ORIGINAL RESEARCH

Evaluating microvascular architecture loss and intraneurial fibrosis: Crucial discovery in ineffective human nerve grafts

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ABSTRACT
Background: Processed nerve allografts are commonly utilized for clinical nerve repair. Nonetheless, chronic discomfort and ongoing loss of function have been linked to allograft failure, which has a high documented occurrence rate. **Aim**: One year after the first operation, the current study sought to evaluate the unsuccessful allograft repair in a sensory human nerve by immunohistochemical and histological investigation. **Methods**: Processed nerve allografts were used for repair in four participants who had suffered superficial radial nerve injuries. Clinical findings at the follow-up visit revealed significant neuropathic pain and no reinnervations of sensory nerves. The removal of the unsuccessful transplant and histologic and immunohistochemical analyses came next. Collagen content, lymphatic and blood vasculature, and the neurofilament network were measured in the middle of the specimens. **Results**: Histologic examination revealed increased fibrosis, fatty degeneration, and disordered growth of nerve fibers. Additionally, a recognizable pattern was observed in the microvascular network of the allografts, with an increase in microvessels and no alteration in the lymphatic vasculature. **Conclusion**: Within the constraints of the research, the current study finds that loss of microvascular and physiologic architecture is linked to human allograft failure. More clinical research is necessary to evaluate the interaction between angiogenesis, lymphangiogenesis, and axonal regeneration, nevertheless, in order to better understand the mechanism underlying the failure of human nerve allografts.

Keywords: Nerve Allograft, Lymphatic Drainage, Peripheral Nerve, Nerve Surgery

INTRODUCTION
Damage to peripheral nerves is a frequent and very prevalent condition that affects a sizable portion of the worldwide human population, including those in India. Severe nerve injuries, however, come with a heavy cost, both socially and financially, as the long-term effects cause the affected individuals to stop working and take time off.[1]

Despite several advances in microsurgery methods, neuropathic pain remains a significant barrier in reconstructive nerve surgical, along with the loss of motor and sensory skills. Nerve continuity can be restored using synthetic/autologous conduits or allogenic/autologous nerve grafts in situations when tension-free neurorrhaphy is not practical. Even though autografts are recognized they found as the gold standard for therapy. The paradigm in peripheral nerve repair changed as a result of a rise in the usage of processed nerve allografts.[2]

However, harvesting the autograft resulted in various drawbacks including neuroma formation, scarring, loss of sensation, and donor site morbidity. Conversely, using allografts has none of these issues or disadvantages. Furthermore, processed nerve allografts do not require further immunosuppression since they do not include antigenicity from the original cell components. Neural microarchitecture is preserved in the allografts, providing guidance and a scaffold for the regeneration of axons between damaged nerve terminals.[3]

Despite the encouraging outcomes and significant recovery shown in earlier research utilizing these allografts, graft failure due to neuroma development and graft resorption in continuity can cause chronic

discomfort, hypesthesia, and persistent paresthesia.[4] The exact biological mechanisms behind transplant failure are unknown, although inadequate blood flow to the allograft—which can result in scarring and graft necrosis—is thought to be a critical issue. According to evidence from the literature now under publication, intraneural angiogenesis promotes Schwann cells in terms of cellular alignment and produces neurotrophic factors, both of which are essential for axonal regeneration.[5]

According to findings from the current literature, peripheral nerve regeneration should be associated with both angiogenesis and lymphangiogenesis, which denotes the restoration of neural lymphatic drainage. By providing more information about the mechanism that may restrict the processed nerve allografts' ability to successfully regenerate their nerves, the results of surgical care can be enhanced.[6,7]

The current clinical investigation was carried out in light of these published findings in order to evaluate the failure of allograft repair using immunohistochemical and histological examination in a sensory human nerve one year after the initial operation.

MATERIAL AND METHODS

The present clinical study was out a year after the first operation to evaluate the failure of the allograft repair using immunohistochemical and histological examination in a sensory human nerve. After receiving approval from the relevant ethical committee, the current study was conducted at Department of General Surgery, K. J. Somaiya Medical College & Research Centre, Sion Mumbai, Maharashtra from march 2016 to march 2017. Prior to beginning the study, informed permission was obtained from each study participant, both verbally and in writing. Four patients who had surgical treatment using processed nerve allografts were evaluated for the research. The radial artery, the extensor pollicis brevis and abductor pollicis longus tendons, and the superficial radial nerve were all impacted related to the trauma in both cases. The brachioradialis tendon, the extensor carpi radialis brevis tendon, the pronator teres tendon, and the superficial radial nerve were the afflicted and severed anatomical components for the other two research participants.

Following the restoration of the research subjects' tendons and arteries, a processed nerve allograft was used to restore the superficial radial nerve. For nerve reconstruction, two participants received an allograft with a dimension of 2-3/30 mm, whereas the other two subjects received an allograft with a diameter of 3-4/50 mm.

For surgical rehabilitation and methodology, nerve repair was performed in two cases on the first day after trauma and in the other two cases on the second day. In order to accomplish the procedure, surgical microscopes were used to examine severed stumps of the superficial radial nerve and evaluate the intraneural morphology. After the excision, an allograft was used to restore the nerve without strain, and fibrin glue and sutures were used to approximate the area.

Following the five days of operation, wrist immobilization was indicated, and mobilization was carried out in accordance with hand treatment guidelines. Multidisciplinary care including the pain experts was initiated during the postoperative phase. All research participants received systemic or local anti-neuropathic pain drugs for three to six months following surgery, depending on the need. Following a 12-week period, each subject was permitted to load to capacity. After a year of follow-up, the subjects were brought back, and the decision to proceed with a surgical reconstruction was based on the results of the radiographic evaluation and clinical examination, which indicated a growing neuropathic pain and advancing Tinel's sign. Perineural lipofilling was performed in all individuals to boost the revascularization of the nerve reconstructions and to obtain mechanical protection after a sample of SRN was collected from a healthy proximal nerve stump.

The healthy proximal superficial radial nerves were sectioned into slices about 2µm thick, and the allografts were fixed in formalin. To be examined under a microscope, paraffin-embedded slides were stained with eosin and hematoxylin stains. To improve the collagen's visibility, an extra Elastin van Gieson stain is created. Monoclonal antibodies were utilized for immunochemical staining utilizing an automated immunostainer. Normal nerve fibers, blood arteries, and lymphatic vessels were considered the positive internal controls. Therefore, there was no need for external positive control.

RESULTS

After the first nerve repair, the research participants had significant and early allodynia with no sensory recovery in the superficial radial nerve area. Excision of the allografts was performed after 12-month follow-up period. The allografts were distinguished morphologically by thickening at the coaptation site and central atrophy. Two participants experienced significant neuropathic pain at the 12-month follow-up after the resurgery, suggesting a risk of morbidity and incapacity, whereas the other two went back to work.

In terms of neurofilament and collagen distribution, histologic evaluation of removed allografts revealed higher levels of pathological neurofilaments and intraneural fibrosis compared to normal samples taken from the superficial radial nerves. Along with the disorderly development of the neurofilaments, deteriorated allografts in two participants showed signs of fibrosis and lipomatosis. The predominant finding in the other two patients was intraneural fibrosis. When comparing immunohistochemistry results for neurofilaments to normal superficial radial

nerve tissues, the results showed fewer and poorly ordered neurofilaments.

The superficial radial nerve's blood and lymphatic vasculature had distinctive hierarchical microvessels, primarily in the epineurium and perineurium. The removed allograft had a greater quantity of randomly distributed micro-vessels. D2-40 was applied to the sections in order to evaluate the intraneural lymphatic network. Lymphatic channels were seen in the perineural and epineural soft tissues of normal superficial radial nerves. Furthermore, little increase in lymphangiogenesis was seen in unsuccessful allografts, and there were few lymphatic channels in the perineural tissue.

DISCUSSION

Reconstruction of the nerve injuries from the upper and lower extremities has shown predictable results clinically. But failure of these grafts can result in persistent discomfort and irreversible loss of function, as recently documented by Lin MY et al[8] and Griffin JW et al.[9] The explanted human allografts' histologic evaluation revealed central necrosis and inadequate axonal regrowth, which is in line with the results of the current investigation and the earlier work by Griffin JW et al.[9] Though the topic is elusive, it is important to keep in mind that the majority of the data on nerve allograft failure comes from case reports and rodent experiments, and there is no standard protocol for performing the histologic examination of failed nerve allografts.

In order to recreate a sensory human nerve, four examples of unsuccessful nerve allografts were evaluated in the current clinical investigation. In addition, the study evaluated the collagen composition, neurofilament network, and lymphatic and blood vasculature in the center of the examined specimens.

According to a 2015 study by Cattin et al[10] the functional integration of the nerve grafts is a complicated process including revascularization and axonal ingrowth. The endothelial cells and axon ingrowth may be halted at the site of coaptation by severe scarring or intraneural fibrosis. Staining revealed core intraneural fibrosis in the current investigation, along with a disordered neurofilament architecture that was similar to a neuroma. These results were in agreement with the case report of Berrocal et al[11] in 2013 where authors reported nearly 6% regeneration at the center and axonal degeneration from 16000 to 1000 fibers in a failed nerve allograft. This was comparable to a research by Nietsvaara et al.8 that showed how decellularized allograft and host rejection were likely contributing reasons to graft failure and how allograft failure led to graft resorption.

Recent research on revascularization of allografts was conducted by Slutsky DJ et al[12] and they found insufficient axonal regrowth along with central graft resorption or necrosis. The revascularization of nerve allografts and autografts is caused by longitudinal inosculation, as shown by Cheng CJ et al[13] illustration, which shows the ingrowing vessels to the remaining microvascular channels in the nerve end.

Because the surviving vascular network in decellularized allografts lacks endothelial and mural cells, the technique is less practical. In addition, Cheng CJ et al[13] found that allografts showed slower vascularization than autografts. The core of the failed allograft in the current investigation revealed a thick, disordered microvascular network that was not longitudinal. Even though the unsuccessful allografts were removed after a year, the current study's findings may aid in understanding the process of central graft necrosis.

The process of nerve regeneration was described by P. Dubový et al. (2011)[14] as a complicated biologic entity involving a variety of cell lines, where VEGF produced by macrophages causes the polarized microvasculature, which aids in the development and migration of Schwann cells' axons. Similar to this, the microvascular system plays a major role in stimulating neural regeneration by aiding in cell metabolism, providing trophic and nutritional factors, and aiding in different stages of the healing process by drawing in stem cells from the bloodstream, as demonstrated by Weber RA et al.[15] in 2019. In addition to the well-established role of angiogenesis, Meek MF et al.[16] in 2020 indicated that lymphangiogenesis and the lymphatic system can play a critical role in peripheral nerve damage and nerve regeneration. Weber RA et al[15] confirmed the lymphatic system's function in peripheral nerve healing comes lately.

The lymphatic vasculature of normal superficial radial nerve and failed nerve allografts was assessed using a D2-40 stain. It was observed that in failed allografts and normal nerve, there was no alteration observed in comparison to blood vasculature. Since the Resurgery was carried out after a year, it is challenging to evaluate the validity of these results, which calls for more clinical longitudinal research to determine the lymphatic system's contribution to peripheral nerve regeneration. As proven by Meek MF et al.[16] tissue edema may be considerably reduced with the activation of lymphangiogenesis in nerve allografts by the clearance of myelin debris, inflammatory cells, and interstitial fluid. This can lead to a better functional recovery and encourage the synthesis of new myelin.

A couple of the study's weaknesses were that it was descriptive in nature and that it used short, thin allografts, which might have led to different outcomes when using long, thick allografts. Additionally, the small sample size and brief follow-up may provide inconsistent findings.

CONCLUSION

Taking into account its limitations, the current study comes to the conclusion that unsuccessful nerve

allografts are linked to aberrant neurofilament organization, fatty degeneration, and increased intraneural fibrosis. In addition, the microvascular network of the allografts is thick and disordered, with relatively few perineural and epineural lymphatic vessels. In order to reduce the likelihood of allograft failure in the future, a deeper knowledge of the interaction between axonal regeneration, lymphangiogenesis, and angiogenesis is needed via more clinical research.

REFERENCES

1. Cho MS, Rinker BD, Weber RV, Chao JD, Ingari JV, Brooks D, Buncke GM. Functional outcome following nerve repair in the upper extremity using processed nerve allograft. J Hand Surg Am. 2012, 37(11):2340-9.
2. Brooks DN, Weber RV, Chao JD, Rinker BD, Zoldos J, Robichaux MR, et al. Processed nerve allografts for peripheral nerve reconstruction: a multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions. *Microsurgery.* 2012;32:1–14.
3. M.S. Cho, B.D. Rinker, R.V. Weber, J.D. Chao, J.V. In gari, D. Brooks, G.M. Buncke. Functional outcome following nerve repair in the upper extremity using processed nerve allograft. J Hand Surg. Am. 2012;37:2340-9.
4. D. Grinsell, C.P. Keating Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. Biomed Res. Int. 2014.
5. Karabekmez F.E, A. Duymaz, S.L. Moran. Early clinical outcomes with the use of decellularized nerve allograft for the repair of sensory defects within the hand. Hand (N. Y.). 2009;4:245-9.
6. Rinker BD, Ingari JV, Greenberg JA, Thayer WP, Safa B, Buncke GM. Outcomes of short-gap sensory nerve injuries reconstructed with processed nerve allografts from a multicenter registry study. J Reconstr Microsurg. 2015 Jun;31(5):384-90.
7. Safa B, Buncke G. Autograft Substitutes: Conduits and Processed Nerve Allografts. Hand Clin. 2016 May;32(2):127-40.
8. Lin MY, Manzano G, Gupta R. Nerve allografts and conduits in peripheral nerve repair. Hand Clin. 2013 Aug;29(3):331-48.
9. Griffin JW, Hogan MV, Chhabra AB, Deal DN. Peripheral nerve repair and reconstruction. J Bone Joint Surg Am. 2013 Dec 4;95(23):2144-51.
10. A.L. Cattin, J.J. Burden, L. Van Emmenis, F.E. Mackenzie, J.J. Hoving, N. et al. Macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. 2015;162:1127-39.
11. Y.A. Berrocal, V.W. Almeida, A.D. Levi. Limitations of nerve repair of segmental defects using acellular conduits. J. Neurosurg. 2013;119:733-8.
12. Slutsky DJ. The management of digital nerve injuries. J Hand Surg Am. 2014;39:6:1208-15.
13. Cheng CJ. Synthetic nerve conduits for digital nerve reconstruction. J Hand Surg Am. 2009;34:9:1718-21.
14. P. Dubový. Wallerian degeneration and peripheral nerve conditions for both axonal regeneration and neuropathic pain induction Ann. Anat. 2011;193:267-75.
15. Weber RA, Breidenbach WC, Brown RE, Jabaley ME, Mass DP. A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. Plast Reconstr Surg. 2000;106:5:1036-45.
16. Meek MF. A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. Plast Reconstr Surg. 2001;108:4:1087-8.