Original Article

Effect of Amniotic Membrane Nerve Wrapping in Final Results of Traumatic Peripheral Nerve Repair

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ABSTRACT

BACKGROUND

Preventing perineural adhesions and scars formation in the traumatic peripheral injuries is very important on the recovery process. We aimed to evaluate the effect of using the amniotic membrane wrapping on the results of surgical treatment of damaged peripheral nerves.

METHODS

This cohort study included 30 patients with symptoms of acute peripheral nerve injuries due to penetrating trauma in the forearm or wrist in January 2019 to November 2020 referred to the Hand and Microsurgery Department, 15 Khordad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. In 15 patients, after nerve repair, amniotic membrane coverage was used around the nerve, all patients were followed for 12 months. Ultrasound study for neuroma formation and nerve regeneration was determined based on EMG and NCV findings. The modified Medical Research Councile classification (MRCC) was used to evaluate of motor and sensory recovery.

RESULTS

In the amniotic membrane wrapping group, all patients had nerve regeneration and functional nerve recovery occurred after 12 months. In the control group, 5 patients (33.4%) did not have nerve recovery and had functional and sensory impairment. In terms of functional capabilities; there was a significant difference in pinch strength, grip power and MRCC scoring between the two groups. Moreover, the mean volume of neuroma in these patients who used amniotic membrane covering was 2.7 mm^3 and in the control group, it was 3.9 mm^3 (P=0.001). Five patients who did not have a damaged nerve, the neuroma volume was 4.8 ± 0.9 mm^3.

CONCLUSION

The use of amniotic membrane covering is effective methods in the improve results of peripheral nerve repair and nerve function recovery.

KEYWORDS

Amniotic membrane; Peripheral nerve; Nerve regeneration; Functional recovery

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INTRODUCTION

Traumatic peripheral nerve lesions due to penetration, laceration or crush injuries are a most common peripheral nerves injury damage that causes complete or incomplete limb dysfunction and long-term morbidity[1]. Frequency of laceration or stab wound lesions with peripheral nerve injuries includes 2.8% all of traumatic patients[1,2].

Following nerve damage, fibrosis and scar formation at the site of injury by disrupting the cell growth process causes deformity, sensory and motor dysfunction. Scar formation at the site of peripheral nerve injury acts as a mechanical barrier and prevents sprouting axons and axonal regeneration[1, 3]. Moreover, fibrosis and scarring in and around nerve

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damage have a negative impact on the recovery process. The ultimate goal in repairing traumatic peripheral nerve damage is to maximize the healing process and prevent neuroma[1- 3]. Post-traumatic neuroma is an irregular tissue of stem cells that do not differentiate in the process of nerve repair and produces an inefficient tissue considered as a pseudotumor that can occur from one to twelve months after the injury. Imbalance in the process of cell differentiation in the microscopic environment and adhesion to surrounding tissue can affect the quality of nerve repair. The amniotic membrane is a vascular membrane of epithelial cells that contains mesodermal cells on the inside that can prevent the formation of inflammatory cytokines[3,4]. In vitro, in addition to preventing adhesion to surrounding tissue, it is involved in the differentiation of nerve cells. The site of injury to the surrounding tissue reduces fibrosis tissue and inflammatory cells[4,5]. It also significantly prevents the formation of scar tissue. This membrane can stimulate fibroblasts to produce collagen and extracellular matrix components[3- 6].
There are very few studies in this field so that the aim of this study was to investigate the effect of using the amniotic membrane wrapping on the results of surgical treatment of damaged peripheral nerves.

METHODS

This cohort study, enrolled 30 traumatic patients with peripheral nerve damage in the upper limb in the wrist or distal forearm (median or ulnar nerves) referred to the Hand and Microsurgery Department, 15 Khordad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran from Jan 2019 to Nov 2020. To estimate the sample size based on a similar study [5] and according to the study of power of 80%, z=1.96, 25 patients and 10% probability of loss of follow up, 30 patients were identified who were divided into two groups. The two groups including 15 patients in each groups were matched in terms of age and sex.
Inclusion criteria included people over 18 yr and under 65 yr, no congenital neurological disorder, no diabetes and other systemic diseases that may increase neuropathy, no previous history of wrist nerve surgery and wrist deformity, absence of cervical discopathy and no history of allergies. Patients who did not seek follow-up during the one year were excluded from the study. In both groups, patients underwent standard nerve repair after complete resection of the neuroma under a microscope guide as end-to-end epineural neurorrhaphy was performed with 8-0 monofilament nylon sutures. All patients were operated on by a hand surgeon who had undertaken a specialist hand surgery fellowship.
In one group, after repair, it was sterilized using an acellular amniotic membrane prepared from Parsa Teb Iran with a thickness of 0.2 mm to 0.5 mm and was wrapped in a size of 5 × 5 (Figures 1 and 2). All amniotic membranes were screened for serological tests including VDRL (RPR), HTLV 1,2Ab, HCV Ab, HBS Ag, HBS Ab, HIV Ab, and microbiological tests (aerobic and anaerobic). After repairing the posterior plaster, the splint is placed in the flexion position of the wrist in the position of 20 to 45 degrees. The rehabilitation program was the same for all patients. In follow-up, patients after 12 months were compared in terms of final treatment results. Restored nerve recovery in both groups was determined based on clinical findings and the use of EMG and NCV nerve regeneration. Moreover, the volume of neuroma in the restored site was determined using ultrasound after 12 months of repair by the Supersonic Ultimate device with a linear converter of 5-18 SL with connection to the ultrasound system. Examined parameters were the form, echo pattern, diameter, and volume of the neuroma, overall integrity of the nerve and nerve bundles, perineurium, epineurium, and peripheral tissues of the median or ulnar nerves.



Fig. 1: Pre-prepared sample of sterile amniotic membrane



Fig. 2: Clinical photo of nerve wrapping with amniotic membrane after end-to-end epineuria neurorrhaphy

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The rate of sensory recovery was determined using the Semmes Weinstein monofilament test in patients at the last follow-up. All patients were assessed post-operatively by either an experienced hand surgeon. The modified Medical Research Council Classification (MRCC) was used to monitor motor and sensory changes during the follow up period that included two-point discrimination testing (2-PD) and motor recovery[6]. Meaningful Recovery defined as having a score of S3-S4 or M3-M5 according to the modified MRCC outcome[6]. Grip power and pinch strength of the hands were measured (Sammons Preston, Warrenville, Illinois, United States) and compared in every patient. Functional outcome was determined by Quick Disabilities of the Arm, Shoulder, and Hand (Quick DASH) at the end of the follow-up period.

All ultrasounds were performed by an experienced musculoskeletal sonographer and this person was uninformed from the results of patients groups, clinical examinations and electrodiagnostic tests. All patients were followed for at least 12 months.

This study was performed under the supervision of the Ethics Committee of Shahid Beheshti University of Medical Sciences with the code IR.SBMU.RETECH.REC.1400.097 after obtaining written consent from patients.

Normality of the distribution of each variable was checked using the Kolmogorov–Smirnov's test. Mann-Whitney U was used to evaluate and compare quantitative variables and Chi-square or Fisher`s exact test was used in the case of qualitative variables. A *P*-value less than 0.05 is considered significant. Data analysis was performed using the SPSS software (ver. 16.0, Inc., Chicago, Ill, USA).

RESULTS

Based on demographic findings, there was no significant difference between the two groups (Table 1). Based on clinical findings and EMG and NCV in the amniotic membrane wrapping group, all patients had nerve regeneration and recovery. In the control group, 5 patients (33.4%) did not have nerve recovery and had functional and sensory impairment in terms of functional capabilities; there was a statistically significant difference between punching and motor strength between the two groups. Patients treated with amniotic membrane wrapping were in a better condition.

In addition, the average volume of neuroma based on ultrasound findings in these patients was lower (Table 2). Based on MRCC scoring in the amniotic membrane wrapping group patients had better scoring compared to the control group. Most patients had scores of S3 or S4 in the amniotic membrane wrapping group. In 5 patients who did not have damaged nerve recovery after 12 months, the neuroma volume was 4.8 ± 0.9 mm^3. There were no complications in patients who used amniotic membrane covering.

DISCUSSION

The peripheral nervous system has the potential for repair and regeneration after injury. However, in most cases, traumatic injuries due to scar tissue and fibrosis impair nerve function recovery and regeneration does not occur[7]. One of the major challenges in the peripheral nerve repair process is the prevention of adhesion and fibrosis[7,8] . Functional nerve recovery depends on several factors such as the type, the severity of nerve damage, and its repair type affect the final results of nerve recovery. Tissue fibrosis and adhesion caused by scarring at the site of injury is one of the disruptive factors in the process of functional nerve recovery that cannot be prevented by surgical methods. The human amniotic membrane is the innermost layer adjacent to the amniotic fluid and the fetus. The stem cells on the amniotic membrane are mesenchymal cells[8,9]. These cells can differentiate into transplanted tissue cells. Amniotic membrane mesenchymal cells can differentiate into keratinocytes (skin epidermis), angiogenesis (vascular), myogenic (muscle building), and nerve cells[8,9]. It also has a variety of biological properties so that it has low preparation and maintenance costs. This curtain has anti-adhesive, antibacterial, low immunogenicity, anti-inflammatory, and anti-scarring properties. It also helps speed up the tissue repair process by producing growth factors[10, 11].

Data from studies in animal models have shown a significant effect of the amniotic membrane in the peripheral nerve recovery process. As Gärtner et al showed improvement in neuronal function after 12 months of repair using the amniotic membrane in peripheral nerves in the animal model[12]. Better peripheral nerve regeneration has been reported in cases where the amniotic membrane has been used[13]. In a similar study[8], on 42 rats in six groups, the use of amniotic membrane and betamethasone alone and simultaneously in the process of repairing damaged peripheral sciatic nerve in rats was investigated. After 8 wk of the injury and initial treatment, electrophysiological examination of rat sciatic nerve showed a positive effect of using amniotic membrane covering on the generation of cut and trimmed sciatic nerve. In our study, in cases where the amniotic membrane was used after peripheral nerve repair, neurodevelopmental recovery occurred in all patients after 12 months, while in patients in the opposite group, 33% (5 patients) had disorders in function and neuroma[8]. Perineural scarring of the ulnar nerve was one of the causes of recurrence and recurrence of symptoms and complications of primary cubital tunnel syndrome. Eight patients with symptoms of ulnar nerve involvement in the cubital tunnel of the elbow underwent release of the ulnar nerve in the elbow and then the amniotic membrane covering the elbow was used as a sheath. Preoperative amount of elbow movements, punching strength, pinch strength,

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pain intensity, and functional ability were recorded using the DASH score (Disabilities of the Arm, Shoulder, and Hand functional outcome score) and re-measured at 30-month follow-up of patients[7]. The findings of this study showed a statistically significant difference in the variables measured before and after treatment with the above method in 8 patients. Moreover, the use of amniotic membrane allograft and covering the ulnar nerve with it after nerve neurolysis has been effective in reducing recurrences of cubital tunnel syndrome[9]. In our study, functional ability, pinch strength, and grip power in patients who used amniotic membrane covering in the nerve repair process had a statistically significant difference with patients in the control group and had better functional ability and more strength in the injured limb, indicating faster recovery of these patients.

In the study on 72 rats divided into three groups, the use of an amniotic membrane in the process of sciatic nerve repair was investigated. After 4 to 12 wk of increasing initial repair, it was re-explored and the results showed the least amount of adhesion and formation of perineural scar tissue in the repaired sciatic nerve and covered with an amniotic membrane[7]. There was a significant difference in nerve adherence in the control group (2.4 ± 0.66 mm) and the group treated with an amniotic membrane (1.75 ± 0.45)[7]. Considering that in our study the peripheral nerve repair in similar patients was done by a treatment team, what is effective is perineural adhesion and scar, which has been effective in the recovery process of patients.

In the study in patients who underwent initial peripheral nerve repair, the volume of neuroma formed in patients who did not have complete neurological recovery was significantly higher than in other patients[2]. Thus, a 5-fold increase in the volume of the repair site relative to the diameter of the nerve was associated with a poor prognosis of nerve recovery [2]. In our patients, the volume of neuroma at the site of repair was 2.7 mm^3 in cases where the amniotic membrane was used and 3.9 mm^3 in cases where the amniotic membrane was not used. Besides, in 5 patients who did not have nerve recovery, the volume of neuroma at the repair site was 4.8 mm^3, which is consistent with the findings of previous studies. The use of amniotic membrane coverage has been effective in preventing the growth of neuroma at the site of nerve repair. A post-traumatic neuroma is an irregular tissue of stem cells that do not differentiate in the process of trying to repair a nerve and creates an inefficient tissue that develops as a pseudotumor

Table 1: Comparison demographic findings between two groups

Variables	Amniotic membrane wrapping N=15	Control group N=15	*P*-value
Age(year)	35.9±9.5	34.9±9.2	0.8
Sex			
Male	11(73.3%)	9(60%)	0.6
Female	4(26.7%)	6(40%)	
Smoking positive(%)			
Positive	3(20%)	2(13.3%)	0.4
Negative	12(80%)	13(86.7%)	
Hand dominance			
Right	8(53.4%)	10(66.6%)	0.2
Left	7(46.6%)	5(33.4%)	
Nerve			
Median	6(40%)	5(33.4%)	0.3
Ulnar	9(60%)	10(66.6%)	

Table 2: Final results of functional and sensorial recovery between two groups

Variables	Amniotic membrane wrapping N=15	Control group N=15	*P*-value
Quick DASH score	7.1±0.9	8.9±2.02	0.03*
MRCC Score	3.5±0.4	2.4±1.1	0.03*
Pinch strength (kg)	5.9±0.7	4.01±1.9	0.04*
Grip power(kg)	23.2±2.08	17.6±6.3	0.003*
Neuroma volume mm^3	2.7±0.3	3.9±1	0.001*

*Significant difference

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that can develop one to twelve months after injury[4,5]. The neuroma occurs as a result of damage to the perineurium of the nerve. The axon grows and leans into the surrounding tissue without covering or with covering damage, and the nerve fibers are placed in a bundle of connective tissue [5-8]. This irregular network of axons is intertwined and does not function, and eventually, the nerve will not function. Autologous sutures and nerve grafts are used for the treatment of primary acute injuries. However, imbalances in the process of cell differentiation in the microscopic environment and adhesion to surrounding tissue can affect the quality of nerve repair. The amniotic membrane is a vascular membrane of epithelial cells that contains mesodermal cells on the inside that can prevent the formation of inflammatory cytokines. In vitro, in addition to preventing adhesion to surrounding tissue, it is involved in the differentiation of nerve cells. Therefore, the use of amniotic membrane coverage is effective in the process of nerve repair[4,5]. The repair method and the surgical team in our study were similar, the recovery of the repaired nerve can be reliably attributed to the use of amniotic fluid.

Limitation of the study

In our study, a comparison was based on functional ability and neurological recovery using clinical examinations and the findings of Electromyography (EMG) and Nerve Conduction Velocity (NCV). However, examination of the number of adhesions and scars requires histopathological examinations, and since it was not possible to explore and re-operate on patients in both groups, it was one of the limitations of our study. The two groups were completely identical, but due to the severity of the injuries, there was no complete matching in this case.

CONCLUSION

The use of amniotic membrane coverage is effective in the process of peripheral nerve repair and recovery of nerve function. Covering the amniotic membrane is a simple way to prevent neuroma at the site of injury and seems to prevent adhesions and perineural scarring.

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CONFLICT OF INTEREST

There is no conflict of interest to be reported.

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