ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Distribution of sympathetic fiber areas in the sensory nerves of forearm: an immunohistochemical study in cadavers

S. CHAKRAVARTHY MARX[1)], P. KUMAR[2)], S. DHALAPATHY[3)], C. ANITHA MARX[4)]

[1)]*Department of Anatomy*
[2)]*Department of Plastic Surgery*
Kasturba Medical College, Manipal, Karnataka, India
[3)]*Department of Endocrine Surgery,*
Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGI), Lucknow, Uttar Pradesh, India
[4)]*Department of Computer Science,*
Manipal Institute of Technology, Manipal, Karnataka, India

Abstract

Purpose: Secondary to peripheral nerve injuries, involvement of sympathetic fibers complications such as Complex Regional Pain Syndrome (CRPS) have been reported. There are no reports available in the distribution of the sympathetic fibers/areas of sensory nerves in the forearm. *Materials and Methods*: The present study aim is an attempt to find the distribution of sympathetic fibers in the anterior branch of medial antebrachial cutaneous nerve of forearm (AMACN), lateral antebrachial cutaneous nerve of forearm (LACN) and superficial branch of radial nerve (SBRN) at cubital fossae. We have studied on 17 fresh human cadaveric AMACN, LACN and SRBN samples. Frozen sections of these nerves were processed by immunohistochemical (tyrosine hydroxylase) method for sympathetic fibers. *Results*: Sympathetic fibers area (Asym) was found to be more in SBRN when compared to AMACN and LACN. The comparison of the sympathetic index (SI = sympathetic fibers area / total fascicular area of the nerve) between AMACN and LACN (p-value <0.001), AMACN and SBRN (p-value <0.001), LACN and SBRN (p-value <0.001) were statistically significant. Sympathetic index (SI) for SBRN was more when compared to AMACN and LACN. SBRN had maximum percentage (5.16%) of Asym when compared with LACN and AMACN. *Conclusions*: Sympathetic fibers area (Asym), sympathetic index (SI) and percentage of sympathetic fibers area (Asym %) were found to be more in SBRN when compared with AMACN and LACN. These results of the study might help to explain sympathetic system-related diseases in the area of distribution of AMACN, LACN and SBRN.

Keywords: medial cutaneous nerve of forearm, lateral cutaneous nerve of forearm, superficial branch of radial nerve, sympathetic fibers, immunohistochemistry.

Introduction

Though a peripheral nerve may have sensory, motor and /or autonomic fibers, sympathetic fibers exclusively supply the upper limbs. The neurons from the lateral horn of the upper thoracic spinal segments T1–T6 constitute the pre-ganglionic sympathetic inflow whereas branches from brachial plexus form the post-ganglionic sympathetic fibers. The anterior branch of the medial antebrachial cutaneous nerve of forearm (AMACN) supplies skin over the volar aspect of the forearm [1–3]. The lateral antebrachial cutaneous nerve of forearm (LACN) is the terminal sensory branch of musculocutaneous nerve which supplies skin over the lateral aspect of the forearm [4, 5]. The superficial branch of the radial nerve (SBRN) supplying the cutaneous aspect on the dorsum of the later al three and a half digits [2].

Complex Regional Pain Syndrome (CRPS), diagnosed clinically can occur after peripheral nerve injuries (entrapment/traumatic) involving the sympathetic system. Based on etiology, CRPS is subdivided into two types: type 1 CRPS precipitated by a noxious event and type 2 CRPS occur due to peripheral nerve injury [6–8]. 1–5% of peripheral nerve injuries may have CRPS as a complication [9]. Investigators estimated that 2–5% of those with peripheral nerve injury [10] and 13–70% of those with hemiplegia [11] would suffer from CRPS. In general, after AMACN, LACN and SBRN injury, occurrence of complications as if CRPS has been reported [12–19]. This could be because of involvement of sympathetic fibers in the AMACN, LACN and SBRN. No reliable clinical study was published regarding these issues. Sympathetic fibers travel along with sensory nerves and very few studies have reported the anatomical basis of the sympathetic fiber distribution in the upper limb [17–23]. Higher sympathetic fiber content in nerves has a crucial role in the etiology of CRPS [24]. CRPS is yet to be fully understood.

The present study aim is an attempt to find the distribution of sympathetic fibers in the AMACN, LACN and SBRN at cubital fossae.

Materials and Methods

Anatomical dissection and materials

Seventeen samples of AMACN, LACN and SBRN were collected from 14 fresh human cadavers (10 male and four female) at cubital fossae. Out of 17 samples, 11 samples were collected from either right (six) or left (five) limbs of the cadavers (11 cadavers) and six samples were collected from both sides (three cadavers). These cadavers were donated to the Department of Anatomy, Kasturba Medical College, Manipal, India. The approval of the Ethics Committee was obtained from Kasturba Medical College, Manipal, India. During their lifetime, these individuals had neither neurologic nor metabolic disorders, nor any other kind of upper limb nerve damage.

In the cubital fossa region, a transverse incision was made in front of the elbow between the two epicondyles. A vertical incision was made from the middle of transverse incision until the apex of the cubital fossa where the brachioradialis overlaps the pronator teres. After roof dissection, median cubital vein, lateral and medial antebrachial cutaneous nerves and bicepital aponeurosis were identified. LACN was traced upwards until the tendon of biceps brachii and obtained just lateral to it. AMACN was collected adjacent to basilic vein (above the cubital fossa crease). The contents were identified and SBRN was obtained under the cover of brachioradialis at cubital fossa [25]. (Figure 1).

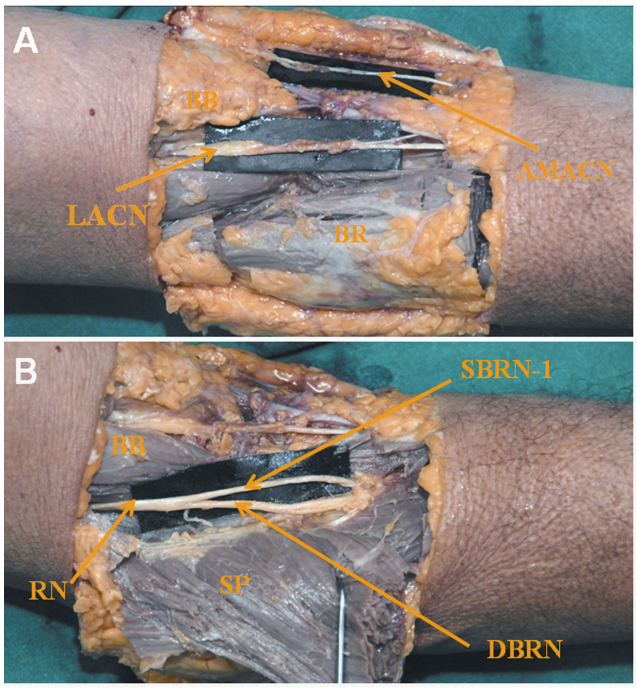

Figure 1 – ***(A) Anterior view of the right cubital fossa shows the sites of AMACN and LACN sample collection: AMACN – anterior branch of medial antebrachial cutaneous nerve of forearm, LACN – lateral antebrachial cutaneous nerve of forearm, BB – biceps brachii muscle, BR – brachioradialis muscle. (B) Anterior view of the right cubital fossa shows the site of SBRN sample collection: SBRN – superficial branch of the radial nerve, DBRN – deep branch of the radial nerve, BB – biceps brachii muscle, SP – supinator muscle.***

Tissue sampling and processing

Two centimeters long (AMACN, LACN and SBRN) tissues were obtained (within 12 hours after death) after the body donated to the Anatomy Department, Kasturba Medical College, Manipal, India. Nerves were fixed immediately with 4% paraformaldehyde solution. The nerves were later treated with 30% sucrose solution for 24 h, and then stored at -70^{0}C.

Immunohistochemistry (IHC)

The study was undertaken to observe the sympathetic fibers in AMACN, LACN and SBRN. Seven-micron cryostat sections were taken on poly-lysine-coated slides. The selected sections for the study were from the mid-region of the samples and they were processed for immunohistochemistry [19–23].

Fund details

The fund for the study was collected from Kasturba Medical College, Manipal, India. The antibodies and chemicals used were purchased from Sigma-Aldrich Chemicals Private Limited, Pennsylvania, USA.

Immunohistochemical procedure

At first, the sections were washed in phosphate buffer saline (PBS), treated with normal goat serum solution for 30 minutes to block the non-specific binding of immunoglobulins. The sections were then incubated overnight at 4^{0}C in a primary antibody (rabbit anti-tyrosine hydroxylase, 1:1000, catalogue no. T 8700, Sigma-Aldrich Chemicals Private Ltd., Pennsylvania, USA).

Next day, the sections were incubated in peroxidase blocking solution for 10 minutes to block the endogenous peroxidase activity. These sections were treated with secondary antibody (goat anti-rabbit IgG, catalogue no. B 8895, Sigma-Aldrich Chemicals Private Ltd., Pennsylvania, USA) and tertiary antibody (HRP–Streptavidin) for 30 minutes and the color was developed by treating the sections with 3,3'-diaminobenzidine (DAB) for 5–10 minutes. Finally, the immunostained sympathetic fibers were identified under the light microscope. Sections were washed in distilled water briefly, dehydrated in graded alcohol, cleared with xylene and mounted with coverslips. All the stained sections were photographed with Motic live image

programme (Version 2.0, Motic China Group Co., Ltd.) for morphometric analysis.

A normal Wistar rat adrenal gland, sciatic nerve and sympathetic plexus of the carotid artery were used as control tissues. For negative controls, the primary antibody was replaced by a non-immune rabbit serum or the secondary antibody was omitted.

Morphometric analysis

The morphometric analysis was performed using light microscope with a projection screen at a magnification of 50×. The images were analyzed using the in house developed software named "Tissue Quant" (TQ, Version 1.0), which is designed for color quantification in Manipal Centre for Information Science (MCIS), Manipal. This software provides the facility to choose a color for selectively measuring the areas in the image with a particular color. For evaluating fascicle areas, circles were drawn manually around each of the fascicles in all the images. The area covered by the circle was then calculated by the software in terms of number of pixels.

Micrometer scale was photographed under the same (50×) magnification for the calibration purpose. Number of pixels representing a length of 1 mm was calculated for both horizontal and vertical arrangements. This provided the calibration for the number of pixels representing one sq mm of area.

The first part of the study included the estimation of the number of fascicles (Nf), total fascicular areas (Af) of AMACN, LACN and SBRN.

The second part of the study (tyrosine hydroxylase immunohistochemistry) included the measurement of the area occupied by the sympathetic fibers in each fascicle (Figure 2). Then, total area occupied by the sympathetic fibers (Asym) in each nerve was calculated.

The third part of the calculation was to estimate the ratio between the sympathetic fiber area (Asym) and the total fascicular area (Af) of the nerve which is termed as sympathetic index of the nerve (SI) (SI = Asym/Af).

The fourth part of the calculation was to estimate the percentage of sympathetic fibers area (Asym %) in AMACN, LACN and SBRN fascicular areas.

Statistical analysis

Data was analyzed using "Graph pad instat" (Version 3.06, Graph pad Software Inc.) and "SPSS" (Version 11.5, The Predictive Analytics Company) statistical packages. Each data was analyzed for range, mean and standard mean of error (SEM). For comparison of Asym, SI and Asym's percentage at three sites, One-way analysis of variance (ANOVA) followed by "post hoc test", e.g. Tukey's test was used.

Results

The age of 17 cadavers' at the time of death ranged between 47 and 87 years, the mean age and standard deviation (SD) was 68.29 and 12.95. The study of the AMACN, LACN and SBRN cross-section showed the difference in number, shape and distribution of the fascicles. In all cases, fascicular pattern belonged to the polyfascicular type. The site at which the 2 cm segment of the nerve collected had no branches.

We found that statistically there was no difference between right and left side nerves. So, we treated them as independent sample. The sympathetic fibers of AMACN, LACN and SBRN fascicles were shown in Figure 2, a–c.

The Nf, Af, Asym and SI of AMACN, LACN and SBRN were obtained during morphometric analysis and range, mean and SEM were given in Tables 1 and 2.

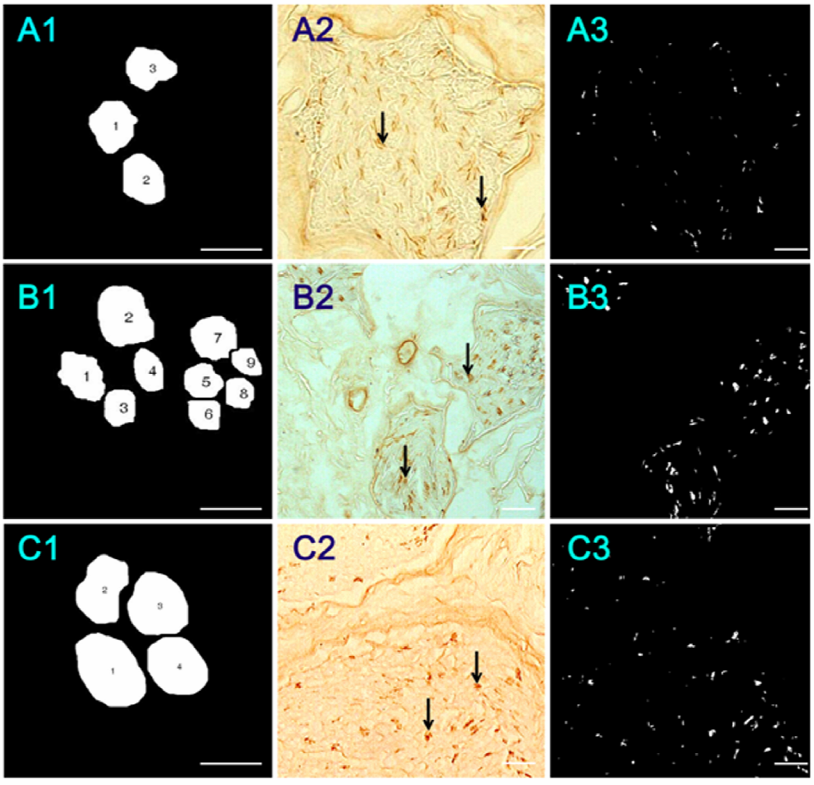

Figure 2 – ***The results of the automated measurement of (A1) the individual fascicular area of a single anterior branch of medial antebrachial cutaneous nerve of forearm (AMACN), (B1) a single lateral antebrachial cutaneous nerve of forearm (LACN) and (C1) a single superficial branch of the radial nerve (SBRN) that are shown in white were calculated by the image analysis software (40×). In 40× magnification, 1 mm^2 sympathetic fibers area = approx. 450×450 pixels. Photomicrograph shows (A2) fascicle of the AMACN, (B2) fascicle of the LACN and (C2) fascicle of the SBRN that were stained with tyrosine hydroxylase (TH) immunohistochemistry (100×). (A3), (B3) and (C3) show the result of the automated measurement of sympathetic fiber area (shown in white) of the same fascicle that was calculated by the image analysis software (100×). In 100× magnification, 1 mm^2 sympathetic fibers area = approx. 712×712 pixels. Scale bar = 100 µm valid for all the images.***

Table 1 – ***Human AMACN, LACN and SBRN sympathetic fibers area (Asym) morphometric absolute values***

Case No.	Age [years]	AMACN					LACN					SBRN				
		Nf	*Af [mm²]*	*Asym [mm²]*	*Asym %*	*SI*	*Nf*	*Af [mm²]*	*Asym [mm²]*	*Asym %*	*SI*	*Nf*	*Af [mm²]*	*Asym [mm²]*	*Asym %*	*SI*
1.	47	3	0.451	0.008	1.779	0.018	12	0.764	0.022	2.880	0.029	3	0.895	0.044	4.916	0.070
2.	48	4	0.309	0.003	1.008	0.010	7	0.833	0.029	3.481	0.035	4	0.683	0.041	6.003	0.070
3.	52	2	0.232	0.002	0.861	0.009	9	0.688	0.019	2.762	0.028	4	0.609	0.026	4.269	0.043
4.	61	3	0.154	0.002	1.362	0.014	8	0.891	0.024	2.694	0.027	3	0.931	0.038	4.082	0.041
5.	68	4	0.337	0.005	1.472	0.015	6	0.963	0.025	2.596	0.026	5	0.594	0.037	6.229	0.063
6.	74	3	0.358	0.007	1.876	0.019	7	0.631	0.031	4.913	0.049	6	0.492	0.024	4.878	0.049
7.	78	2	0.282	0.006	2.078	0.021	8	0.776	0.027	3.479	0.035	4	0.918	0.037	4.031	0.063
8.	82	3	0.267	0.005	1.927	0.019	9	0.857	0.028	3.267	0.033	3	0.973	0.039	4.008	0.061
9.	84	4	0.307	0.005	1.498	0.015	10	0.849	0.024	2.827	0.028	4	0.826	0.032	3.874	0.063
10.	87	3	0.393	0.009	2.188	0.022	7	0.894	0.025	2.796	0.028	3	0.633	0.043	6.793	0.069
11.	57	2	0.324	0.007	2.101	0.021	8	0.713	0.021	2.945	0.029	6	0.492	0.034	6.911	0.069
12.	62	3	0.275	0.005	1.793	0.018	7	0.682	0.045	6.598	0.066	4	0.608	0.035	5.757	0.058
13.	69	3	0.302	0.005	1.713	0.017	10	0.723	0.047	6.501	0.065	5	0.827	0.049	5.925	0.059
14.	74	4	0.453	0.008	1.676	0.017	7	0.674	0.039	5.786	0.058	4	0.918	0.037	4.031	0.040
15.	82	2	0.352	0.006	1.755	0.018	8	0.943	0.042	4.454	0.045	3	0.973	0.039	4.008	0.040
16.	58	3	0.282	0.006	2.078	0.021	9	0.956	0.034	3.556	0.036	4	0.588	0.049	8.333	0.083
17.	78	3	0.324	0.007	2.101	0.021	10	0.634	0.041	6.467	0.065	4	0.828	0.031	3.744	0.037

AMACN – anterior branch of medial cutaneous nerve of forearm; LACN – lateral antebrachial cutaneous nerve of forearm; SBRN – superficial branch of radial nerve; Nf – number of fascicles; Af – total facicular area; Asym % – sympathetic fibers area in total fascicular area; SI – sympathetic index (Asym / Af).

Table 2 – ***Descriptive statistics of 17 evaluated cases AMACN, LACN and SBRN mean morphometric parameters at cubital fossa***

Name	Nf		Af [mm²]		Asym [mm²]		Asym %		SI = Asym / Af	
	Range	*Mean ±SEM*	*Range*	*Mean ±SEM*	*Range*	*Mean ±SEM*	*Range*	*Mean ±SEM*	*Range*	*Mean ±SEM*
AMACN	2–5	3.00 ±0.172	0.154–0.453	0.318 ±0.018	0.002–0.009	0.006 ±0.001	0.86–2.19	1.72 ±0.09	0.009–0.022	0.017 ±0.001
LACN	6–12	8.35 ±0.373	0.631–0.963	0.792 ±0.028	0.019–0.047	0.031 ±0.002	2.60–6.60	4.00 ±0.36	0.026–0.066	0.040 ±0.004
SBRN	3–6	4.06 ±0.243	0.492–0.973	0.752 ±0.042	0.024–0.049	0.037 ±0.002	3.74–8.33	5.16 ±0.33	0.037–0.083	0.058 ±0.003
Sample (N)	17	17	17	17	17	17	17	17	17	17

AMACN – anterior branch of medial cutaneous nerve of forearm; LACN – lateral antebrachial cutaneous nerve of forearm; SBRN – superficial branch of radial nerve; Nf – number of fascicles; Af – total facicular area; Asym % – percentage of sympathetic fibers area; SI – sympathetic index (Asym / Af); SEM – standard mean of error.

The LACN was observed to have more fascicles and fascicular area, when compared to AMACN and SBRN. The number of fascicles and fascicular area was more in SBRN when compared to LACN.

The anatomical distribution of the TH-positive sympathetic fibers inside the each fascicles and each nerve was arranged in the form of groups. The sympathetic fibers area (Asym) in SBRN fascicular area was more when compared to AMACN and LACN fascicular area. The sympathetic fibers area (Asym) was more in LACN when compared to AMACN. Sympathetic index (SI) in SBRN was more when compared to AMACN and LACN (Figure 3).

Mean percentage of sympathetic fibers area (Asym %) in SBRN was more when compared to AMACN and LACN (Figure 4).

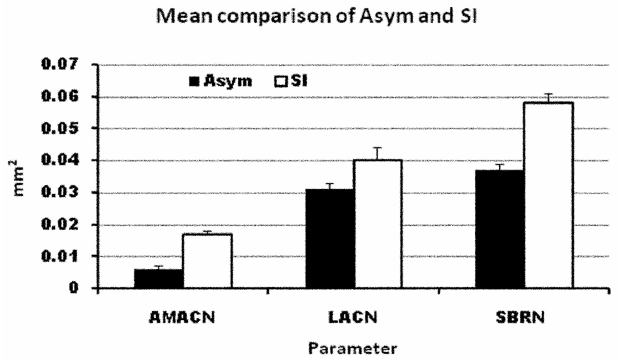

Figure 3 – ***The graph contains mean of sympathetic fiber area (Asym) and sympathetic index (SI) of AMACN, LACN and SBRN. Each bar represents Mean with SEM (standard error of mean).***

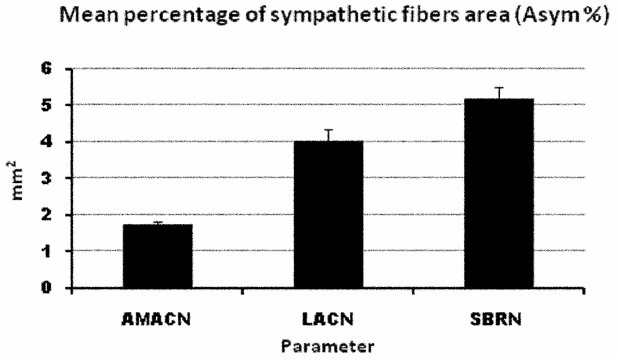

Figure 4 – ***The graph compares percentage [%] of mean sympathetic fiber area in AMACN, LACN and SBRN. Each bar represents Mean with SEM (standard error of mean).***

For comparison of Asym, SI, and Asym's percentage at three sites (AMACN, LACN and SBRN), One way Analysis of Variance (ANOVA) followed by "*post hoc* test" (Tukey's test) was used. The comparisons of the Asym of AMACN and LACN (p-value <0.001), AMACN and SBRN (p-value <0.001), LACN and SBRN (p-value =0.015) were statistically significant. The comparisons of the SI between AMACN and LACN (p-value <0.001), AMACN and SBRN (p-value <0.001), LACN and SBRN (p-value <0.001) were statistically significant. The comparison of the Asym's percentage (Asym %) between AMACN and LACN (p-value <0.001), AMACN and SBRN (p-value <0.001), LACN AND SBRN (p-value =0.016) were statistically significant (Table 3).

There was no significant change in the sympathetic nerve fiber area (Asym) with age and sex in AMACN, LACN and SBRN.

Table 3 – ***AMACN, LACN and SBRN morphometric parameters comparison by using One-way analysis of variance ANOVA – Tukey test***

No.	Group A	Group B	Asym		Asym %		SI	
			Mean difference (A - B)	*Sig.*	*Mean difference (A - B)*	*Sig.*	*Mean difference (A - B)*	*Sig.*
1.	*AMACN*	*LACN*	0.0251*	<0.001	2.2785*	<0.001	0.0227*	<0.001
2.	*AMACN*	*SBRN*	0.0317*	<0.001	3.4427*	<0.001	0.0401*	<0.001
3.	*LACN*	*SBRN*	0.0065*	0.015	1.1641*	0.016	0.0174*	<0.001

AMACN – anterior branch of medial cutaneous nerve of forearm; LACN – lateral antebrachial cutaneous nerve of forearm; SBRN – superficial branch of radial nerve; Asym – sympathetic fibers area; Asym % – percentage of sympathetic fibers area; SI – sympathetic index (Asym / fascicular area); Sig. – significance; * The mean difference is significant at the 0.05 level.

Discussion

The CRPS-II caused by sympathetic system involvement due to peripheral nerve injury is reported by very few studies using immunohistochemistry [18–23], which identifies TH-positive sympathetic fibers mainly on the ulnar and median nerves at the wrist, radial nerve at the cubital fossa and its superficial branch and finally the digital nerves in the hand. Due to lack of studies on sympathetic fibers content in AMACN, LACN and SBRN distribution, the present study was undertaken to find out the sympathetic fibers area in AMACN, LACN and SBRN.

The lateral antebrachial cutaneous nerve of forearm (LACN) was observed to have more fascicles and fascicular area, when compared to AMACN and SBRN. The number of fascicles and fascicular area was more in SBRN when compared to AMACN. Sunderland S [26] and Campero M *et al.* [27] reported three fascicles in their respective studies in the forearm. Higgins JP *et al.* [28] have reported the mean fascicle number of the AMACN at mid forearm as 3.8 in nine cases. Chiarapattanakom P *et al.* [29] have mentioned that LACN fascicles are located in the lateral part of the musculocutaneous nerve. The present study results were comparable to these.

Morgan RF *et al.* [18] in his study found the sympathetic fibers were more in the median nerve when compared to the ulnar nerve at the wrist. Balogh B *et al.* [19] identified sympathetic fibers in the nerve of Henlé, which is a branch of the ulnar nerve of the forearm. Also, Balogh B *et al.* [20] have compared the sympathetic fibers of the ulnar and median nerves at wrist, and branches of superficial branch of radial nerve and digital nerves in hand for 10 cases by using immunohistochemistry and concluded that proximal to the wrist, the median nerve had more sympathetic fibers than the ulnar nerve. The digital nerves showed significant differences between radial and ulnar nerves supplying various digits. Chakravarthy Marx S *et al.* [21] identified the sympathetic fiber area (0.050 mm^2) of the radial nerve in 21 cases at cubital fossa. Chakravarthy Marx S *et al.* [22] have also identified and compared the sympathetic fiber area (Asym) in course of the radial nerve (RN) and superficial branch of radial nerve (SBRN) in 19 cases and concluded that sympathetic fibers area (Asym) was more in RN when compared to SBRN. This may be due to some amount of sympathetic fibers passing through deep branch of the radial nerve (DBRN). The above studies did not include the identification and comparison of sympathetic fiber area (Asym) in the course of AMACN, LACN and SBRN. The present study aim is to identify and compare the sympathetic fiber area (Asym) in the course of AMACN, LACN and SBRN at cubital fossa. In the present study, in order to compare the sympathetic fiber area in all three different sensory nerves (AMACN, LACN and SBRN) of the forearm, 14 fresh cadavers were used. The present study's result regarding the sympathetic fiber area (Asym) in SBRN (0.037 mm^2) at cubital fossa confirms the findings of the previous study. In the present study, when we compared the sympathetic area in cross section, it was more in SBRN than in AMACN and LACN.

Complex regional pain syndrome (CRPS) is a very complex syndrome, which is not understood. Previously known as reflex sympathetic dystrophy (RSD), the change in the term was actually due to the lack of evidence of the malfunction of the sympathetic nervous system [30]. CRPS is estimated to occur at least in 1% to 5% of patients who have incurred a peripheral nerve injury [9]. Investigators estimated that 2–5% of those with peripheral nerve injury [10] and 13–70% of those with hemiplegia [11] would suffer from CRPS. The AMACN, LACN and SBRN are mostly composed of sensory and sympathetic fibers in its fascicles. Sympathetic fibers distribution to the skin and its appendages travel mainly through sensory nerves. In general, after AMACN, LACN and SBRN injury, occurrence of complications as if CRPS-II has been reported [12–16]. This could be because of involvement of sympathetic fibers. No reliable clinical study was

published regarding these issues. The sympathetic nervous system is more involved in CRPS than in other neuropathic pain syndromes [31]. There is still considerable disagreement as to the mechanisms underlying CRPS. This is probably related to the lack of quantitative clinical data, which would allow the formulation of precise testable hypotheses, and to the lack of animal models and experimental approaches using the human patient as the model [32]. Observations on CRPS patients clearly show that sympathetic, somatomotor, and somatosensory systems contribute to this pain syndrome. Characteristic sign and symptom of CRPS are abnormal pain (such as allodynia and hyperalgesia) and sympathetic disturbance (such as abnormal sweating and non-symmetric skin temperature) [33]. Although the pathogenesis of CRPS-II remains controversial, many theories have been purported. Some continue to propose that CRPS is a sympathetically mediated phenomenon after nerve injury [9]. These changes may lead to sweating abnormalities and poor blood flow due to vasoconstriction. One possible mechanism is that loss or inhibition of sympathetic vasoconstrictor neurons provokes an adrenergic super-sensitivity in affected tissue, which in turn encourages the release of nociceptive mediators, or directly enhances the excitability of nociceptive afferents [34].

Chémali KR *et al.* [35] study concluded that the abnormal higher sweat response in patients with acute CRPS is most likely mediated by an axon reflex and that alpha-adrenoreceptor supersensitivity occurs in the presynaptic portion of the postganglionic sudomotor axon. Wakisaka S *et al.* [36] have demonstrated that chronic injured sciatic nerve of the rat causes a neuropathic pain syndrome. They processed injured sciatic nerves using histofluorescence method to visualize catecholamines. There was a gradual loss of nor-epinephrine (NE)-containing sympathetic efferents on the nerve-injured side. Abnormal skin temperature and abnormal sympathetic vasomotor innervation was found in experimental painful peripheral neuropathy [36].

Several investigations studied the relationship between nerve compression and CRPS [37, 38]. Monsivais JJ *et al.* [38] showed 30 of 35 patients presenting with CRPS had compression of one or more peripheral nerves. In entrapment syndrome, sympathetic dysfunction occurs distal to the site of trauma along with the motor and sensory loss [39, 40]. The sympathetic fibers carrying pain sensation travel with sensory nerve, which is at risk to trauma like the sensory fibers. The location and distribution of these pain fibers is crucial in conditions like CRPS where pain with retention of all other sensations in the area supplied by a peripheral nerve indicate an entrapment syndrome with involvement of the sympathetic fibers [17].

As pain is the cardinal symptom being disproportionate to the inciting event, in the new classification appears the terms sympathetically maintained pain (SMP) and sympathetically independent pain (SIP), using sympathetic blocking to distinguish both. Injury to the sensory nerve produces less severe pain, swelling, stiffness and dysfunction in CRPS than found in major mixed nerves [24]. Higher sympathetic fiber content in nerves has a crucial role in the etiology of CRPS. In the present study, sympathetic fibers area (Asym) was more in SBRN when compared to AMACN and LACN. Hence, SBRN involvement may be expected to produce major CRPS when compared with AMACN and LACN involvement.

We also calculated the ratio between sympathetic fibers area and total fascicular area in the AMACN, LACN and SBRN as sympathetic index (SI). The SBRN total fascicular area was less when compared to LACN. SBRN sympathetic index was more when compared to LACN due to less total fascicular area. The AMACN sympathetic index was less when compared to LACN and SBRN.

Studies have shown that fracture of distal radius might lead to CRPS [41, 42]. SBRN with high sympathetic fibers ratio (SI) when compared to RN being in proximity might be injured or stretched leading to damage of sympathetic fibers, which may explain the association between CRPS and distal radius fracture.

Conclusions

Sympathetic fibers area (Asym), sympathetic index (SI) and percentage of sympathetic fibers area (Asym %) were found to be more in SBRN when compared with AMACN and LACN. These results of the study might help to explain sympathetic system-related diseases in the area of distribution of AMACN, LACN and SBRN.

Acknowledgements

We thank Prof. K. Ramachanra Bhat, Department of Anatomy, Kasturba Medical College, Manipal for the valuable guidance and help.

References

[1] Schwartzman RJ, *Brachial plexus traction injuries*, Hand Clin, 1991, 7(3):547–556.
[2] Standring S, *Gray's anatomy: the anatomical basis of clinical practice*, 40th edition, Churchill Livingstone, New York, 2008, 829–856.
[3] Race CM, Saldana MJ, *Anatomic course of the medial cutaneous nerves of the arm*, J Hand Surg Am, 1991, 16(1):48–52.
[4] Sunderland S, *The musculocutaneous nerve*, 1st edition, Williams & Wilkins, Baltimore, 1968.
[5] De Bisschop G, De Bisschop E, Commandré F, *Les syndromes canalaires*, Masson, Paris, 1997.
[6] Hassantash SA, Maier RV, *Sympathectomy for causalgia: experience with military injuries*, J Trauma, 2000, 49(2):266–271.
[7] Horowitz SH, *Venipuncture-induced causalgia: anatomic relations of upper extremity superficial veins and nerves, and clinical considerations*, Transfusion, 2000, 40(9):1036–1040.
[8] Wong GY, Wilson PR, *Classification of complex regional pain syndromes. New concepts*, Hand Clin, 1997, 13(3):319–325.
[9] Bryant PR, Kim CT, Millan R, *The rehabilitation of causalgia (complex regional pain syndrome-type II)*, Phys Med Rehabil Clin N Am, 2002, 13(1):137–157.
[10] Veldman PH, Reynen HM, Arntz IE, Goris RJ, *Signs and symptoms of reflex sympathetic dystrophy: prospective study of 829 patients*, Lancet, 1993, 342(8878):1012–1026.
[11] Yu D*, Shoulder pain in hemiplegia*, Phys Med Rehabil Clin N Am, 2004, 15(3):vi–vii, 683–697.
[12] Schmid C, Tjan TD, Scheld HH, *Severe complex regional pain syndrome type II after radial artery harvesting*, Ann Thorac Surg, 2002, 74(4):1250–1251.

[13] Mori H, Watanabe M, Akahane M, Yajima C, Ohtomo K, *A case of venipuncture-induced complex regional pain syndrome*, Nippon Igaku Hoshasen Gakkai Zasshi, 2002, 62(14):834–835.
[14] Rikli DA, Businger A, Babst R, *Dorsal double-plate fixation of the distal radius*, Oper Orthop Traumatol, 2005, 17(6):624–640.
[15] Kim G, Jeong Y, Cho Y, Lee J, Cho J, *Endoscopic radial artery harvesting may be the procedure of choice for coronary artery bypass grafting*, Circ J, 2007, 71(10):1511–1515.
[16] Yamada K, Yamada K, Katsuda I, Hida T, *Cubital fossa venipuncture sites based on anatomical variations and relationships of cutaneous veins and nerves*, Clin Anat, 2008, 21(4):307–313.
[17] Weiner RS (ed), *Pain management: a practical guide for clinicians*, 6th edition, CRC Press, New York, 2002, 220–221.
[18] Morgan RF, Reisman NR, Wilgis EF, *Anatomic localization of sympathetic nerves in the hand*, J Hand Surg Am, 1983, 8(3):283–288.
[19] Balogh B, Valencak J, Vesely M, Flammer M, Gruber H, Piza-Katzer H, *The nerve of Henle: an anatomic and immunohistochemical study*, J Hand Surg Am, 1999, 24(5):1103–1108.
[20] Balogh B, Auterith A, Behrus R, Maier S, Vesely M, Piza-Katzer H, *The sympathetic axons of the nerves of the hand*, Handchir Mikrochir Plast Chir, 2002, 34(6):369–373.
[21] Chakravarthy Marx S, Kumar P, Dhalapathy S, Prasad K, Anitha Marx C, *Microanatomical and immunohistochemical study of the human radial nerve at the antecubital fossa*, Ann Anat, 2009, 191(4):389–398.
[22] Chakravarthy Marx S, Kumar P, Dhalapathy S, Anitha Marx C, D'Souza AS, *Distribution of sympathetic fiber areas of radial nerve in the forearm: an immunohistochemical study in cadavers*, Surg Radiol Anat, 2010, 32(9):865–871.
[23] Chakravarthy Marx S, Kumar P, Dhalapathy S, Anitha Marx C, *Microanatomical and immunohistochemical study of the human anterior branch of the medial antebrachial cutaneous nerve of forearm at the antecubital fossa and its clinical implications*, Rom J Morphol Embryol, 2010, 51(2):337–346.
[24] Lankford LL, Reflex sympathetic dystrophy. In: Evarts CM (ed), *Surgery of the musculoskeletal system*, Churchill Livingstone, New York, 1983, 145–174.
[25] Romanes GJ, Upper and lower limbs. In: Romanes GJ (ed), *Cunningham's manual of practical anatomy. Vol. 1: The arm*, Oxford University Press, Oxford, 2000, 69–70.
[26] Sunderland S, *Nerves and nerve injuries*, Churchill Livingstone, Baltimore, 1978, 827–828.
[27] Campero M, Serra J, Ochoa JL, *Peripheral projections of sensory fascicles in the human superficial radial nerve*, Brain, 2005, 128(Pt 4):892–895.
[28] Higgins JP, Fisher S, Serletti JM, Orlando GS, *Assessment of nerve graft donor sites used for reconstruction of traumatic digital nerve defects*, J Hand Surg Am, 2002, 27(2):286–292.
[29] Chiarapattanakom P, Leechavengvongs S, Witoonchart K, Uerpairojkit C, Thuvasethakul P, *Anatomy and internal topography of the musculocutaneous nerve: the nerves to the biceps and brachialis muscle*, J Hand Surg Am, 1998, 23(2):250–255.
[30] Stanton-Hicks M, Jänig W, Hassenbusch S, Haddox JD, Boas R, Wilson P, *Reflex sympathetic dystrophy: changing concepts and taxonomy*, Pain, 1995, 63(1):127–133.
[31] Baron R, Levine JD, Fields HL, *Causalgia and reflex sympathetic dystrophy: does the sympathetic nervous system contribute to the generation of pain?*, Muscle Nerve, 1999, 22(6):678–695.
[32] Jänig W, Baron R, *Experimental approach to CRPS*, Pain, 2004, 108(1–2):3–7.
[33] Sandroni P, Low PA, Ferrer T, Opfer-Gehrking TL, Willner CL, Wilson PR, *Complex regional pain syndrome I (CRPS I): prospective study and laboratory evaluation*, Clin J Pain, 1998, 14(4):282–289.
[34] Arnold JMO, Teasell RW, MacLeod AP, Brown JE, Carruthers SG, *Increased venous alpha-adrenoceptor responsiveness in patients with reflex sympathetic dystrophy*, Ann Intern Med, 1993, 118(8):619–621.
[35] Chémali KR, Gorodeski R, Chelimsky TC, *Alpha-adrenergic supersensitivity of the sudomotor nerve in complex regional pain syndrome*, Ann Neurol, 2001, 49(4):453-459.
[36] Wakisaka S, Kajander KC, Bennett GJ, *Abnormal skin temperature and abnormal sympathetic vasomotor innervation in an experimental painful peripheral neuropathy*, Pain, 1991, 46(3):299–313.
[37] Grundberg AB, Reagan DS, *Compression syndromes in reflex sympathetic dystrophy*, J Hand Surg Am, 1991, 16(4):731–736.
[38] Monsivais JJ, Baker J, Monsivais D, *The association of peripheral nerve compression and reflex sympathetic dystrophy*, J Hand Surg Br, 1993, 18(3):337–338.
[39] Kurvers HA, Hofstra L, Jacobs MJ, Daemen MA, van den Wildenberg FA, Kitslaar PJ, Slaaf DW, Reneman RS, *Reflex sympathetic dystrophy: does sympathetic dysfunction originate from peripheral neuropathy?*, Surgery, 1996, 119(3):288–296.
[40] Teasdall RD, Smith BP, Koman LA, *Complex regional pain syndrome (reflex sympathetic dystrophy)*, Clin Sports Med, 2004, 23(1):145–155.
[41] Dürr W, *Sudeck's disease after radius fracture*, Langenbecks Arch Chir Suppl II Verh Dtsch Ges Chir, 1990:693–699.
[42] Hove LM, *Nerve entrapment and reflex sympathetic dystrophy after fractures of the distal radius*, Scand J Plast Reconstr Surg Hand Surg, 1995, 29(1):53–58.

Corresponding author
S. Chakravarthy Marx, PhD, Department of Anatomy, Kasturba Medical College, Manipal, 576104 Karnataka, India; Phone +91 9739867221, e-mail: marxmanipal@gmail.com

Received: September 29th, 2010

Accepted: April 24th, 2011