Assessment of Functional Outcomes of Distal Nerve Transfers in Brachial Plexus and Peripheral Nerve Injuries Using Histomorphometric Parameters As Prognostic Indicators

Prem S. Bhandari[1] Prabal Deb[2]

[1] Department of Plastic and Reconstructive Surgery, Brijlal Superspeciality Hospital and Research Center, Haldwani, Nainital, Uttarakhand, India
[2] Department of Pathology and Neuropathology, Army Hospital (R&R), Delhi Cantt, India

Address for correspondence Prem S. Bhandari, MCh, Brijlal Superspeciality Hospital and Research Center, Anandi Tower Nainital Road, National Highway 87, Haldwani, Uttarakhand 263139, India (e-mail: doctorbhandari@hotmail.com).

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Abstract

Introduction In brachial plexus and peripheral nerve injuries, distal nerve transfers provide an early return of function with minimal donor nerve-related morbidity. In the present study, various histomorphometric parameters have been evaluated to prognosticate the surgical outcomes of distal nerve transfers.

Materials and Methods Thirty patients with brachial plexus and peripheral nerve injuries were treated by distal nerve transfers. Prior to the nerve transfer approximately 0.5 cm segment from the distal end of donor nerve and proximal end of recipient nerve were sectioned and evaluated for histomorphometry. Relevant histopathological parameters were used to predict the functional outcomes.

Results Donor nerve specimens showing good axonal counts had better functional gains. A lack of fibrosis in the suprascapular and spinal accessory nerves resulted in better shoulder functions with majority of patients having upper brachial plexus injury restoring more than 150 degree of active shoulder abduction and abduction strength of at least M4, which was in contrast to patients displaying fibrosis. Inflammation was present in the donor and recipient nerves in majority of cases, though its correlation with functional gains was not as significant as the number of axons and amount of fibrosis. Presence of demyelination and vasculitis had little bearing in the ultimate functional outcomes.

Conclusions This study revealed that the histomorphometric factors have an important role in predicting the functional outcomes in nerve transfers. An understanding of the histomorphometric status of the donor and recipient nerves can form a basis for early prediction of functional outcomes.

Keywords
- distal nerve transfers
- brachial plexus and peripheral nerve injuries
- histomorphometry
- prognostic indicators

Introduction

Brachial plexus and peripheral nerve injuries are challenging and if left untreated often result in a lifelong disability. The preferred treatment in an injured nerve is a direct coaptation of nerve ends. However, in the late repairs nerve ends are retracted and a nerve graft is required to bridge the gap where the growing axons cross two coaptation sites. It is often noted that during this phase

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several axons are misdirected and lost at each of the two coaptation sites. In proximal level and long-standing injuries, nerve grafts are less successful since the motor end plates tend to degenerate by the time the regenerated axons make their way in to the muscle. Further in root avulsion injuries of the brachial plexus, distal repair is not feasible. In these circumstances, nerve transfer, where a healthy expendable nerve is coapted with the denervated recipient nerve, is used to restore the desired function. In the last few years, nerve transfers are being performed more distally close to the target muscle.[1–3] The advantages are numerous: anastomosis is performed close to the target muscles, which assists an early return of function with less donor nerve morbidity, and the procedure is quick and safe to perform. However, there is little information on certain aspects of these nerve transfers:

- The number of axons required in the donor nerve to activate the denervated muscle.
- Relevance of the presence and extent of fibrous tissue in the donor and recipient nerves in relation to the functions achieved.
- Prognostic significance of inflammation, demyelination, and vasculitis component of the target nerves.

Disappointing outcomes of nerve repairs have put forth a challenge to identify various factors that can predict the functional outcomes. In peripheral nerve injuries the extent of trauma goes well beyond the macroscopically observed injury. Histomorphometric assessment of the affected nerve is expected to have a deeper insight into the extent of trauma and serve as a surrogate marker in predicting the outcome of a particular repair. Available literature reveals that a majority of the histomorphometric studies have been conducted in animal[4] and cadaver[5] models, with very little work on human beings. This study has thus been designed to evaluate the usefulness of various histomorphometric parameters in microenvironment of injured nerves at predicting the functional outcomes of distal nerve transfers in patients with brachial plexus and peripheral nerve injuries.

Materials and Methods

Thirty patients (26 adults and 4 children) with upper brachial plexus and peripheral nerve injuries were treated by distal nerve transfers in a tertiary care hospital. These patients had suffered injuries to the suprascapular nerve (SSN), axillary nerve (AXN), and the musculocutaneous nerve (MCN), either as an isolated lesion or as a component of devastating posttraumatic or birth-related brachial plexus injury. The interval between period of injury and time of definitive surgical repair was considered as denervation period. In the posttraumatic group the denervation period was between 3 and 12 months. Patients with prolonged denervation period (more than 12 months) were excluded from this study, whereas in the obstetric brachial plexus patients, this denervation period was extended up to 21 months.

In the adult posttraumatic group, all patients were males with the average age of 26.3 years (range, 20–34 years). Twenty-one cases occurred following road traffic accidents with involvement of C5 and C6 spinal nerve roots, and thus affecting the functions of SSN, AXN, and MCN. Three cases had suffered isolated injuries to the MCN following penetrating trauma along the course of the nerve. Two patients sustained axillary nerve injury as a consequence of fractures around the surgical neck of humerus. In the obstetric palsy group with C5 and C6 root involvement, the average age of the patients was 18 months (range, 6–21 months).

Surgical Technique

Exploration of the brachial plexus and distal nerve transfers were performed as described in the literature.[1–3,6–8] Prior to nerve anastomosis, specimens were obtained from stumps of spinal accessory nerve (SAN), SSN, ulnar and median nerve fascicles, biceps and brachialis branches, long head triceps branch, and the anterior branch of AXN for histopathological evaluation.

Histopathological Processing

All the nerve biopsy samples were received in 2% paraformaldehyde solution. After an initial fixation in 2% paraformaldehyde in phosphate buffer (pH 7.4) for 3 to 4 hours, the tissue was washed overnight in PBS containing 6.8% sucrose (pH 7.4) at 4°C. It was then dehydrated in 100% acetone for 60 minute at 4°C. Acetone was changed a few times during first 5 minutes till the solution was clear. Infiltration solution was prepared by mixing 100 mL base (Liquid Technovit 8100, M/S Kulzer, Germany) and hardener I, at a ratio of 0.06 g hardener I for every 10 mL base. Tissue was kept in infiltration solution for 7 to 8 hours at 4°C. Embedding solution was prepared using infiltration solution and hardener II at a ratio of 40 µL hardener II for every 1 mL infiltration solution. Tissue was transversely oriented and placed in embedding mold (plastic pellets) with embedding solution overnight at 4°C.

The plastic embedded tissue block thus formed was then removed from the mold. Serial semi-thin sections (2–3 µm) were cut using an ultramicrotome, and sections were collected on separate glass slides coated with poly-L-lysine. These glass slides were serially numbered and stored in dust-free container at room temperature. The semi-thin sections thus obtained were kept overnight in xylol alcohol mixture before bringing down to water for the staining procedures.

Staining Methods

Serial sections of the nerve biopsy thus obtained were stained by routine hematoxylin and eosin (H&E) for evaluating number of fascicles, inflammation (►**Fig. 1**), and vasculitis (►**Fig. 2**). Masson trichrome was used for evaluation of the degree of fibrosis (►**Fig. 3**). Luxol fast blue was used for evaluation of the extent of demyelination (►**Fig. 4**), and immunohistochemistry using neurofilament antibody was used for assessing the axonal status (►**Fig. 5**, **6**).

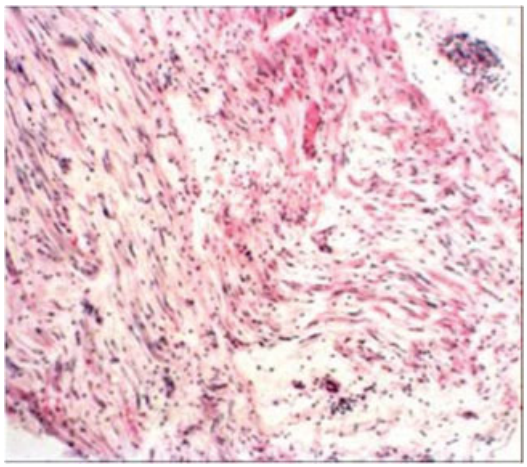

Fig. 1 Nerve biopsy showing inflammation (hematoxylin and eosin stain).

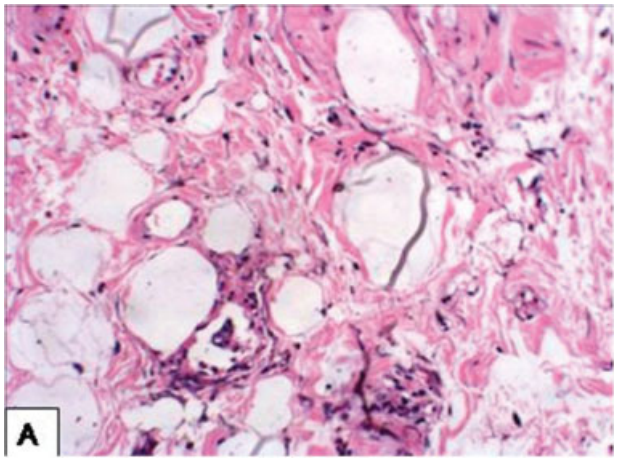

Fig. 2 Nerve biopsy showing evidence of vasculitis.

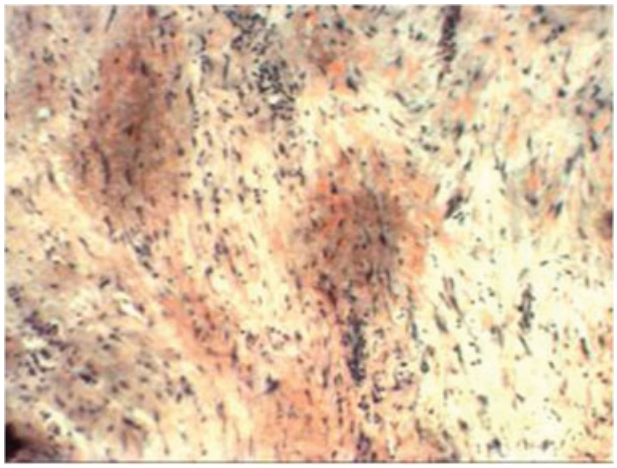

Fig. 3 Nerve biopsy showing fibrosis (Masson's Trichrome Stain).

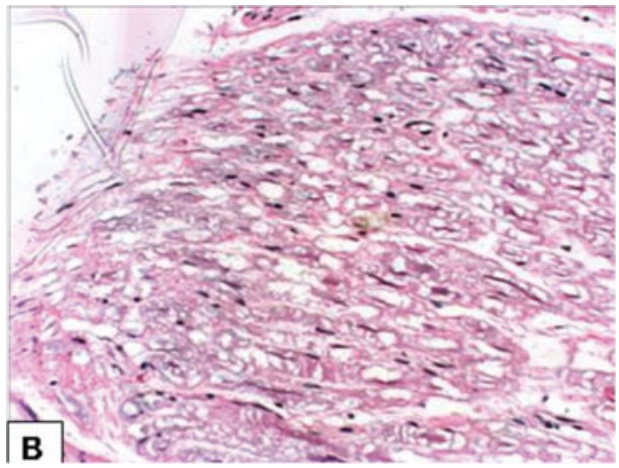

Fig. 4 Nerve biopsy showing demyelination.

Evaluation of Histomorphology

Axonal count by image morphometry: all stained slides were analyzed for axonal count by image morphometry using both manual and digital methods (►**Fig. 1**, **2**). The endoneurial tubes devoid of axons in the damaged nerve were also included in the axonal count.

Manual histomorphometry: for manual counting, a high-resolution microphotograph of the transverse section of the nerve (×400) using a computerized digital photomicrograph system was obtained. The images were then pasted on Microsoft PowerPoint slides. A 24 × 9 chamber grid was superimposed on the images and manual counting performed. The same step was repeated at least three times on each of the three sets of sections and the average count recorded.

Digital histomorphometry: for digital counting, a high-resolution microphotograph of the transverse section of the

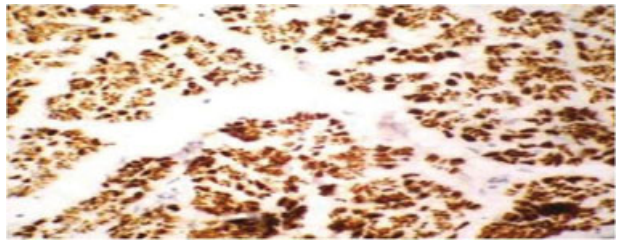

Fig. 5 Patent axons as evidenced by immunohistochemistry.

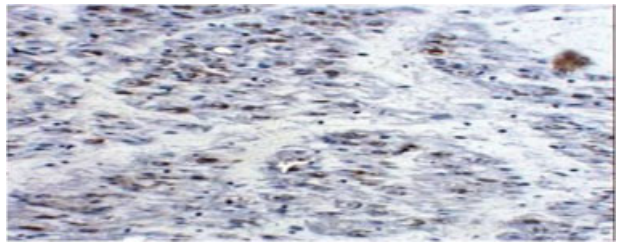

Fig. 6 Axonal loss as evidenced by immunohistochemistry.

nerve (×400) using a computerized digital photomicrograph system was obtained. The images were then evaluated using the Biowizard 4.2 Image analysis software. The same step was repeated at least three times on each of the three sets of sections, and the average count recorded. The status of vasculature of each nerve biopsy was assessed by H&E-stained serial sections and noted for patency of blood vessels and presence of vasculitis.

Follow-Up after Nerve Repairs

Following nerve repairs, the patients were followed-up every month. At every visit the patients were evaluated initially by electromyography for evidence of recovery of the involved muscles and subsequently by clinical examination for evaluating the motor and sensory recovery.

The average follow-up was 19 months (range, 16–28 months). Clinically shoulder functions were assessed by measuring the abduction and external rotation with a goniometer. Abduction was recorded by measuring the angle formed between the arm axis and parallel to the spinal cord axis. External rotation was measured with patient standing and fully internally rotating the shoulder and forearm placed transversally over the abdomen. Any rotation from this position was measured and recorded as the range of external rotation. Similarly, elbow flexion strengths and range of movements were measured.

The motor and sensory recovery commencing from 6 months after surgery was assessed according to Medical Research Council (MRC) scoring system. Motor or sensory function greater than a particular grade but not meeting the criteria of the next higher grade were assigned the lower grade suffixed by "+".

Clinicopathological Correlation

The results of clinical recovery were correlated with the clinical profile of each case in conjunction with results of histopathological evaluation, including image morphometry and immunohistochemistry.

Results

In the adult posttraumatic group, 21 cases occurred following road traffic accidents involving the C5 and C6 nerve roots and their outflows into the suprascapular, axillary, and musculocutaneous nerves. Three cases had suffered isolated injuries to the MCN following penetrating trauma along the course of the nerve. Two patients had sustained AXN injury as a consequence of fractures around the surgical neck of humeus. Four patients with Erb's palsy presented at the age of 9 months (range, 4–21 months).

Clinical Profile of Representative Cases

Patient A (Case no. 22 in ►Table 1)

A 34-year-old man sustained right-sided upper brachial plexus injury in a motor bike accident (►**Fig. 7**). Five months later he underwent multiple nerve transfers; distal SAN into the SSN (►**Fig. 8**), ulnar nerve fascicle to the biceps branch of the MCN (►**Fig. 9**), and transfer of long head triceps branch of radial nerve to the anterior branch of AXN (►**Fig. 10**). At 24 months follow-up he had restored good range of shoulder function (abduction 180 degrees, external rotation 70 degrees) (►**Fig. 11**) and a full range of elbow flexion (120 degrees and M4 strength) (►**Fig. 12**). The histomorphometric findings are summarized in ►**Table 2**.

Patient B (Case no. 14 in ►Table 1)

A 28-year-old man presented with right-sided upper brachial plexus injury (C5, C6 fibrosis) sustained in a road traffic accident. Three months later he underwent distal nerve transfers consisting of distal SAN into the SSN and fascicular transfer of ulnar nerve fascicle to the biceps branch of the MCN. The histomorphometric findings are summarized in ►**Table 3**.

Patient C (Case no. 21 in ►Table 1)

A 21-month-old male child presented with restricted shoulder abduction and external rotation of shoulder following right-sided obstetric brachial plexus palsy. He was treated with distal transfer of SAN into the SSN. The histomorphometric findings are summarized in ►**Table 4**.

Summary of Histomorphological Evaluation

Axonal Counts

Axonal count for both donor and recipient nerves were obtained by image and manual morphometry. Since a large number of sections had to be counted, digital morphometry using computer software was found to be more preferable and less time consuming in comparison to manual morphometry. The average axonal counts in donor and recipient nerve are depicted in ►**Tables 2–4**.

Fibrosis

Histomorphometry of SSN revealed elements of fibrosis in 6 of 25 nerve transfers in contrast to SAN that displayed mild fibrosis in 2 patients. Biceps motor branch revealed fibrosis in a single case of penetrating injury to the MCN. Interestingly fibrosis was absent in obstetric palsy patients.

Inflammation

Inflammation was detected in 8 of the 25 SSNs, 3 SANs, and in 2 patients of biceps motor branch, as against none in the obstetric palsy group.

Demyelination

Demyelination in recipient nerve was present in 22 of the 30 cases. The extent of demyelination was more in patients with adult brachial plexus injuries. Only a single case of Erb's palsy revealed demyelination in the SSN.

Correlation between clinical and histomorphometric parameters: clinical recovery of all the patients in the study group is summarized in ►**Table 1**.

Clinical outcomes and axonal counts: a definite correlation was found in the number of axons in the donor nerve and the functional outcomes in the shoulder and

Table 1 Clinical grading of recovery ($N = 30$)

Case number	Diagnosis	Follow-up (mo)	Abduction (degree)	External rotation (degree)	Elbow flexion (degree)	Elbow flexion strength (MRC grade or equivalent)
1	C5,C6 root avulsions	27	160	50	110	M3+
2	C5,C6 root fibrosis	24	140	40	110	M3+
3	Isolated MCN injury	14	NA	NA	120	M4+
4	C5,C6 root fibrosis	21	180	70	120	M4
5	C5,C6 root avulsions	24	170	50	110	M3
6	Erb's palsy	4	160	30	110	M3+
7	Isolated MCN injury	7	NA	NA	120	M4+
8	C5,C6 root avulsions	24	160	50	110	M3+
9	Erb's palsy	11	140	30	110	M3
10	C5,C6 root fibrosis	14	120	40	110	M3
11	C5,C6 root avulsions	21	170	40	110	M3+
12	C5,C6 root avulsions	24	160	40	110	M3
13	C5,C6 root fibrosis	20	120	40	100	M3
14	C5,C6 root fibrosis	26	120	50	110	M3+
15	C5,C6 root avulsions	24	150	50	110	M3+
16	Erb's palsy	21	90	25	100	M3
17	C5,C6 root avulsions	25	170	60	120	M4
18	Isolated AXN injury	24	180	70	NA	NA
19	C5,C6 root fibrosis	20	110	70	40	M2+
20	C5,C6 root avulsions	24	160	60	120	M4
21	Erb's palsy	9	110	30	NA	NA
22	C5,C6 root fibrosis	24	180	70	120	M4+
23	C5,C6 root avulsions	20	150	40	110	M3+
24	C5,C6 root avulsions	24	150	40	110	M3
25	Isolated MCN injury	20	NA	NA	120	M4
26	C5,C6 root avulsions	24	150	40	110	M3
27	C5,C6 root fibrosis	20	180	70	120	M4+
28	C5,C6 root fibrosis	24	170	60	120	M4
29	C5,C6 root avulsions	20	160	60	110	M3+
30	Isolated AXN injury	20	180	70	NA	NA

Abbreviations: AXN, axillary nerve; C, cervical; MCN, musculocutaneous nerve.

elbow. Donor nerve specimens with good axonal counts (SAN, ulnar nerve, and median nerve or triceps branch of radial nerve) had better functional gains.

Clinical outcomes and fibrosis: patients without evidence of fibrosis in the SSN and SAN revealed better results in shoulder functions with majority of them restoring more than 150 degree of active shoulder abduction. The shoulder abduction strength, in these patients, measured at least M4. Contrary to this, patients with elements of fibrosis measured less than 150 degree of abduction and a poorer abduction strength (M3 or less). Two patients with fibrosis in SAN could achieve only 90 degree of shoulder abduction.

Clinical outcomes and inflammation. Though inflammation was present in the donor and recipient nerves in most cases, its correlation with functional gains was not as significant as the axonal count and degree of fibrosis.

Clinical outcomes and demyelination/vasculitis. Presence of demyelination/vasculitis had little bearing in the ultimate functional results.

Discussion

Nerve transfer to a recipient site close to the target muscle has proved to be more effective than a repair in the proximal

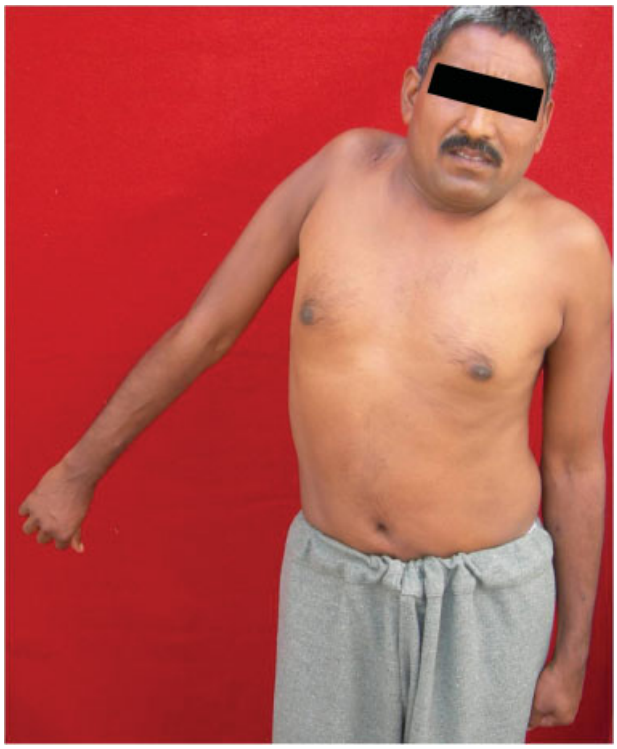

Fig. 7 Upper plexus palsy with lack of shoulder abduction.

part of the brachial plexus.[9,10] In the latter situation the regenerating fibers of the donor nerve are dispersed through branches to several nerves. Scattering of donor fibers over a large area not only makes neurotization insufficient but also causes simultaneous contraction of antagonistic muscles. Nerve transfer or neurotization includes three major categories, extraplexal neurotization, intraplexal neurotization, and end-to-side neurorrhaphy. Extraplexal neurotization[11] is the transfer of a non-brachial plexus component nerve to the brachial plexus for neurotization of an avulsed nerve. Donor nerves commonly used include SAN,

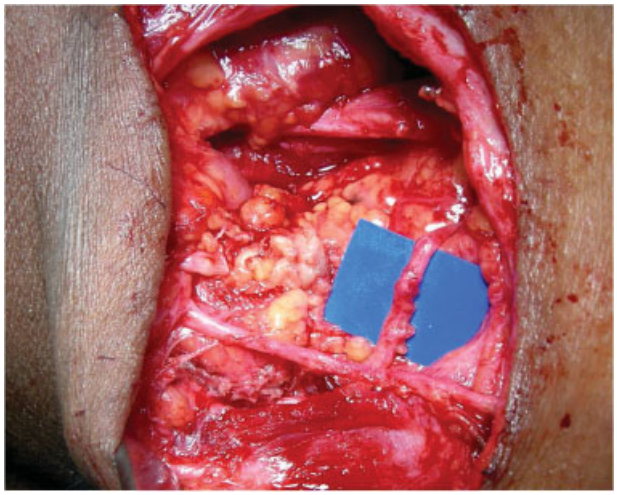

Fig. 8 Spinal accessory nerve to suprascapular nerve transfer by posterior approach.

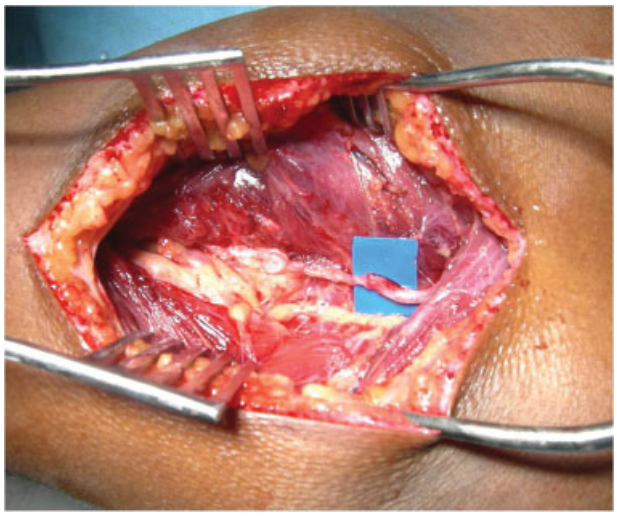

Fig. 9 Nerve transfer between long head triceps branch and anterior branch of axillary nerve.

intercostal nerves, phrenic nerve, deep cervical motor branches, and contralateral C7 transfer.[12] Intraplexal neurotization[1–3] is the transfer of a spinal nerve or more distal plexus component with intact spinal cord connections to a more important denervated nerve. Most striking examples are ulnar and median fascicular nerve transfers to the biceps and brachialis branches of the MCN.

In neurotization an attempt is made to reinnervate the recipient nerve as close to the target muscle as possible.[13] These distal nerve transfers have consistently resulted in good functional out comes.

In the last two decades many advances have been made in the field of neurobiology and nerve regeneration.[14] The practice of neuropathology has moved far ahead from microscopic evaluation of histopathological slides to the application of ancillary techniques in the form of immunohistochemistry and histomorphometry.[15] The use of these adjuvant modalities have not only helped in better understanding of the biological behavior of nerve injuries,

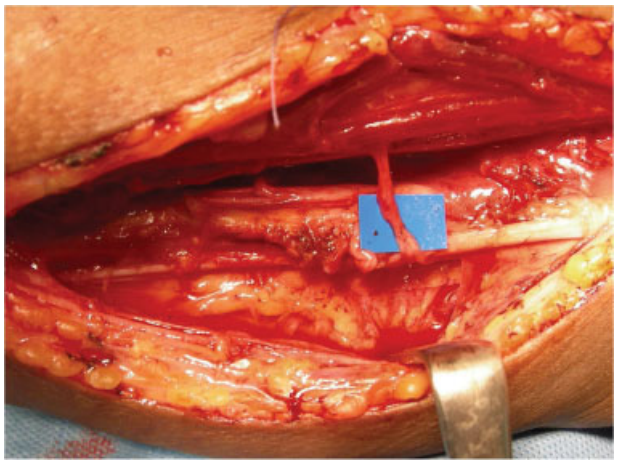

Fig. 10 Fascicular nerve transfer between ulnar nerve and biceps branch of musculocutaneous nerve.

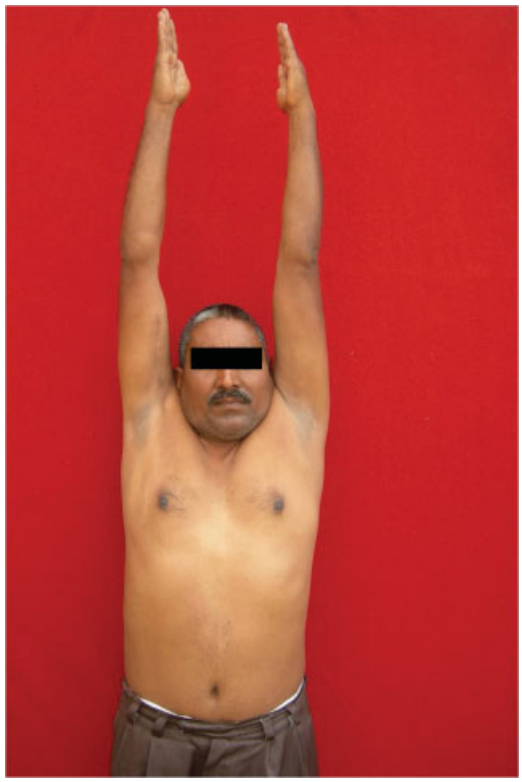

Fig. 11 Restoration of a full range of shoulder abduction.

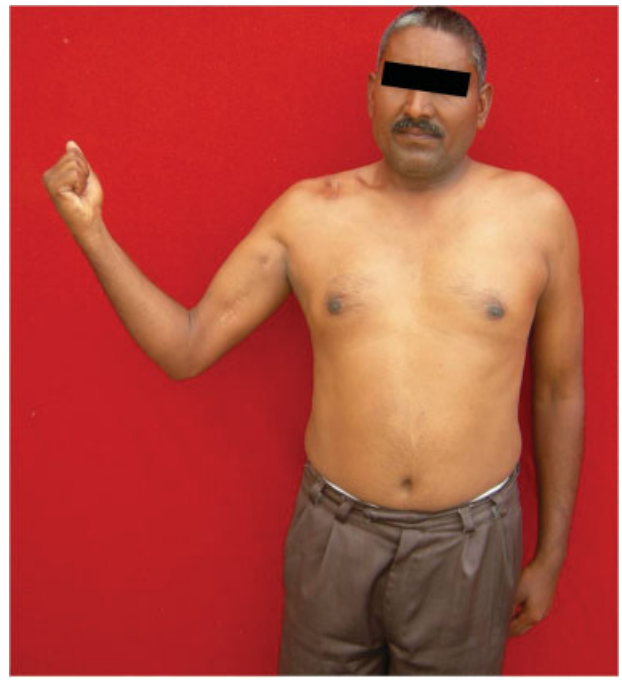

Fig. 12 Elbow flexion strength M4 + .

but have also aided in assessing the severity of the lesion, thus facilitating the treatment options.

There is increasing evidence that clinical features like age of patient, site of injury, time of denervation, and type of injury alone may not be sufficient to correctly determine the outcome of peripheral nerve repair.[16–19] These observations clearly indicate that nerve regeneration is the result of a complex interaction of many factors,[20–22] the gamut of which includes clinical parameters along with histomorphology of the donor and recipient nerves. Therefore, analyzing the histomorphology will enable a pathologist to comment on the prognosis, assist in

Table 2 Histomorphometric details

Nerve	No. of fascicles	Axonal count	Fibrosis	Inflammation	Demyelination
SAN	2	1,950	Nil	Nil	Present
SSN	2	3,450	Present	Present	Present
UN	1	802	Nil	Nil	Nil
BMN	1	960	Nil	Nil	Present
AAN	3	1,850	Nil	Nil	Present
LHT	3	1,100	Nil	Nil	Nil

Abbreviations: AAN, anterior branch of axillary nerve; BMN, biceps branch of musculocutaneous nerve; LHT, long head of triceps; SAN, spinal accessory nerve; SSN, suprascapular nerve; UN, ulnar nerve.

Table 3 Histomorphometric details

Nerve	No. of fascicles	Axonal count	Fibrosis	Inflammation	Demyelination
SAN	2	1,850	Nil	Nil	Nil
SSN	1	3,500	Present	Present	Present
UN	1	850	Nil	Nil	Nil
BMN	1	980	Nil	Present	Present

Abbreviations: BMN, biceps branch of musculocutaneous nerve; SAN, spinal accessory nerve; SSN, suprascapular nerve; UN, ulnar nerve fascicle.

Table 4 Histomorphometric details

Nerve	No. of fascicles	Axonal count	Fibrosis	Inflammation	Demyelination
SAN	2	2,016	Nil	Nil	Nil
SSN	2	2,918	Nil	Nil	Nil

Abbreviations: SAN, spinal accessory nerve; SSN, suprascapular nerve.

therapeutic decision making, and predict the treatment outcomes, when possible.[23] In the present study, an attempt has been made to find a correlation between various histomorphometry parameters and the functional results achieved following distal nerve transfers in brachial plexus and peripheral nerve injuries. It was noted that the frequency and degree of fibrosis in the injured nerve was more prevalent in patients with poor functional outcomes. It is also pertinent to mention that in some cases with severe injuries, fibrosis was observed in donor nerves as well.

Demyelination is an important component of Wallerian degeneration.[14] Myelin is thought to be an inhibitor of axonal regeneration by releasing various myelin-associated molecules. In this study demyelination was evident in majority of the cases.

Regeneration of axons from the proximal end to the distal end is one of the most important factors in reinnervation of peripheral tissue. When a nerve is injured the proximal stump sends out numerous sprouts to the endoneurial tubes in the distal nerve stump.[24] Hence, the residual axons in the proximal nerve stump and the endoneurial tubes into which the regenerative axons grow greatly influence the clinical outcome. In the present study, high axonal counts in donor nerves were associated with good functional outcomes.

The microvascular supply of the peripheral nerve trunk may get affected, particularly if they are associated with direct injury to the epineurial blood supply. Xu et al[25] measured epineural red blood cell flux using quantitative multiple sampling laser Doppler flowmetry, and endoneurial blood flow by microelectrode hydrogen clearance polarography in crush injuries and concluded that regenerative sprouting can sustain prolonged alterations in the epineurial circulation. In the present study, all cases revealed patent vasculature of both donor and recipient nerves.

The maximum duration of follow-up in the present study was 28 months. To accurately determine the actual recovery, a longer and closer follow-up involving larger number of cases is warranted, which is a limitation of the present study.

This study gives an insight that histomorphometric analysis of target nerves has a definite bearing on prediction of clinical outcomes in distal nerve transfers.

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