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The use of computer-assisted image analysis in measuring the histological structure of the human median nerve

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Background and aim: *The aim of this study was to assess the histological structure of the median nerve and its motor branch (number and arrangement of nerve bundles) and the cross-sectional area (CSA) of the median nerve (on the level of the carpal tunnel).*

Material and methods: *This study has been conducted using median nerves dissected from cadavers stored in a 10% solution of formaldehyde at the Department of Anatomy of the Jagiellonian University Medical College and cadavers from the Department of Forensic Medicine of the Jagiellonian University Medical College. After dissection the median nerves were stained with haematoxylin and eosin and histological slides were prepared. These were later photographed (16× magnification) and analysed using ImageJ software. The research protocol was approved by the Jagiellonian University Ethics Committee (registry KBET/209/B/2002).*

Results: *The studied group comprised 8 women and 22 men (age between 23–92 years), yielding a total of 60 median nerves (30 right vs. 30 left). In 4 (6.67%) cases an accessory motor branch was found. The mean CSA of the median nerve was 0.19 cm^2. The median nerves from the right hand had a statistically larger CSA ($p = 0.017$). The number of nerve bundles in the median nerve varied between 13 to 38 and in the motor branch of the median nerve between 4 to 14.*

Conclusions: *The nerve bundles of the median nerve, at the level of the carpal tunnel, display no particular type of arrangement. ImageJ software proved useful in the assessment of the histological structure of the human median nerve and its motor branch.* (Folia Morphol 2012; 71, 2: 82–85)

Key words: nerve bundles, motor branch, carpal tunnel

INTRODUCTION

The median nerve is a mixed motor and sensory nerve [3, 6]. It descends beneath the flexor digitorum superficialis, lying on the flexor digitorum profundus in the forearm, within 5 cm of the transverse carpal ligament, and then it becomes more superficial, situated between the tendons of the flexor digitorum superficialis and the flexor carpi radialis [3]. It lies behind and radial to the side of the palmaris longus tendon just before entering the carpal tunnel [3]. The median nerve is located between the flexor retinaculum and the tendons of the middle

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finger flexor digitorum superficialis muscle in wrist extension [3].

The median nerve normally divides into 6 branches at the distal terminus of the flexor retinaculum [3, 6]. These include the recurrent motor branch innervating the opponens pollicis, abductor pollicis brevis, and the flexor pollicis brevis.

The literature lacks data on the histology of the median nerve. This might be because most studies focusing on the median nerve are conducted during surgery or by using ultrasound or magnetic resonance imaging.

The aim of this study was to assess the histological structure of the median nerve and its motor branch (number and arrangement of nerve bundles) and the cross-sectional area of the median nerve (on the level of the carpal tunnel) using ImageJ software.

MATERIAL AND METHODS

This study has been conducted using median nerves dissected from cadavers stored in a 10% solution of formaldehyde at the Department of Anatomy (Jagiellonian University Medical College) as well as cadavers from the Department of Forensic Medicine (Jagiellonian University Medical College).

There were no restrictions as to gender or age concerning inclusion into the study. Exclusion criteria included extensive damage to the median nerve preventing proper sample acquisition.

The research protocol was approved by the Jagiellonian University Ethics Committee (registry KBET/ /209/B/2002).

Dissection technique

The incision was made starting at the distal 1/3 of the forearm, continuing between the tendon of the flexor carpi radialis and the tendon of the palmaris longus muscle, and along the thumb flexion line. After dissecting the skin and the subcutaneous tissue, the median nerve was exposed and the flexor retinaculum was cut on the ulnar side. Next the median nerve was dissected (in or above the carpal tunnel) together with the motor branches innervating the muscles of the thumb. The existing incision was closed using a running intradermal suture.

Preparing histological slides

First a fragment of the main stem of the median nerve was excised (between the distal 1/3 part of the forearm to the site where the common palmar digital nerves branch out) together with the motor branch innervating the muscles of the thumb. This fragment of the median nerve was then placed in a glass container with a 10% solution of formaldehyde (pH 7.4). After 2–5 days a part of the main stem of the median nerve was excised (right above the motor branch) as well as the motor branch itself. Tissues were dehydrated, embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin.

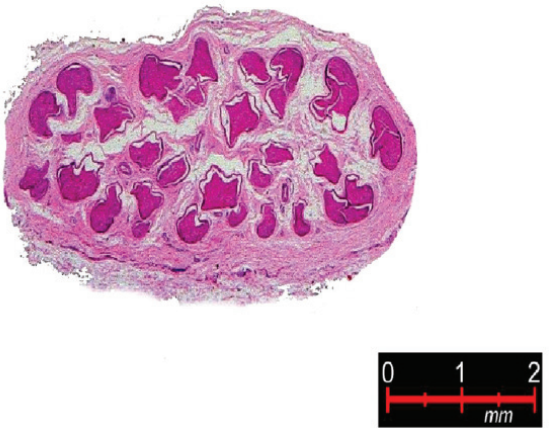

Figure 1. Median nerve cross section (haematoxylin and eosine staining).

Micromorphometry

The number of nerve bundles in the main stem of the median nerve and in the motor branch was assessed using light microscopy (100× magnification). Next, each main stem and motor branch cross-section was photographed (16× magnification) and the images were analysed using Java ImageJ (version 1.46d) developed by Wayne Rasband (National Institute of Health). The cross-sectional area, transverse and longitudinal dimensions, and the number of nerve bundles in the median nerve as well as the number of nerve bundles in the motor branch were calculated (Fig. 1).

Statistical analysis

Statistical analysis was conducted using computer software Statistica 9.0 PL by StatSoft Poland. To analyse the data, elements of descriptive statistics were used (mean, standard deviation, percentage distribution, minimum value, maximum value). To assess differences between groups the Students' t-test was used. The significance level was set at $p < 0.05$.

Table 1. The median nerve and its motor branch measurements

Measurement	Mean value	Standard deviation	Minimal value	Maximal value
Cross-sectional area of the median nerve [cm^2]	0.19	0.06	0.098	0.36
Transverse dimension [cm]	0.59	0.095	0.40	0.79
Longitudinal dimension [cm]	0.40	0.09	0.28	0.56
Number of nerve bundles in the median nerve	24.97	5.90	13.00	38.00
Number of nerve bundles in the motor branch	6.88	2.34	4.00	14.00
Number of nerve bundles in the accessory motor branch	2.70	1.09	1.00	3.00

Table 2. The median nerve and its motor branch measurements — comparison by gender

Measurement	Women		Men		P
	Mean value	Standard deviation	Mean value	Standard deviation	
Cross-sectional area of the median nerve [cm^2]	0.188	0.04	0.20	0.06	0.79
Transverse dimension [cm]	0.57	0.04	0.59	0.10	0.64
Longitudinal dimension [cm]	0.41	0.09	0.40	0.09	0.64
Number of nerve bundles in the median nerve	27.57	7.18	24.41	5.56	0.20
Number of nerve bundles in the motor branch	7.06	2.05	6.82	2.45	0.72

Table 3. The median nerve and its motor branch measurements — comparison by side

Measurement	Right side		Left side		P
	Mean value	Standard deviation	Mean value	Standard deviation	
Cross-sectional area of the median nerve [cm^2]	0.216	0.06	0.173	0.05	0.017
Transverse dimension [cm]	0.60	0.10	0.57	0.09	0.32
Longitudinal dimension [cm]	0.43	0.08	0.38	0.09	0.06
Number of nerve bundles in the median nerve	26.11	5.73	23.90	6.01	0.25
Number of nerve bundles in the motor branch	6.93	2.21	6.84	2.56	0.88

RESULTS

The studied group comprised 8 women and 22 men (age span between 23 and 92 years), yielding a total of 60 median nerves (30 right vs. 30 left). The median nerve and its motor branch measurements are presented in Table 1. In 4 (6.67%) cases an accessory motor branch was found (type IV according to Lanz). It branched out from the stem of the median nerve above the retinaculum flexorum, entered the carpal tunnel, and pierced the retinaculum flexorum. The median nerve and its motor branch measurements (comparison by gender) are presented in Table 2. No statistically significant differences between men and women were found. The median nerve and its motor branch measurements, comparison by side, are presented in Table 3. There was a statistically significant difference when comparing the cross-sectional area of the median nerve. The median nerves from the right side had a larger cross-sectional area (0.216 vs. 0.173 cm^2; $p = 0.017$).

DISCUSSION

This study presents data obtained, using ImageJ software, from the analysis of the histological structure of the median nerve and its motor branch. To

the authors knowledge, this is the first study assessing the histological structure of the median nerve at the level of the carpal tunnel.

At the assessed level there was no particular type of nerve bundle arrangement in the median nerve or in its motor branch. This finding is consistent with other studies assessing the cross-sections of other human nerves such as the oculomotor nerve [7]. We also determined that gender has no significant impact on median nerve histological structure, although median nerves in women had a tendency to have more nerve bundles than those in men (27.57 vs. 24.41; $p = 0.20$). This might possibly explain the fact that women tend to have superior hand stability compared with men at low force levels [4].

When comparing sides, the right median nerves had a significantly larger cross-sectional area (on the level of the carpal tunnel), which might be contributed to the fact that the majority of the Polish population is right-handed.

The mean cross-sectional area of the median nerve obtained in our study is significantly larger (0.19 vs. 0.10 cm^2; $p < 0.0001$) than in the study by Yao and Gai [8]. This might relate to anatomic differences in the perceived boundary of the nerve in magnetic resonance imaging. Such problem disappears when using computer-assisted histological image analysis.

Using ImageJ software proved to be the right choice for our study. ImageJ not only shortened the time needed to assess a single histological slide but also increased the accuracy of the conducted measurements. Going through literature concerning the use of computer-assisted image analysis techniques one can rarely find a study that assesses cross-sectional area without the use of such software. That is also the case in our study, as well as other studies assessing nerve structure [1, 2, 5, 7].

CONCLUSIONS

The nerve bundles of the median nerve, at the level of the carpal tunnel, display no particular type of arrangement. The number of nerve bundles in the median nerve varies between 13 to 38 and in the motor branch of the median nerve between 4 to 14. ImageJ software proved useful in the assessment of the histological structure of the human median nerve and its motor branch.

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