Morphometric Data of Normal Sural Nerve in Thai Adults

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Morphometry has an important role in the interpretation of sural nerve biopsies. It is used for early detection of structural abnormalities in peripheral neuropathies. This application requires a comparison with data of normal population. However, most data in the literature were of Western subjects with a small number of samples. In this study the authors reported the morphometric data of sural nerve harvested within 24 hours after death from 78 Thai subjects without known causes of neuropathy. The samples were transversely sectioned and analyzed for the number and area of fascicles, the total number of myelinated axons, myelinated fiber diameter, myelinated axon diameter, myelin sheath thickness, g ratio and myelinated axon density. Results were discordant in some measurement parameters compared to previous reports. These data are valuable for the early recognition of peripheral nerve diseases from biopsied sural nerve of Thai subjects.

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Evaluation of nerve biopsies is an important diagnostic tool in peripheral nerve disorders. Apart from diagnosis, this method can be used to evaluate the progression of diseases or response to treatments. Sural nerve, which is located between the lateral malleolus and Achilles tendon, is the most commonly used for nerve biopsy due to its superficial position and easily identifiable anatomy.

Morphometric analysis under light microscopy has been introduced to provide more objective and quantitative data compared to the conventional histologic evaluation. Various image analysis software has been used to facilitate the quantitative evaluation of peripheral nerve[1-3]. This computer-assisted morphometric study can be used to detect early abnormalities by comparing the results with the data of normal population. Several studies have reported the morphometric data from normal human sural nerve[4-8]. Common measurement parameters were, fascicular area, number of myelinated axons, density of myelinated axons, myelinated fiber diameter, myelinated axonal diameter, myelin thickness and g ratio. Since most of the know data is from studies of Western subjects, the data from Asian populations, including Thai, are still lacking data. These data are essential, as they are presumably more suitable to be used for the diagnosis of peripheral nerve diseases in Thailand. Therefore, the present study was carried out to provide the morphometric data of sural nerve from subjects included in Thailand.

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Material and Method

Subjects

Sural nerves were obtained from 78 subjects within 24 hours of death. These cases were included with approval from the Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University. There were 66 males and 12 females and the age range was 20-60 years. This age range was used due to the mature morphometric details of sural nerve suggested[5]. The included subjects must have no known causes or conditions of peripheral neuropathy. Delay duration until removal of nerve after death was 1 hour at minimum and 22 hours at maximum.

Collection of specimens and tissue processing

In each subject, 2 cm segment of sural nerve was harvested at the site between the lateral malleolus and Achilles tendon. The nerve was stretched by hanging with suitable weight in 2.5% glutaraldehyde for 12 min. The nerve was then divided into several short segments for better penetration and left in the same fixative for 12-24 hours. The specimens were subsequently washed with 0.1M cacodylate buffer pH 7.4, postfixed in 1% osmium tetroxide and dehydrated in increasing concentration of alcohol. The specimens were embedded in Epoxy resin and processed to transverse semi-thin section yielding 1 mm sections. Finally, the sections were stained with 1% paraphenylenediamine and subjected to nerve morphometric analysis.

Morphometric analysis

All the sections were screened for artifacts and pathologic appearance under light microscopy before the analysis. For each section, the number of fascicles was noted and the area of all fascicles was calculated using Image-Pro Plus software (under x4 objective). Subsequently, several images under x40 objective were imported to cover each fascicle and the number of myelinated axons, myelinated axon diameter, and myelinated fiber diameter were determined for each fascicle. The g ratio and myelin thickness were then calculated from the corresponding myelinated axon and fiber diameters of the same axon. Finally, the data from all fascicles of each sural nerve section were obtained and myelinated axon density was calculated by dividing the total number of myelinated axons by the fascicular area. Moreover, the frequency distribution of myelinated fiber diameter from all subjects was studied.

Results

Subjects

The average age of all 78 subjects was 37 years. The most common cause of death was an accident, found in 33 cases (6 females and 27 males). The mean duration after death until the removal of nerve was approximately 10 hours.

Morphometric data of sural nerve

Ranges,means and standard deviations are shown in Table 1.

Number of fascicles

The number of fascicles varied from 4 to 16 with an average of 9.2. When the number of fascicle was low, the size of each fascicle was usually large.

Fascicular area

The range of fascicular area was 0.34-1.38 mm^2 and the mean $\pm$ SD of all subjects was 0.85 $\pm$ 0.23 mm^2.

Number of myelinated axons

The average number of myelinated axons was 5,672.8 $\pm$ 1,753.7. However, the values varied greatly from 2,155 to 10,750.

Myelinated fiber diameter

The diameter of fibers varied from 5.65 to 8.92 mm with an average of 6.87 $\pm$ 0.77 mm. Distribution of the fiber diameter is shown as a histogram (Fig.1). Bimodal distribution was observed with the range of 3-5 mm for the lower mode and 8-10 mm for the upper mode.

Myelinated axon diameter

The range of axonal diameter was 2.23-4.54 mm with 3.21 $\pm$ 0.48 mm as the average.

Myelin sheath thickness

Myelin thickness values ranged from 1.23 to 2.79 mm. The average was 1.83 $\pm$ 0.34 mm.

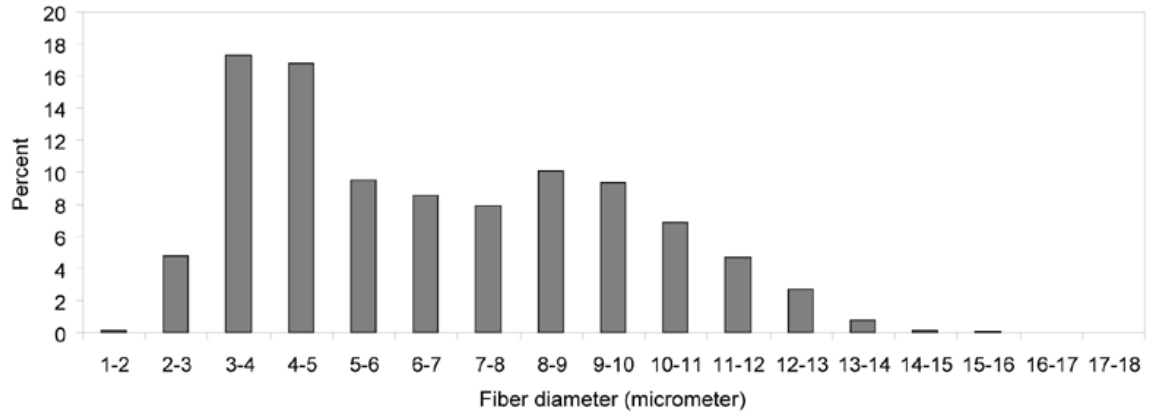

Fig. 1 The distribution of myelinated fiber diameter derived from 78 samples

Table 1. Morphometric data of human sural nerve from this and previous studies

Measurement	This study (n = 78)		Jacobs & Love, 1985 (n = 6)*		Behse, 1990 (n = 8)**	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Number of fascicle	4-16	9.20±2.8	5-10#	-	7-18	-
Fascicular area (mm^2)	0.34-1.38	0.85±0.23	0.58-0.63	0.70±0.10	0.65-1.26	1.04±0.20
Number of axon	2,155-10,750	5,672.8±1,753.7	4,730-7,950	5,918.3±1,201.9	5,200-9,460	7,252.5±1,242.8
Fiber diameter (m)	5.65-8.92	6.87±0.77	-	-	-	-
Axon diameter (m)	2.23-4.54	3.21±0.48	-	-	-	-
Myelin thickness (m)	1.23-2.79	1.83±0.34	-	-	-	-
g ratio	0.33-0.61	0.48±0.06	0.55-0.80#	-	-	-
Density of axon (/mm^2)	3,585.5-10,872.3	6,714.2±1,560.7	7,460-10,190	8,466±1,157.6	5,500-8,000	7,075±1,084.6

* Total number of cases in the study was 27 but only 6 were in the age range of 20-60
Therefore, fascicular area, no. of axon and density of axon were derived from 6 cases

** Total number of cases in the study was 10 but only 8 were in the age range of 20-60
Therefore, data were derived from 8 cases

\# the range with high frequency, not the true range

g ratio

The g ratio, ratio of axon to fiber diameters, varied from 0.33 to 0.61 with a mean of 0.48 ± 0.06.

Myelinated axon density

Density of myelinated axons varied considerably from 3,585.5 to 10,872.3 /mm^2 and the average was 6,714.2 ± 1,560.7/mm^2.

Relationship between the age of subject and any measurement parameters after the scatter plots were not analyzed (data not shown).

Discussion

To the authors' knowledge, this was the first report of the morphometric data of sural nerve from Thai subjects. The age range the authors chose to study (20-60 years) is considered mature in the development of sural nerve. Previous report has demonstrated that myelin sheath thickness and axonal diameter were increased until the second decade of life[5]. Furthermore, the same study also showed that although the density of myelinated axons tended to decrease with increasing age, the value remained constant between 10 and 60 years. Similarly, g ratio was found to remain almost unchanged until about 60 years. After 60 years, most parameters started to change markedly, for example, decreased myelinated axon density, increased variations in myelin thickness and g ratio including axonal degeneration-regeneration profiles. Therefore, the morphometric data from the age range used in the present study are supposed to be relatively less scattered and presumably more optimal for accurate interpretation of nerve biopsies. However, according to the above data, aged altered morphometric parameters should be kept in mind during an examination of sural nerve biopsies in cases older than 60 years. The number of cases included in the present study was higher than in previous reports. This may better represent the normal variation and may be more accurate in discriminating diseased from normal nerves.

Despite assuming the less dispersed values as mentioned above, some measurement parameters in the present study showed high variations. The total number of myelinated axons ranged from 2,155 to 10,750 and the density of myelinated axons varied from 3,585.5 to 10,872.3/mm^2. This was unlikely due to a wide range of ages since the scatter plots showed no relationship between these two parameters with age. Previous studies have reported smaller variations[5,6]. This inconsistency may be due to the higher number of samples used in the present study (n = 78) compared to those studies (n < 10). With more cases, variations are likely to increase and may explain the wider ranges of the two parameters observed in the present study.

In comparison with the previous studies, there were some similarities and differences (Table 1.). The range of fascicular area in the present study was more similar to Behse's than Jacob's and Love's

studies[5,6]. Additionally the mean figures were comparable among the studies. Regarding the number of myelinated axons, the authors found the wider range of values compared to the previous reports, as mentioned earlier. Despite differences in the range, means of this measurement data between the present study and Jacob's and Love's studies were identical and lower than that observed in Behse's study. For the fiber diameter, the presented mean was similar to that of Cai et al[7] (6.87 $\pm$ 0.77 vs 6.32 $\pm$ 3.21, respectively). When considering the distribution of fiber diameter, two peaks observed here were the same as what has been reported by Behse (3-5 and 8-10 m vs 4 and 10 m, respectively). For g ratio, the presented values were slightly lower than those found by Jacobs and Love. This might be because of the difference in the races of subjects. The density of myelinated axons in the present study was with higher variation in comparison with that of other studies. Nevertheless, the density means were not much different among the three studies (Table 1.)

In conclusion, the authors reported the first morphometric data of sural nerve from normal Thai subjects. The age range of the presented cases is relatively free from the influence of normal development and aging-related degeneration of sural nerve. The measurement data in the present study were similar in the majority of parameters previously reported. The discordances in some parameters may be due to the different races and/or number of samples and should be noted. These data are clinically useful for early detection of neuropathy from sural nerve biopsies, especially in Thai subjects.

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ข้อมูลวิเคราะห์เชิงโครงสร้างของเส้นประสาท sural ในคนไทยวัยผู้ใหญ่

วิไล ชินธเนศ, พินิดดา ชะอุ่มผล, อทิตยา แก้วเสมา, สิทธิพร แอกทอง, ธนศิลป์ หวลมานพ

การวิเคราะห์เชิงโครงสร้างของเส้นประสาท sural มีความสำคัญในการวินิจฉัยโรคทางระบบประสาทส่วนปลาย ซึ่งจำเป็นต้องใช้การเปรียบเทียบกับข้อมูลของประชากรปกติ แต่ข้อมูลที่มีรายงานไว้เป็นของชาวตะวันตกและใช้จำนวนตัวอย่างน้อย ในรายงานนี้ได้ทำการศึกษาข้อมูลเชิงโครงสร้างของเส้นประสาท sural ที่เก็บมาจากผู้เสียชีวิตคนไทยภายใน 24 ชั่วโมง ทั้งหมด 78 ราย โดยทำการตัดชิ้นเนื้อตามขวางและวิเคราะห์หาจำนวนและพื้นที่ทั้งหมดของ fascicle, จำนวนของ myelinated axon, ความยาวเส้นผ่านศูนย์กลางของ myelinated fiber และ axon, ความหนาของเยื่อหุ้ม myelin, g ratio และความหนาแน่นของ myelinated axon ผลการศึกษาพบว่ามีความแตกต่างในบางค่าระหว่างรายงานนี้กับการศึกษาก่อนหน้านี้ ข้อมูลเหล่านี้จะเป็นประโยชน์ในการตรวจพบความผิดปกติของระบบประสาทส่วนปลายตั้งแต่ระยะเริ่มแรกจากเส้นประสาท sural โดยเฉพาะในคนไทย