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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

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Abstract

Purpose: Poor prognosis of nerve repair in patients may be due to changes in intraneural anatomy with age. Also, chances of Complex Regional Pain Syndrome–Type I (CRPS–I) secondary to peripheral nerve injury are comparatively high. The present study is to find the fascicular pattern of the anterior branch of the medial antebrachial cutaneous nerve of forearm (MACN) (at antecubital fossa), microanatomic morphometric characteristics of its connective tissue components (adipose tissue) and changes with age and study of intraneural sympathetic fiber content. *Material and Methods*: Sixty six human (37–88-year-old) cadaveric anterior branch of MACN have been collected from antecubital fossa and the study has been performed at magnifications (5×, 10×, 20×, and 40× objective) after routine histological (Hematoxylin & Eosin stain, Masson's trichrome stain) processing was done for morphometric analysis (total cross-sectional, fascicular and non-fascicular area) and immunohistochemical (tyrosine hydroxylase) processing for sympathetic fibers. *Results and Conclusions*: The anterior branch of the MACN's average total cross section area was 1.150 mm^2 on right side and 1.156 mm^2 on left side. There was significant increase in non-fascicular connective tissue area. In non-fascicular area, there was very less amount of adipose tissue in 86.37% of cases and more adipose tissue in 13.63% (elderly) cases. The average sympathetic fiber area is 0.0109 mm^2 without definite relationship with age. Our study makes an attempt to build a normal data base for MACN which might be helpful during the application of diagnostic and surgical nerve graft procedures.

***Keywords*:** medial cutaneous nerve of forearm, fascicle, adipose tissue, morphometry, sympathetic fiber.

Introduction

A thorough knowledge of nerve fascicular and non-fascicular morphology is necessary for the diagnostic application of ultrasound as well as for its successful surgical nerve repair [1–3]. The medial antebrachial cutaneous nerve is a branch from the medial cord of the brachial plexus that consists of sensory fibers from the first thoracic level. The nerve accompanies the basilic vein down the arm and pierces the deep fascia in the middle of the proximal part of the arm. The nerve then splits into anterior and posterior branches in the distal third of the arm. The anterior branch supplies sensation to the volar aspect of the forearm including the antecubital fossa and proximal anterior part of the forearm [4, 5]. The anterior branch of the medial antebrachial cutaneous nerve of forearm (MACN) has been reported as a potential donor nerve for repair of sensory nerves [6]. In nerve repair, axonal regeneration depends upon amount of intervening perineural tissue [7]. In peripheral nerves, higher percentage of connective tissue in relation to nervous tissue plays an important role in predicting prognosis after nerve repair [8, 9]. Also, after peripheral nerve injuries, complications such as Complex Regional Pain Syndrome–Type I (CRPS–I) are yet to be fully understood.

In this study, we observed the cross-sectional anatomy and microanatomy of the anterior branch of the MACN at the antecubital fossa in relation to age, and the changes in fascicular, non-fascicular components (adipose tissue) and the sympathetic fiber content.

Material and Methods

Anatomical dissection and materials

Sixty-six fresh human cadaveric MACN were collected for the study from 41 cadavers (34 males and seven females) of the antecubital fossae. Out of 41 cadavers, samples were collected from both sides in 25 cadavers (24 males and one female), and samples were collected from only one side from the remaining

16 cadavers (10 males and 6 females) due to injury to the area of study (Table 1).

Table 1 – ***Distribution of human MACN samples***

No.	Sex	No. of cadavers	Bilateral (right & left)	Right only	Left only	Total nerves	Overall nerves	
							Right	*Left*
1.	Male	34	24	7	3	58	31	27
2.	Female	7	1	3	3	8	4	4
	Total	*41*	*25*	*10*	*6*	*66*	*35*	*31*

The cadavers' age ranged from 37 to 88 years, the mean age and standard deviation (SD) was found to be 62±14.82 years (right side) and 61.06±15.10 (left side). These cadavers were donated to the Department of Anatomy, Kasturba Medical College, Manipal. The ethical clearance was obtained from the Manipal University, India. During their lifetime, these individuals had neither neurologic nor metabolic disorders, nor any other kind of MACN damage.

In cubital fossa region, a transverse incision was made on front of the elbow between the two epicondyles. A downward vertical incision was made from the middle of transverse incision extended until the apex of the cubital fossa where the brachio radialis overlaps the pronator teres. The skin was reflected. Then in the roof, median cubital vein, medial antebrachial cutaneous nerves and bicepital aponeurosis were identified and they were superficial to deep fascia of the forearm [10].

Tissue sampling

Two centimeters of anterior branch of MACN was obtained within 6–10 hours after death. These nerve tissues were collected adjacent to basilic vein (above the antecubital fossa crease) (Figure 1).

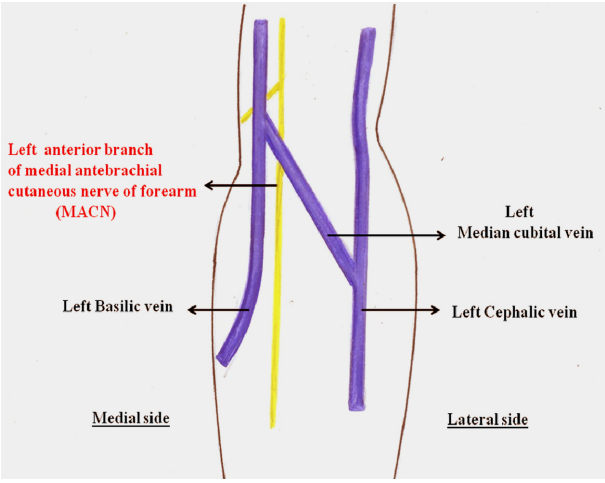

Figure 1 – ***Anterior view of the left antecubital fossa shows the site of the anterior branch of medial antebrachial cutaneous nerve of forearm (MACN) collection (adjacent to basilic vein).***

Tissue processing

The obtained anterior branch of MACN tissues were fixed immediately with 4% paraformaldehyde solution and embedded in paraffin. For frozen section immunohistochemical studies, fifteen pieces (10 males and five females) of nerve samples were used. The cadavers' age ranged from 56 to 86 years, the mean age and standard deviation (SD) was found to be 69.53±7.31 years. These nerves were treated with 30% sucrose solution for 24 hours, and then stored at -70^{0}C. Nerve tissue was processed for histological (paraffin sections) and immunohistochemical staining (frozen sections) in the Anatomy Department, Kasturba Medical College, Manipal, India.

Histological staining

Six-micron thick paraffin sections were placed on gelatin-coated slides. Selected sections were processed for histological Hematoxylin and Eosin (H&E) [11] and Masson's trichrome staining for connective tissue (collagen fibers) [12].

Immunohistochemical (IHC) staining for sympathetic fibers

Fifteen nerve tissues were processed for cryostat sectioning. Seven-micron cryostat sections were taken on polylysine-coated slides. Randomly selected sections were processed for immunohistochemistry.

IHC procedure [13–15]: 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 immunoglobulin. Then the sections were incubated overnight at 4^{0}C in a primary antibody (monoclonal rabbit anti-tyrosine hydroxylase, 1:1000) (Catalogue number T8700, Sigma-Aldrich Chemicals Private Ltd., Pennsylvania, USA). The next day, the sections were incubated in peroxidase blocking solution for 10 minutes to block the endogenous peroxidase activity. Then they were treated with secondary antibody (goat anti-rabbit IgG, 1:200) (Catalogue number B8895, Sigma-Aldrich Chemicals Private Ltd., Pennsylvania, USA) for 45 minutes, then with HRP–Streptavidin for 30 minutes and the color was developed by treating the sections with 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.

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.

All the histological and immunohistochemical (IHC) stained sections were focused under trinocular microscope (Olympus Company), photographed with Motic live image programme (Version 2.0, Motic China Group Co., Ltd.) for morphometric analysis (Figures 1–4).

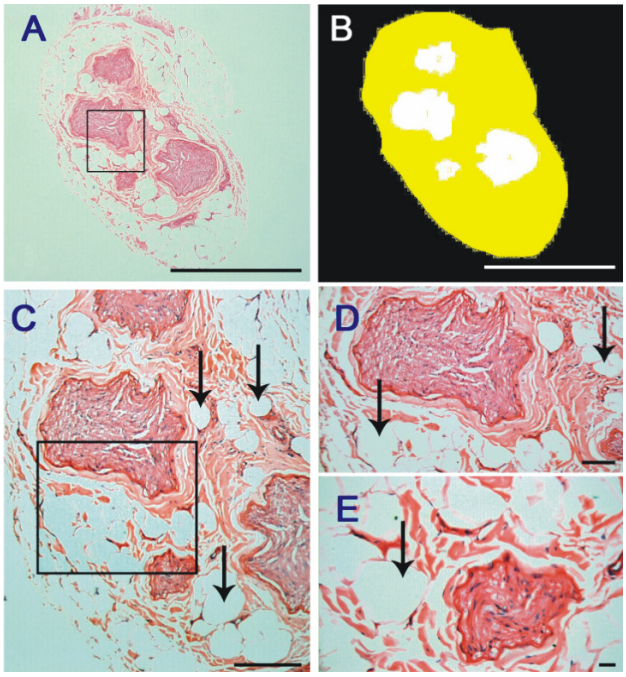

Figure 2 – ***Photomicrograph of the anterior branch of medial antebrachial cutaneous nerve of forearm (MACN) at the antecubital fossa H&E stained shows fascicular and non-fascicular tissue (adipose and non-adipose tissue) pattern during aging process. (A) Anterior branch of medial antebrachial cutaneous nerve of forearm of a 88-year-old male (right side) containing a large amount of adipose tissue (fat) in the epifascicular and interfascicular connective tissue region (50×); (B) The results of the automated measurement of the individual fascicular area shown as white and the non-fascicular area shown as yellow of a single anterior branch of MACN were calculated by the image analysis software in (50×). In magnification (50×), 1 mm^2 fascicular or non-fascicular area = approx. 490×490 pixels. Figures (C)–(E) shows the selected boxed area of Figure (A) in (100×), (200×) and (400×) respectively. Figures (A)–(E) show the increased amount of adipose tissue in non-fascicular region of the anterior branch of MACN in an individual. Arrows indicate more amount of adipose tissue (fat) present in non-fascicular area of the MACN. Scale bar = 100 mm valid for all the images.***

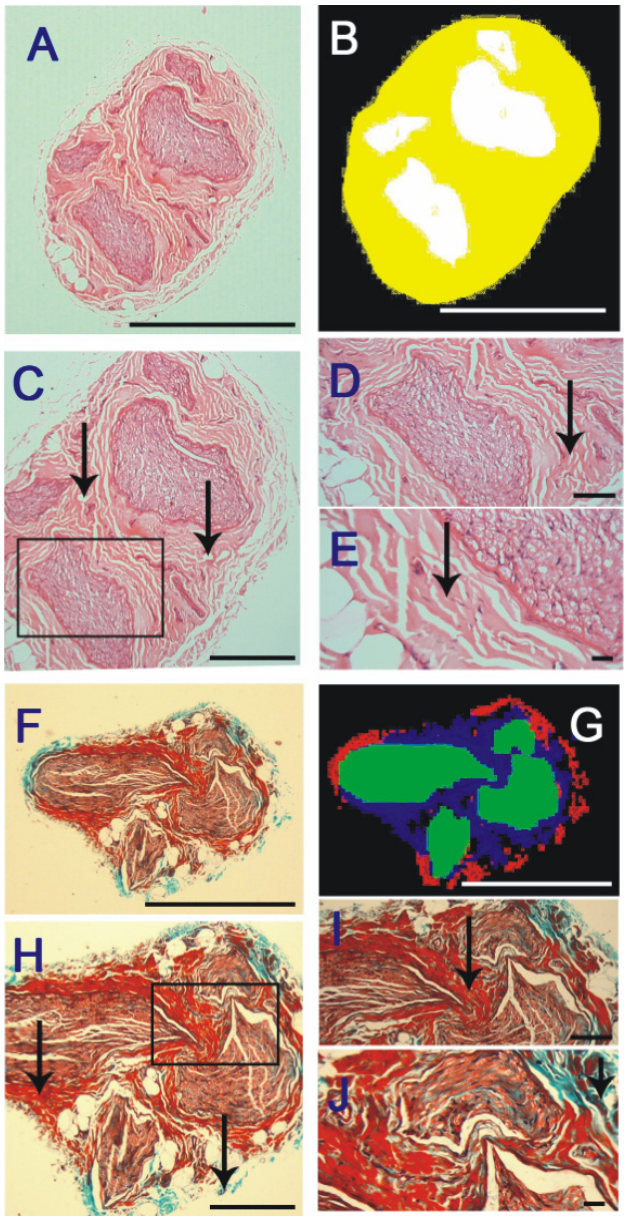

Figure 3 – ***Photomicrograph of the anterior branch of medial antebrachial cutaneous nerve of forearm (MACN) at the antecubital fossa H&E stained shows fascicular and non-fascicular tissue (adipose and non-adipose tissue) pattern during aging process. (A) The anterior branch of medial antebrachial cutaneous nerve of forearm (MACN) of a 37-year-old female (left side) containing a clearly lesser amount of adipose tissue (fat) in the epifascicular and interfascicular connective tissue region (50×); (B) The results of the automated measurement of the individual fascicular area shown as white and the non-fascicular area shown as yellow of a single anterior branch of MACN were calculated by the image analysis software in (50×). In magnification (50×), 1 mm^2 fascicular or non-fascicular area = approx. 490×490 pixels. Figures (C), (D) and (E) shows the selected boxed area of Figure (A) in (100×), (200×) and (400×) respectively. Figures (A)–(E) show the clearly lesser amount of adipose tissue in non-fascicular region of the anterior branch of the MACN. (F) The anterior branch of medial antebrachial cutaneous nerve of forearm (MACN) of a 38-year-old male (left side) stained with Masson's trichrome shows fascicular and non-fascicular tissue (mainly non-adipose tissue components – collagen fibers and connective tissue) pattern, which has less amount of adipose tissue (fat) in the epifascicular and interfascicular connective tissue region (green and red area shows the non-adipose area) (50×). (G) The results of the automated measurement of the individual fascicular area shown as green and the non-fascicular area (collagen fibers and connective tissue) shown as red and blue of a single anterior branch of MACN were calculated by the image analysis software in 50×. In magnification (50×), 1 mm^2 fascicular or non-fascicular area = approx. 490×490 pixels. Figures (H)–(J) shows the selected boxed area of Figure (F) in (100×), (200×) and (400×) respectively. Figures (F)–(J) confirms clearly lesser amount of adipose tissue in non-fascicular region of the anterior branch of MACN. The non-fascicular part is rich in collagen fibers and connective tissue. Arrows indicate less amount of adipose tissue (fat) present in non-fascicular area. Scale bar = 100 mm valid for all the images.***

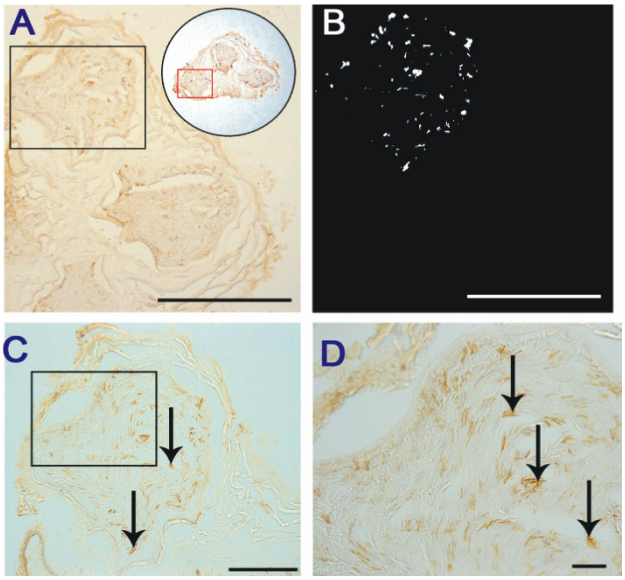

Figure 4 – *(A) Photomicrograph show fascicles of the anterior branch of MACN at antecubital fossa that was stained with tyrosine hydroxylase (TH) immunohistochemistry (100×). In (A), inside the circle anterior branch of MACN was shown (50×). Figure (B) shows the result of the automated measurement of sympathetic fiber area (white) of the same fascicle that was calculated by the image analysis software (100×). Figures (C) and (D) shows the selected area of Figure (A) under higher magnification (200×) and (400×) respectively with arrows pointing to sympathetic fibers. In magnification (100×), 1 mm^2 sympathetic fibers area = approx. 712×712 pixels. Scale bar = 100 mm valid for all the images.*

Morphometric analysis

The morphometric analysis was performed by three authors, randomly and blind foldedly under the light microscope with projection screen with a magnification of 50×, 100×, 200× and 400×. Then, the main author was organized and analyzed the data. In-house developed image analysis software named Tissue Quant (TQ, Version 1.0) was used for the automated measurement of total nerve cross sectional area, fascicular area, non-fascicular area, adipose and non-adipose tissue area and sympathetic fiber area [16].

The first part of the study (Hematoxylin and Eosin staining) included the estimation of the total number of fascicles (total Nf) present in the total anterior branch of MACN cross-sectional area, the measurement of total anterior branch of MACN cross-sectional area (Asc), individual and total fascicular area (Af) and non-fascicular area (Anonf). The number of anterior branch of MACN fascicles (Nf) per square millimeter was obtained by total number of fascicles in anterior branch of MACN divided by total cross-sectional area of the anterior branch of MACN (Nf = Total Nf/Asc) (Figures 2B and 3B).

The second part of the study (Masson's trichrome staining) included the measurement of the non-adipose area (nFAT) in the non-fascicular area of the anterior branch MACN. The adipose tissue area (FAT) in the anterior branch of MACN non-fascicular area was calculated by taking the difference between non-fascicular area (Anonf) and non-adipose area (nFAT) (FAT = Anonf - nFAT) (Tables 2 and 3, and Figure 3G).

The third part of the study (tyrosine hydroxylase immunohistochemistry) included the counting of sympathetic fibers and the measurement of the area occupied by the sympathetic fibers (Figure 4B).

Table 2 – *Human anterior branch of medial antebrachial cutaneous nerve of forearm (MACN) – right side morphometric parameters absolute values*

Age [years]	Sex	Total Nf	Asc [mm^2]	Af [mm^2]	Anonf [mm^2]	FAT [mm^2]	nFAT [mm^2]	Nf [1/mm^2]
38	M	3	0.900	0.218	0.683	0.065	0.618	3
38	M	5	0.898	0.264	0.633	0.082	0.551	5
38	M	4	0.987	0.232	0.754	0.077	0.678	4
40	M	5	1.179	0.072	1.106	0.173	0.933	4
41	M	7	1.264	0.322	0.942	0.065	0.877	6
43	F	4	0.705	0.282	0.423	0.081	0.342	6
46	M	6	1.162	0.358	0.804	0.299	0.505	5
47	M	4	1.483	0.279	1.204	0.196	1.008	2
47	M	4	0.893	0.309	0.584	0.056	0.528	5
49	M	5	1.046	0.183	0.864	0.071	0.793	5
55	M	2	1.082	0.453	0.629	0.103	0.525	2
56	M	4	0.916	0.327	0.588	0.083	0.506	4
60	F	6	1.047	0.207	0.840	0.207	0.633	6
60	M	7	0.944	0.299	0.646	0.045	0.601	7
62	M	4	1.284	0.227	1.057	0.520	0.537	3
62	M	3	1.098	0.352	0.747	0.131	0.615	3
63	M	4	0.983	0.315	0.668	0.071	0.597	4
63	M	5	1.120	0.274	0.845	0.582	0.264	4
64	M	2	1.376	0.417	0.959	0.186	0.773	2

Age [years]	Sex	Total Nf	Asc [mm^2]	Af [mm^2]	Anonf [mm^2]	FAT [mm^2]	nFAT [mm^2]	Nf [1/mm^2]
65	M	4	1.326	0.344	0.982	0.472	0.510	3
67	M	6	1.219	0.238	0.981	0.094	0.888	5
67	M	5	1.294	0.360	0.934	0.325	0.609	4
68	M	4	1.264	0.248	1.016	0.349	0.667	3
68	M	5	1.320	0.334	0.986	0.231	0.755	4
69	F	5	1.266	0.283	0.983	0.087	0.896	4
71	M	4	1.652	0.343	1.309	0.265	1.044	3
73	M	4	1.156	0.474	0.682	0.090	0.592	4
75	F	3	1.295	0.292	1.003	0.112	0.890	2
78	M	4	0.813	0.262	0.551	0.143	0.408	5
78	F	4	0.961	0.289	0.672	0.074	0.597	4
80	M	5	1.316	0.267	1.048	0.847	0.202	4
81	M	4	1.114	0.305	0.810	0.235	0.574	4
84	M	5	1.750	0.303	1.447	0.806	0.641	3
86	M	4	1.270	0.344	0.926	0.184	0.742	3
88	M	4	0.862	0.206	0.656	0.344	0.312	5

Total Nf – total number of fascicles per anterior branch of MACN, Asc – total (whole) cross sectional area, Af – fascicular area, Anonf – non-fascicular area, FAT – adipose tissue area (fat) occupied in non-fascicular area, nFAT – non-adipose tissue area occupied in non-fascicular area, Nf/mm^2 – number of fascicles per mm^2 (total number of fascicles divided by total cross sectional area). The non-fascicular area (Anonf) is higher than the fascicular area in all cases. In maximum number of cases, non-adipose tissue (nFAT) area is predominantly higher than the adipose tissue (FAT) area. 62, 63, 80, 84 and 88-year-old males had increased amount of adipose tissue in the non-fascicular area. Nf/mm^2 – number of fascicles per mm^2 ranges from 2 to 7.

Table 3 – ***Human anterior branch of medial antebrachial cutaneous nerve of forearm (MACN) – left side morphometric parameters absolute values***

Age [years]	Sex	Total Nf	Asc [mm^2]	Af [mm^2]	Anonf [mm^2]	FAT [mm^2]	nFAT [mm^2]	Nf [1/mm^2]
37	F	3	0.847	0.225	0.622	0.038	0.585	4
38	M	2	0.906	0.299	0.607	0.108	0.499	2
38	M	2	1.123	0.302	0.821	0.043	0.779	2
40	M	3	0.903	0.275	0.628	0.047	0.581	3
41	M	3	0.688	0.196	0.491	0.057	0.434	4
44	M	4	1.229	0.201	1.029	0.083	0.946	3
46	M	4	1.585	0.337	1.248	0.210	1.038	3
47	M	3	1.428	0.233	1.195	0.103	1.092	2
49	F	5	1.151	0.211	0.941	0.431	0.509	4
51	M	7	1.089	0.262	0.827	0.321	0.506	6
55	M	4	1.167	0.204	0.962	0.360	0.602	3
56	M	5	1.500	0.377	1.123	0.442	0.681	3
60	M	5	1.201	0.476	0.725	0.488	0.237	4
62	M	6	1.284	0.251	1.033	0.626	0.407	5
62	M	3	1.253	0.451	0.802	0.110	0.692	2
63	M	4	0.983	0.315	0.668	0.071	0.597	4
63	F	4	1.170	0.424	0.746	0.199	0.547	3
64	M	6	1.002	0.410	0.592	0.077	0.515	6
65	M	3	1.299	0.272	1.027	0.135	0.892	2
67	M	4	1.126	0.286	0.841	0.078	0.762	4
68	M	2	1.181	0.167	1.014	0.587	0.427	2
68	M	2	1.120	0.437	0.684	0.241	0.443	2
70	M	3	0.997	0.344	0.653	0.114	0.539	3
71	M	5	1.462	0.432	1.030	0.705	0.325	4
73	M	6	1.461	0.438	1.023	0.349	0.675	4
78	M	5	0.919	0.234	0.685	0.120	0.565	5
78	F	4	1.043	0.315	0.727	0.054	0.674	4
81	M	6	1.329	0.463	0.866	0.152	0.714	5
84	M	8	1.194	0.288	0.907	0.304	0.603	7
86	M	3	1.182	0.393	0.789	0.115	0.674	3
88	M	6	1.027	0.257	0.770	0.167	0.603	6

The non-fascicular area (Anonf) is higher than the fascicular area in all cases. In maximum number of cases, non-adipose tissue (nFAT) area is predominantly higher than the adipose tissue (FAT) area. 60, 62, 68 and 71-year-old males had increased amount of adipose tissue in the non-fascicular area. Nf/mm^2 – number of fascicles per mm^2 ranges from 2 to 7.

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. Mutual correlation between all the anterior branch of MACN morphometric parameters (total MACN cross-section, number of fascicles, total fascicular area, non-fascicular area, adipose and non-adipose area, size of the fascicles and sympathetic fibers area) was carried out. Each data was analyzed for range, mean and standard mean of error (SEM). Right and left side MACN were compared by using "paired *t*-test".

Results

The study of the anterior branch of MACN cross-section showed the difference in number, size and distribution of the fascicles. In all cases, anterior branch of MACN fascicular pattern belonged to the polyfascicular type. It also showed the difference in the amount of connective and adipose tissues in non-fascicular area. The anterior branch of medial antebrachial cutaneous nerve of forearm's total cross-sectional area, fascicular area, non-fascicular area, adipose area, non-adipose area and sympathetic fiber area were obtained during morphometric analysis (Tables 2–5, and Figures 5–7).

The anterior branch of medial antebrachial cutaneous nerve of forearm's total cross-sectional area ranged from 0.705 to 1.179 mm^2 (1.150±0.039, mean ± SEM) on the right side and 0.688 to 1.585 mm^2 (1.156±0.037) on the left side.

The number of fascicles per square millimeter of cross-sectional area ranged from 2 to 7 (4±0.209) on the right side and 2 to 7 (3.6±0.247) on the left side. The fascicular area ranged from 0.183 to 0.474 mm^2 (0.294±0.013) on the right side and 0.167 to 0.463 mm^2 (0.315±0.016) on the left side.

Table 4 – ***Descriptive statistics of all evaluated cases MACN (right side) morphometric parameters***

	Age	Total Nf	Asc [mm^2]	Af [mm^2]	Anonf [mm^2]	FAT [mm^2]	nFAT [mm^2]	Nf [1/mm^2]
Mean	62.00	4.40	1.15	0.29	0.86	0.22	0.63	4
SEM	2.51	0.19	0.04	0.01	0.04	0.03	0.03	0.21
95% LCL	56.90	4.01	1.07	0.27	0.78	0.15	0.57	3.58
95% UCL	67.10	4.79	1.23	0.32	0.93	0.29	0.70	4.43
Count	35	35	35	35	35	35	35	35

LCL – lower limit of confidence interval; UCL – upper limit of confidence interval.

Table 5 – ***Descriptive statistics of all evaluated cases MACN (left side) morphometric parameters***

	Age [years]	Total Nf	Asc [mm^2]	Af [mm^2]	Anonf [mm^2]	FAT [mm^2]	nFAT [mm^2]	Nf [1/mm^2]
Mean	61.06	4.19	1.16	0.32	0.84	0.22	0.62	3.68
SEM	2.71	0.28	0.04	0.02	0.03	0.03	0.03	0.25
95% LCL	55.52	3.62	1.08	0.28	0.77	0.15	0.55	3.17
95% UCL	66.61	4.77	1.23	0.35	0.91	0.29	0.69	4.18
Count	31	31	31	31	31	31	31	31

LCL – lower limit of confidence interval; UCL – upper limit of confidence interval.

Table 6 – ***Descriptive statistics of 15 evaluated cases MACN (Af, Asym and SI = Asym/Af) morphometric parameters***

Case no.	Af [mm^2]	Asym [mm^2]	SI = Asym/Af
1.	0.451	0.008	0.018
2.	0.309	0.003	0.010
3.	0.232	0.002	0.009
4.	0.154	0.002	0.014
5.	0.337	0.005	0.015
6.	0.358	0.007	0.019
7.	0.282	0.006	0.021
8..	0.267	0.005	0.019
9.	0.307	0.005	0.015
10.	0.393	0.009	0.022
11.	0.324	0.007	0.021
12.	0.275	0.005	0.018
13.	0.302	0.005	0.017
14.	0.453	0.008	0.017
15.	0.352	0.006	0.018
Mean	0.32	0.005	0.016
SEM	0.02	0.0005	0.0009
95% LCL	0.280	0.004	0.014
95% UCL	0.360	0.006	0.018
Count	15	15	15

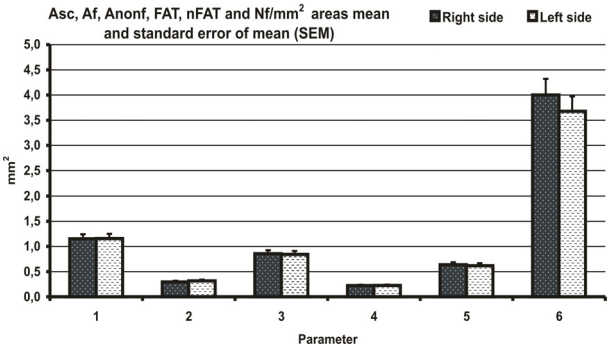

Figure 5 – ***The graph shows the anterior branch of MACN mean of total cross-sectional area (1 = Asc), fascicular area (2 = Af), non-fascicular area (3 = Anonf), adipose tissue area (fat) (4 = FAT), non-adipose tissue area (5 = nFAT) and number of fascicles per square millimeter (6 = Nf/mm²) for comparison. All the parameters were compared both sides (right and left) by using "paired t-test". Statistically they were not significant. There was increased non-fascicular tissue compared to fascicular tissue in all age cases. There is very less amount of adipose tissue compared to non-adipose tissue of the anterior branch of MACN at the antecubital fossa. The number of fascicles per square millimeter of cross-sectional area was increased. Each bar represents mean + SEM (standard error of mean).***

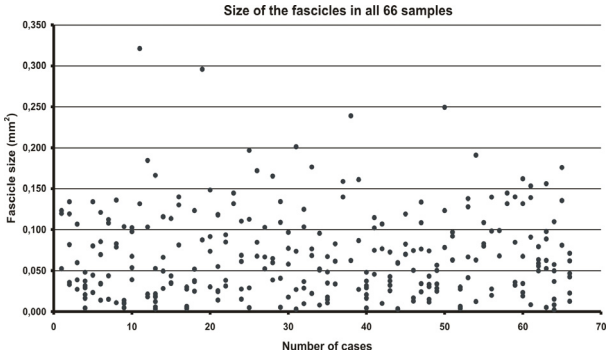

Figure 6 – ***XY (scatter) chart shows variety of fascicles size, which were present in all 66 anterior branch of MACN of different age groups; 95% of the fascicles sizes was below 0.150 mm². Majority of the fascicles were medium sized.***

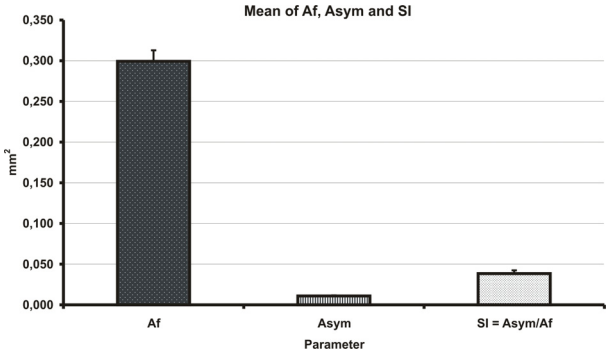

Figure 7 – ***The graph contains the mean of fascicular area (Af), sympathetic fiber area (Asym) and sympathetic index (SI) and compares the area occupied by the sympathetic fibers in a fascicular area. Each bar represents mean + SEM (standard error of mean).***

The non-fascicular area ranged from 0.423 to 1.447 mm² (0.856±0.038) on the right side and 0.491 to 1.248 mm² (0.841±0.034) on the left side. The adipose tissue area ranged from 0.045 to 0.847 mm² (0.221±0.035) on the right side and 0.038 to 0.705 mm² (0.224±0.034) on the left side. The non-adipose tissue area ranged from 0.202 to 1.008 mm² (0.635±0.034) on the right side and 0.237 to 1.092 mm² (0.618±0.034) on the left side (Tables 2–5). All the above parameters were compared between right and left side by using "paired *t*-test". They were statistically not significant (Figure 5).

The anterior branch of medial antebrachial cutaneous nerve of forearm's non-fascicular area and fascicular area were well developed in all age cases. The anterior branch of MACN's non-fascicular area was predominantly increased compared to fascicular area in age cases (Tables 2 and 3, and Figure 2, A–E).

In all age cases, there was decreased amount of adipose tissue (fat) deposition compared to the non-adipose tissue in the non-fascicular area. Whereas in 62-year-old male (both sides), 63, 80, 84, 88-year-old males of right side and 60, 68, 71-year-old males of left side anterior branch of MACN had more amount of the adipose tissue (fat) in the non-fascicular area (Tables 2 and 3) (Figure 3, A–E). About 85.72% on right side and 87.10% on left side anterior branch of MACN had very less amount of adipose tissue (fat) in the non-fascicular area. 14.28% of cases on right side and 12.90% of cases on left side had more amount of adipose tissue (fat) in the non-fascicular area. The increased adipose tissue in non-fascicular area was in elderly cases. Overall (in 66 cases), 13.63% had increased adipose tissue (fat) in non-fascicular area and 86.37% had decreased adipose tissue (fat) in non-fascicular area. Masson's trichrome stained anterior branch of MACN sections confirmed the rich connective tissue (collagen fibers) and decreased amount of adipose tissue in the non-fascicular area (Figure 3, F–J).

Also, the sizes of the individual fascicles of the anterior branch of MACN that ranged from 0.004 to 0.321 mm² (0.067±0.003) was measured. In the majority of instances, the fascicular size (95%) was below 0.150 mm² (Figure 6).

The sympathetic fiber area ranged from 0.002 to 0.009 mm² (0.005±0.0005). The ratio between the sympathetic fiber area and the total fascicular area of the anterior branch of MACN ranged from 0.009 to 0.022 mm² (0.017±0.0009). For our convenience, the ratio was termed as sympathetic index of the nerve (SI) (SI = Asym/Af) (Figure 4, A–D and Figure 7). These values might be used for the sympathetic nerve-related problems (Table 6).

Discussion

The morphologists focused on the fascicularisation of the peripheral nerves during the early twentieth century. Soon after, the neurosurgeons and the orthopedists that usually perform microsurgical interventions on peripheral nerves realized its significance in the diagnosis and treatment of the nerve lesions [17]. Hence, they started implementing fascicularisation, as a

recovery of nerve lesions mainly focusing on the nerve, as they are important especially in the elderly due to alterations with increase in age.

We focused on the connective tissue that undergoes change during the aging process, which is similar to that of the nerve fiber. Various studies have shown that the amount of connective tissue varies in different peripheral nerves, even in different parts of the same nerve [18, 19].

In the available literature, there are only data for the changes in the fascicular and non-fascicular area in relation to age. A study on human sciatic nerve revealed about the presence of more connective tissue and adipose tissue in the course of aging [8]. In elderly cases, there is significant increase in total radial nerve cross-sectional area due to an increase in its non-fascicular connective tissue area and increased adipose tissue deposition in interfascicular domains [16]. Azcoitia I *et al.* [20] reported that rat sciatic nerve cross sectional area increases with aging. In the human facial nerve, the number of fascicles and the rate of connective tissue is increased in the proximo-distal way along the course of the facial nerve, especially in the extra-temporal part [21]. Study on rat tibial nerve, showed the accumulation of collagen and lipid droplets in the perineurium with aging [22]. A study on human sural nerve showed the axonal degeneration and an increase in endoneurial collagen in the older age groups [23]. Authors of late have found that there is a statistically insignificant increase in the total nerve cross-sectional area but a higher percentage of connective tissue in relation to nervous tissue.

Fascicular component

In present study, the anterior branch of MACN at antecubital fossa was observed to have a polyfascicular pattern in all cases. Higgins JP *et al.* [24] have reported the mean fascicle number of the anterior branch of MACN at mid forearm as 3.8 in nine cases. McCormick SU *et al.* [25] have reported the fascicular area of the anterior branch of MACN above antecubital fossa in three cases.

Non-fascicular components

The non-fascicular area was predominantly increased compared to fascicular area due to the increased amount of non-adipose tissue or adipose tissue (fat) deposition in it. In most of the cases, the non-adipose tissue area (connective tissue) was increased in the non-fascicular area. In few elderly cases, there was more adipose tissue in the non-fascicular area. In the present study, the amount of adipose tissue in the non-fascicular area is found to be high in most of the elderly cases (especially in the ages from 60 to 90). McCormick SU *et al.* [25] have compared the non-fascicular area of the anterior branch of MACN (above antecubital fossa) in three cases with sural nerve.

Total cross-sectional area

Higgins JP *et al.* [24] reported the mean total cross sectional area of the anterior branch of MACN was 0.6856 mm^2 in nine cases (at mid forearm). In our study, the mean total cross section area of anterior branch of MACN was 1.150 mm^2 on right side and 1.156 mm^2 on left side (at antecubital fossa). This difference in total cross sectional area explains that the anterior branch of MACN has more branches and connective tissue before reaching to the mid forearm. McCormick SU *et al.* [25] have reported the total cross section area of the anterior branch of MACN above cubital fossa in three cases.

In previous reports, there were no data regarding the age, sex and condition of the cadavers [24–26]. We could not trace the data for the same parameter in the available literature for comparison.

Sympathetic fiber area

Complex Regional Pain Syndrome–Type I (CRPS–I) is a complication in MACN injury involving sympathetic system [27, 28]. There are limited studies, which quantify sympathetic fiber content in the upper limb. Morgan RF *et al.* [29] compared sympathetic fiber concentration between median and ulnar nerves and concluded that the sympathetic fibers are more in the median nerve as compared to the ulnar nerve at the wrist. Balogh B *et al.* [14] have identified sympathetic fibers in the palmar cutaneous branch of the ulnar nerve. Also, Balogh B *et al.* [15] have compared the sympathetic fibers of median nerve, ulnar nerve, the superficial branch of the radial nerve, and all digital nerves. Chakravarthy Marx S *et al.* [16] identified the sympathetic fiber area of the radial nerve in antecubital fossa. In the present study, we have identified sympathetic fibers area and there is no significant change with age.

Limitations of the study

To carry out this study, we took samples only from the donated bodies of the Anatomy Department, Kasturba Medical College, Manipal, India during the daytime. Also, the samples were collected within 6–10 hours after death to avoid the degradation of tyrosine hydroxylase enzyme for immunohistochemistry. The study represents Indian population, which differs in physical build from the west. The data in the available literature study were carried out by histological method in United States of America (USA) and United Kingdom (UK).

Clinical importance

It is clear that conditions such as entrapment, hereditary neuropathies, acquired neuropathies, trauma, partial or full nerve transections and nerve tumors result in an increase nerve cross-sectional fascicular and/or non-fascicular area. Various authors have commented on cross-sectional, fascicular and non-fascicular areas of the anterior branch of MACN, the exact anatomical reference value is not yet available in the literature [24–26]. Our study makes an attempt to build normal data base for the anterior branch of the MACN at antecubital fossa. This data can be extrapolated for clinical use. Any deviation from this reference value captured on image analysis may help the clinician to diagnose underlying problems.

The following authors have used anterior branch of MACN as a potential donor graft for digital nerves [6, 24, 30–32], facial nerve [33, 34], alveolar and lingual nerves [25, 26] repair. Our values on cross sectional area, fascicular area and non-fascicular area (adipose and non-adipose area) changes with age may be of use during selection of this nerve as nerve graft for a particular nerve with similar or different fascicular area. In elderly cases, larger amount of adipose tissue in interfascicular area of the anterior branch of the MACN may lead to poor outcome after surgery.

Sympathetic index might help to diagnose/predict the sympathetic system-related problem CRPS–I in the area of distribution of the anterior branch of the MACN.

Our study contributes to the knowledge of the anterior branch of MACN's normal fascicular pattern, size, area and non-fascicular area arrangement (adipose and non-adipose area) and sympathetic fiber area in relation to change with advancement of age.

Conclusions

In the present study, the amount of adipose tissue in the non-fascicular area is found to be high in most of the elderly cases (especially, in the ages from 60 to 90 years). This may help clinicians to diagnose a condition, better planning during repair and predicting more accurate outcome. There was no significant change in the sympathetic nerve fiber area with age.

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