells with remarkable vacuolization (spongious appearance), congested blood vessels (red arrows) and absence of some nerve fibers.(d,e,f) showed well recognized Schwann cells surrounding the axons of neurons that are free of the spongious appearance and perineurium (red arrows) ,epineurium (yellow stars) and few missing neural fibers (red stars) were also recognized (g,h,i) showed well defined neural fascicles formed of nerve axons surrounded by myelin sheath, perineurium (red arrows), well-formed epineurium with remarkable decrease in inflammatory cells (yellow stars) and few nerve fibers were lost (red stars).

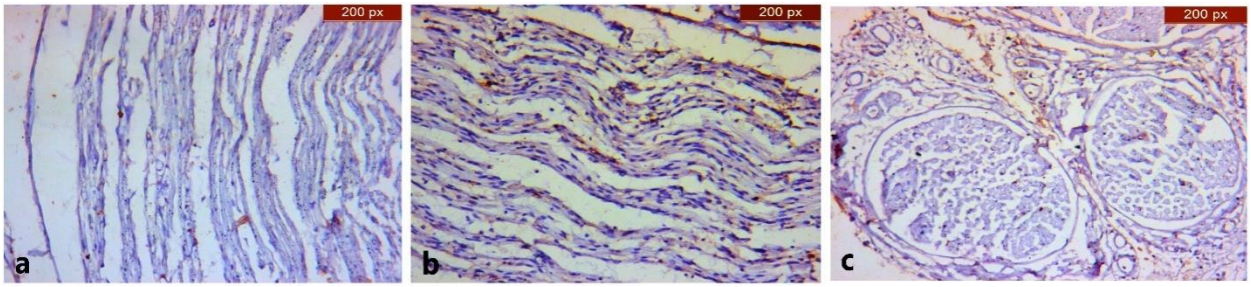


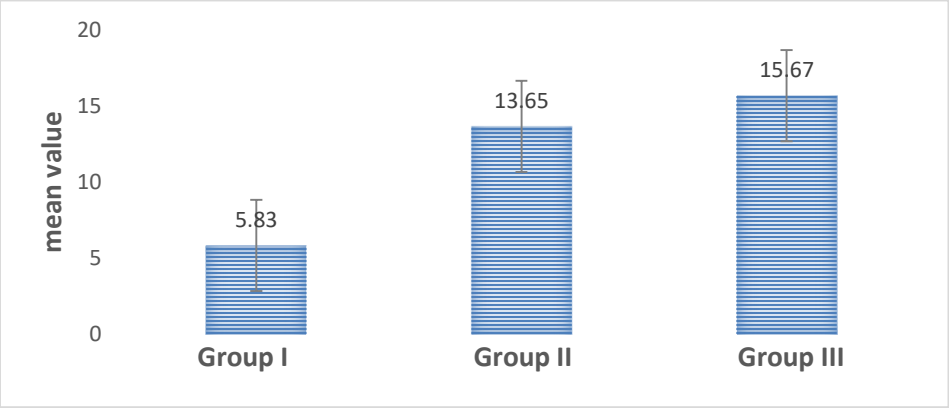
Fig.3 shows the immunohistochemical examination of PCNA expression in sciatic nerve tissues of all groups, PCNA x 200 as (a) for group I, (b) for group II and (c) for group III. The percentage of PCNA positive cells was calculated showing least values in compressed group I while group II and III showed high values with insignificant differences.

Table 2 shows the statistical results between all studied groups.

	Group I	Group II	Group III	F test	P value
Mean	5.83[b]	13.65[a]	15.67[a]	14.19	0.0016 **
SD	1.51	3.23	3.18		
Min	4.07	11.07	11.51		
Max	7.12	18.34	19.19		

**** and different superscript letters means significant difference between groups at P<0.05**

Fig.4 shows the mean values for PCNA expression for all studied groups.



Discussion

The causes of peripheral nerve injury include compression, focal contusion, traction, or transection of the nerve. Ischemic injury, inflammation, and oxidative stress follow peripheral nerve injury, which explains the infiltration of inflammatory cells at the epineurium. (Ghayour, Abdolmaleki et al. 2017). Simultaneously, immune responses occurring in the region of injury result in the production of pro-inflammatory messengers surrounding the injured tissue and aid in the formation of scar tissue and nerve fibrosis.(Lemke, Penzenstadler et al. 2017), neuromas, and cytoplasmic vacuolization that result in nerve damage and less neural conduction following the injury (Ngeow 2010) that is compatible with results of present study in group I.

The gold standard for healing a damaged nerve is anastomosis with epineural suturing, but using tissue adhesives for microneural anastomosis appears promising in order to get over the drawbacks of epineural suturing. (Suri, Mehta et al. 2002, Breshah, Sadakah et al. 2013).

Histopathological results in group III treated with fibrin glue showed lower neural damage than group I. Furthermore, the PCNA localizations were the highest among all groups (15.67±3.18) as a result of regeneration of Schwann cells and reconstruction of myelin sheath.

Schwann cells stimulate the production of neurotrophic factors, including ciliary neurotrophic factor (CNTF), fibroblast growth factor (bFGF), and nerve growth factor (NGF).(Ebadi, Bashir et al. 1997, Elfar, Jacobson et al. 2008). These factors, encourage the generation of new Schwann cells (Birchmeier and Nave 2008, Vrbova, Mehra et al. 2009) and, in conjunction with acetylcholine, stimulate their continued proliferation (Stoll, Griffin et al. 1989). They also release neuregulin and ATP from the proximal nerve endings (Pellegrino and Spencer

1985). The integrity of the fascicles was changed, and regenerative neural epineural connective tissue was formed in both treated samples but less connective tissue growth was found at the suture site in group II, that agreed with other study when FG did not affect the change of nerve fascicles and no inflammatory leukocyte infiltrations (Ghayour, Abdolmaleki et al. 2017, Masgutov, Masgutova et al. 2019). Preservation of endoneuraltubes is essential for nerve regeneration because regenerating nerve fibers adhere to endoneural fibroblasts, collagen and other extracellular matrix proteins that may explain privilege of FG over suturing that was represented in lower values of PCNA localization in group III (13.65±3.23) than group II however the values were significantly different when compared with group I (5.83±1.51).

In the present study, instance of anastomotic failure in group II and group III at the end of the experimental period were not found and this is compatible with the results of Attar et al. (Attar, Zalzali et al. 2012) who revealed that suture and fibrin glue were nearly serve to maintain the nerves during regeneration while results reached by Feldman et al. and Wang (Feldman, Sataloff et al. 1987, Wang, Hua et al. 2007) who found that the alignment and axonal regeneration were better with the fibrin adhesive technique beside, less fibrosis compared with epineural suturing.

Although the morphological and histological findings showed that neural degeneration was evident in group I, both treated groups were well formed with less sites of vacuolized fibers after two months. Breshah et al. (Breshah, Sadakah et al. 2013) reported that after three months of nerve injury, incomplete axonal regeneration in the repaired groups was found, regarding the studies of Elgazzar et al. (Elgazzar, Abdulmajeed et al. 2007) and Sandrini et al. (Sandrini, Pereira-Júnior et al. 2007) on suture and fibrin glue reanastomosis respectively that showed less nerve regeneration after three months from reanastomosis, suggesting a partial regeneration of the nerve fibers.

Therefore, further studies should be done to approach the complete recovery of injured nerves and axonal regeneration concerning other recent promising techniques.

Conclusion

Although the sciatic nerve tissue was transected, approach of normal neural structure, increase in PCNA and Schwann cell proliferation may depend upon the tissue regeneration stimulated by means of application of fibrin glue to the area of nerve injury allowed a significant improvement through stimulation of angiogenesis and neuroprotection.

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