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EVALUATING INTRANEURIAL FIBROSIS AND MICROVASCULAR ARCHITECTURE LOSS: AN ESSENTIAL FINDING IN INEFECTIVE HUMAN NERVE GRAFTS

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ABSTRACT

Background: Clinical nerve repair frequently makes use of processed nerve allografts. Allograft failure, however, has a high recorded incidence rate and has been associated with persistent pain and loss of function.

Aim: Using immune-histochemistry and histological analysis, the current study aimed to assess the failed allograft healing in a sensory human nerve one year following the first procedure.

Methods: Four patients who had superficial radial nerve damage were repaired using processed nerve allografts. At the follow-up visit, the clinical data showed no reinnervations of sensory nerves and considerable neuropathic pain. The failed transplant was removed, and then histologic and immune-histochemical tests were performed. The midpoint of the specimens was examined for collagen content, lymphatic and blood vasculature, and the neurofilament network.

Results: Increased fibrosis, fatty degeneration, and abnormal nerve fibre development were all seen upon histologic investigation. The microvascular network of the allografts also showed a discernible pattern, with an increase in microvessels and no change in the lymphatic vasculature.

In summary, the current study concludes that loss of microvascular and physiologic architecture is associated with human allograft failure, within the limitations of the investigation. To further understand the mechanism behind the failure of human nerve allografts, more clinical research is needed to assess the interplay between angiogenesis, lymphangiogenesis, and axonal regeneration.

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

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INTRODUCTION

Peripheral nerve damage is a common and widespread disorder that affects a significant section of the global human population, including people living in India. However, severe nerve injuries have a high social and financial cost since the long-term repercussions force the affected people to take time off from work.[1]

Neuropathic pain and loss of motor and sensory abilities continue to be major obstacles in reconstructive nerve surgery, despite considerable advancements in microsurgery techniques. When tension-free neurorrhaphy is impractical, nerve continuity can be restored using allogenic/autologous nerve grafts or synthetic/autologous conduits. Autografts are acknowledged as the gold standard in treatment, despite this.

The paradigm in peripheral nerve repair changed as a result of a rise in the usage of processed nerve allografts.[2]

Nevertheless, there were a number of negative effects from harvesting the autograft, such as neuroma development, scarring, loss of feeling, and morbidity at the donor site. On the other hand, none of these problems or drawbacks exists when employing allografts. Additionally, since processed nerve allografts lack the antigenicity of the original cell components, they do not require additional immunosuppression. The allografts' maintained neural microarchitecture serves as a scaffold and a guide for the regeneration of axons connecting injured nerve terminals.[3]

Even while earlier research employing these allografts showed encouraging outcomes and significant healing, graft failure brought on by neuroma development and graft resorption in continuity can result in chronic discomfort, hypesthesia, and persistent paresthesia.[4] Although the precise biological factors underlying transplant failure remain unclear, insufficient blood supply to the allograft is believed to be a major problem since it can lead to graft necrosis and scarring. Evidence from the literature that is currently being published suggests that intraneural angiogenesis creates neurotrophic factors and aligns Schwann cells cellularly, two processes that are critical for axonal regeneration.[5]

Peripheral nerve regeneration should be linked to both angiogenesis and lymphangiogenesis, which indicates the restoration of neural lymphatic drainage, based on results from the present research. 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, Shree Krishna Medical College and Hospital,

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Muzaffarpur, Bihar in the year 2022-23, after obtaining clearance from the concerned Ethical committee. Prior to beginning the study, informed consent was obtained from each study participant, both verbally and in writing.

The study analysed four patients who had surgical therapy using processed nerve allografts. The trauma in both cases affected the superficial radial nerve, the radial artery, and the tendons of the extensor pollicis brevis and abductor pollicis longus. For the other two research participants, the affected and severed anatomical components were the brachioradialis tendon, the extensor carpi radialis brevis tendon, the pronator teres tendon, and the superficial radial nerve.

The superficial radial nerve was restored using a processed nerve allograft after the tendons and arteries of the study participants were restored. Two individuals got an allograft measuring 2-3/30 mm for nerve repair, whereas the other two subjects received an allograft measuring 3-4/50 mm in diameter.

Nerve repair was done in two cases on the first day following trauma and in the other two cases on the second day for surgical rehabilitation and technique. Surgical microscopes were utilised to assess the intraneural morphology and analyze severed stumps of the superficial radial nerve in order to complete the treatment. Following the resection, fibrin glue and sutures were utilised to approximate the region and reattach the nerve without putting tension on it.

After the five days after surgery, it was decided to immobilize the wrist and proceed with mobilization in compliance with hand therapy protocols. During the postoperative period, multidisciplinary treatment was started, involving the pain specialists.

Depending on their needs, all research subjects were given anti-neuropathic pain medications either locally or systemically for three to six months after surgery. After twelve weeks, each subject was allowed to fill to capacity. When the participants were brought back after a year of follow-up, the decision to proceed with a surgical reconstruction was made based on the findings of the clinical examination and radiographic evaluation, which showed increasing Tinel's sign and neuropathic pain. After a sample of SRN was taken from a healthy proximal nerve stump, perineural lipofilling was done on each patient to improve the revascularization of the nerve reconstructions and to provide mechanical protection.

Formalin was used to fix the allografts after the healthy proximal superficial radial nerves were cut into slices that were around 2µm thick. Hematoxylin and eosin stains were applied to paraffin-embedded slides so they could be studied under a microscope. An additional Elastin van Gieson stain is made in order to enhance the visibility of the collagen. An automated immunostainer was used to perform immunochemical staining with monoclonal antibodies. Blood arteries, lymphatic vessels, and normal nerve fibres were regarded as the positive internal controls. Thus, external positive control was not required.

RESULTS

The research subjects experienced severe and early allodynia with no sensory recovery in the superficial radial nerve region following the first nerve restoration. The allografts were excised following a 12-month observation period. Thickness at the coaptation location and central atrophy were two morphological characteristics that set the allografts apart. At the 12-month

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follow-up following the resurgery, two of the participants reported considerable neuropathic pain, indicating a potential risk of morbidity and disability, while the other two resumed their jobs.

Histologic analysis of excised allografts showed increased levels of diseased neurofilaments and intraneural fibrosis in comparison to normal samples collected from the superficial radial nerves in terms of neurofilament and collagen distribution. In addition to the disorganised growth of the neurofilaments, two individuals' failing allografts had indications of lipomatosis and fibrosis. Intraneural fibrosis was the main finding in the other two cases. Neurofilaments were seen to be less numerous and ill-organized in the neurohistochemistry results when compared to normal superficial radial nerve tissues.

There were characteristic hierarchical microvessels in the blood and lymphatic vasculature of the superficial radial nerve, mainly in the perineurium and epineurium. There were more randomly scattered micro-vessels in the excised allograft. The sections were treated with D2-40 to assess the intraneural lymphatic network. The soft tissues of the perineural and epineural sections of normal superficial radial nerves showed the presence of lymphatic channels. Moreover, there were few lymphatic channels in the perineural tissue and ineffective allografts showed minimal growth in lymphangiogenesis.

DISCUSSION

Clinical outcomes for nerve injury reconstruction from the upper and lower limbs have been consistent. However, failure of these grafts can cause irreparable loss of function and chronic pain, as recently reported by Safa B, Buncke G et al,[7] Lin MY et al[8] and Gryphon JW et al.[9]

The histologic inspection of the explanted human allografts showed insufficient axonal regrowth and core necrosis, which is consistent with the findings of the current study and the previous research by Gryphon JW et al.[9] Even if the subject is obscure, it's vital to remember that case studies and rodent trials provide the majority of the information on nerve transplant failure, and there is no set procedure for histologically examining failed nerve allografts.

In the current clinical inquiry, four cases of failed nerve allografts were assessed in an attempt to reconstruct a sensory human nerve. The collagen composition, neurofilament network, and lymphatic and blood vasculature in the centre of the tissues under examination were also assessed by the research.

According to a 2015 study by Cattin et al[10] Revascularization and axonal ingrowth are two difficult steps in the nerve grafts' functional integration. Severe scarring or intraneural fibrosis may arrest the proliferation of endothelial cells and axons at the site of coaptation. In the current study, staining identified a disrupted neurofilament architecture resembling a neuroma in addition to core intraneural fibrosis. These findings were consistent with a 2013 case report by Berrocal et al.[11] in which the authors described axonal degradation from 16000 to 1000 fibres in a failed nerve transplant and approximately 6% regrowth at the centre. This was similar to a study by Nietsvaara et al.[8] that demonstrated how graft failure was likely caused by decellularized allograft and host rejection, and how allograft failure resulted in graft resorption.

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Slutsky DJ et al[12] recently studied the revascularization of allografts and discovered central graft resorption or necrosis in addition to inadequate axonal regeneration. According to Cheng CJ et al.'s representation, longitudinal inosculation is the cause of the revascularization of nerve allografts and autografts. It depicts the ingrowing vessels to the residual microvascular channels in the nerve end.

The approach is less useful in decellularized allografts because the remaining vascular network in these samples lacks endothelial and mural cells. Furthermore, allografts exhibited slower vascularization than autografts, according to Cheng CJ et al. (2013). In the current analysis, the centre of the failed allograft showed a dense, non-longitudinal, disordered microvascular network. Although the failed allografts were removed after a year, the results of this investigation might help clarify how central graft necrosis occurs.

P. Dubovþ et al. (2011)[14] characterised nerve regeneration as a complex biologic phenomenon comprising several cell lines, wherein the polarised microvasculature caused by macrophage-produced VEGF facilitates the formation and migration of axons from Schwann cells. Similar to this, Weber RA et al.[15] in 2019 showed that the microvascular system plays a significant role in stimulating neural regeneration by supporting cell metabolism, offering trophic and nutritional factors, and supporting various stages of the healing process by attracting stem cells from the bloodstream.

Meek MF et al.[16] in 2020 suggested that lymphangiogenesis and the lymphatic system can be crucial in both peripheral nerve injury and nerve regeneration, in addition to the well-established function of angiogenesis. The lymphatic system's role in peripheral nerve repair has recently been proven by Weber RA et al.[15]

A D2-40 stain was used to evaluate the lymphatic vasculature of both unsuccessful nerve allografts and normal superficial radial nerves. When compared to blood vasculature, it was found that unsuccessful allografts and normal nerve showed no alterations. It is difficult to assess the validity of these results because the Resurgery was performed a year later. This necessitates more clinical longitudinal study to ascertain the lymphatic system's role in peripheral nerve regeneration.

Tissue edema may be significantly decreased with the induction of lymphangiogenesis in nerve allografts by the removal of inflammatory cells, interstitial fluid, and myelin debris, as demonstrated by Meek MF et al.[16] This may promote the synthesis of new myelin and result in a more complete functional recovery.

The descriptive character of the study and the use of short, thin allografts may have produced different results when employing long, thick allografts—were two of its shortcomings. Furthermore, the limited sample size and short follow-up period can yield contradictory results.

CONCLUSION

Considering its limitations, the current study concludes that greater intraneural fibrosis, fatty degeneration, and abnormal neurofilament organisation are associated with failed nerve allografts. Furthermore, the allografts' microvascular network is disorganised and dense, with comparatively few perineural and epineural lymphatic vessels. Further clinical research is

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necessary to have a greater understanding of the relationship between angiogenesis, lymphangiogenesis, and axonal regeneration in order to lower the risk of allograft failure in the future.

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