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Phrenic Nerve Distribution in the Rabbit Diaphragm and Morphometric Analysis of Nerve Branches

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With 2 figures and 3 tables

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Summary

The best method to evaluate the pathogenesis of diaphragmatic disorders is to demonstrate the distribution pattern of the phrenic nerve in the diaphragm. For this purpose the branching pattern and the microanatomic features of the phrenic nerve were observed in six rabbits. All diaphragms were stained by using Sihler's stain method. The phrenic nerve divided into three to four branches when entering the diaphragm. These branches were classified as sternal, anterolateral, posterolateral and crural. The crural branches were the thickest whereas the anterolateral branches were the thinnest. Knowledge about the distribution pattern of the phrenic nerve may be important in surgical approach to the diaphragm.

Introduction

The diaphragm is a thin and flat musculomembraneous structure that separates the thorax from the abdomen (Skandalakis, 2004). The diaphragm is innervated by a single nerve. Therefore, the diaphragm can be used as an innervation model (Laskowski and Sanes, 1987).

Knowledge about the development of the diaphragm is important. Initial contact and innervation of the pleuroperitoneal fold (PPF) by the phrenic nerve occur on E13. However, the actual initiation of phrenic nerve intramuscular branching commences approximately 24 h after initial contact, when the nerve and developing PPF have descended caudally towards the level of the middle to lower thoracic spinal cord (Allan and Greer, 1997). Once intramuscular branching commences within the diaphragm, there are precise, but as-yet-unidentified, guidance mechanisms for establishing the characteristic bilateral entry points of the phrenic nerves and the intramuscular branching pattern consisting of crural, sternal and dorsolateral primary branches (Laskowski and Sanes, 1987; Laskowski and Owens, 1994). Bennett and Pettigrew (1974) initially reported that primary myotubes formed in the diaphragm in a sequential fashion, which paralleled the extent of outgrowth of primary phrenic intramuscular branches. Phrenic nerve intramuscular branching and concomitant diaphragmtic myotube formation continue to progress through to embryonic day 17, at which time the mature pattern of innervation and muscle architecture are approximated (Lindsay et al., 1991; Greer et al., 1999). At day 17, the primary trunks of the left and right phrenic nerve divide into three secondary nerve trunks at the centre of the sternal costal portion. Two of these secondary nerve trunks traverse the sternal costal portions at right angles to the myotubes. Smaller tertiary nerve bundles branch from the secondary nerve trunks and pass between and parallel to the long axis of the myotubes. The third pair of nerve trunks grows from the centre of the sternal costal portions to the centre of the crural portions located more mediodorsally. At their point of entry, these trunks divide into two tertiary nerve trunks which grow through the crural portions at right angles to the myotubes (Noakes et al., 1983).

Studies indicate that there is not sufficient information that could constitute a branching model of phrenic nerve within the diaphragm. Earlier studies of the innervation of the diaphragm have prominently evolved around anatomical dissections (Collis et al., 1954; Botha, 1956; Shehata, 1966). Nevertheless, anatomical dissections carry the risk of cutting the peripheric branches as nerve caliper reduces while advancing from the primary trunk that innervates the organ up to the peripheric end branches. A special nerve staining method developed by Sihler (1895) was used to eliminate this risk in the present study. We believe that a thorough knowledge of the innervation and branching pattern of the phrenic nerve in the diaphragm will contribute to surgical intervention.

Materials and Methods

After obtaining ethics committee approval, six New Zealand rabbits (*Oryctolagus cuniculus*) were studied for the phrenic nerve distribution pattern in both hemidiaphragms. The diaphragms were totally removed and fixed in 10% formalin. They were then dyed to achieve nerve staining through the Sihler's staining method designed by Sihler (1895) first and modified by some researchers in recent years (Liem and Van Willigen, 1988; Wu and Sanders, 1992; Mu et al., 1994; Liu et al., 1997; Peker et al., 2001, 2003). The modified Sihler's stain method was used in the present study. The connective tissue around the muscle was removed under operating microscope (Zeiss Opmi Pico, Oberkochen, Germany). Afterwards, the nerve distribution pattern was studied under an Olympus SZ 40 Stereo microscope (Tokyo, Japan) and photographed. The images obtained with CCD camera from the operating microscope were transferred onto PC for morphometric analyses of the nerve trace and the measurements. The statistical evaluations were performed through SPSS for Windows (Release 11.5 package program).

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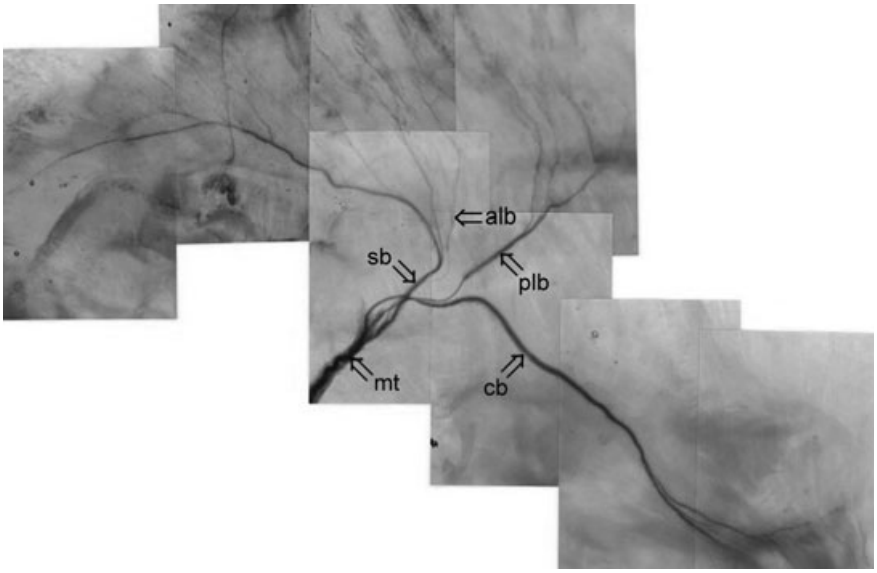


Fig. 1. General view of the right hemidiaphragm under the stereomicroscope. The main trunk divides into three primary branches (sb, plb, cb). Anterolateral branches emerge from the sternal branch. Haematoxylin ×6.7. mt, main trunk; sb, sternal branch; alb, anterolateral branch; plb, posterolateral branch; cb, crural branch.

Results

Microanatomic evaluation

The phrenic nerve branched off into three to four branches upon its entry into the diaphragm. These were the sternal, anterolateral, posterolateral and crural branches. The anterolateral and posterolateral branches varied among the diaphragms. Of all the branches, the crural branch was the thickest. The thinnest branches, if existed, were the anterolateral branches. The sternal branch, despite being thinner than the crural branch, was thicker than the anterolateral branch (Figs 1 and 2).

In all examinations, each phrenic nerve innervated its own hemidiaphragm and except in one case, in which it traversed the midline and anastomosed with the phrenic nerve branches off the opposite side. On the other hand, in each diaphragm, there were anastomoses among the secondary branches of the phrenic nerve. These anastomoses were marked particularly among the anterolateral branches. In some cases, however, there were anastomoses between the anterolateral and sternal branches. When the course of the phrenic nerve in the diaphragm was evaluated, despite the contractile structure of the diaphragm, strikingly the phrenic nerve did not follow a tortuous course to adapt this pattern. While the phrenic nerve was branching off in the diaphragm after its entry, it coursed near the abdominal surface within the muscular layer of the diaphragm. The main trunk ramified just after its entry in the diaphragm.

Statistical analysis

Chi-square and Spearman rank correlation analyses were used to investigate the relation of the diameters of the phrenic nerve and its branches. The Mann–Whitney *U*-test was performed to determine the relationship between diameters of the phrenic nerve and its diameters in the presence or absence of anastomoses. Descriptive statistics are given as mean ± SD, minimum–maximum values for continuous variables and frequencies, and percentages for categorical variables (Tables 1–3).

The general evaluation of the diaphragms presented the following results: absence of the primary anterolateral branch accounted for 16.7%; the sternal branch as the origin of the anterolateral branch accounted for 76.9%; the main trunk (phrenic nerve) as the origin of the anterolateral branch accounted for 7.7%; absence of the primary posterolateral

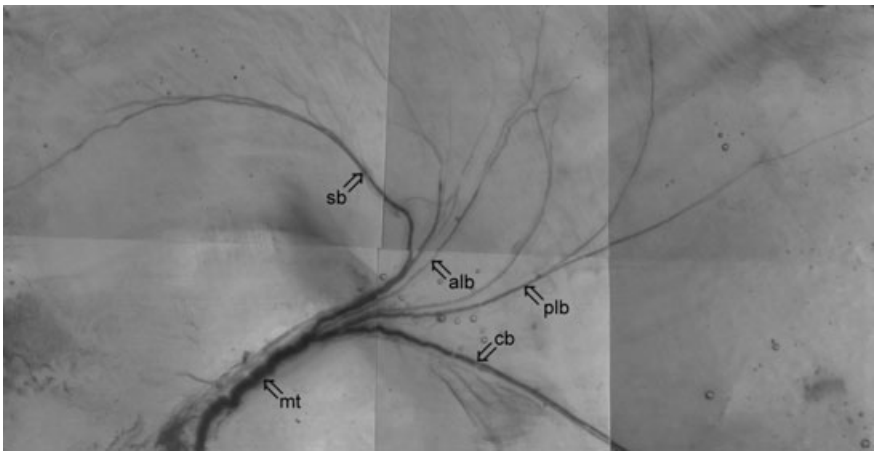


Fig. 2. Three primary branches (sb, alb, cb) of the phrenic nerve (main trunk). Posterolateral branches emerge from the crural branch. One of the anterolateral branches arises from the main trunk whereas the other one arises from the sternal branch. There are anastomoses among the primary and secondary branches of the phrenic nerve. Haematoxylin ×6.7. mt, main trunk; sb, sternal branch; alb, anterolateral branch; plb, posterolateral branch; cb, crural branch.

Table 1. Diameter of the main trunk, primary and secondary branches of the phrenic nerve

	Mean ± SD	Min	Max
Main trunk	406.67 ± 79.69	320.00	560.00
Primary branch (sternal)	220.00 ± 49.73	160.00	320.00
Primary branch (anterolateral)	100.00 ± 69.28	00.00	240.00
Primary branch (posterolateral)	110.00 ± 70.58	00.00	240.00
Primary branch (crural)	280.00 ± 53.94	160.00	320.00
Secondary branch (sternal)	72.92 ± 24.91	50.00	100.00
Secondary branch (anterolateral)	52.08 ± 37.63	00.00	150.00
Secondary branch (posterolateral)	56.25 ± 37.12	00.00	125.00
Secondary branch (crural)	101.39 ± 41.10	50.00	150.00

Table 2. Number of primary branches of the phrenic nerve

No. primary branches	Number	Rabbit (%)
Sternal	Absence	0 (0)
	1	12 (100)
	Total	12 (100)
Anterolateral	Absence	2 (16.7)
	1	9 (75.0)
	2	1 (8.3)
	Total	12 (100)
Posterolateral	Absence	2 (16.7)
	1	9 (75.0)
	2	1 (8.3)
	Total	12 (100)
Crural	Absence	0 (0)
	1	12 (100)
	Total	12 (100)

Table 3. Number of secondary branches of the phrenic nerve

No. secondary branches	Number	Rabbit (%)
Sternal	2	10 (83.4)
	3	1 (8.3)
	4	1 (8.3)
	Total	12 (100)
Anterolateral	0	2 (16.7)
	2	8 (66.6)
	4	2 (16.7)
	Total	12 (100)
Posterolateral	0	2 (16.7)
	2	9 (75.0)
	3	1 (8.3)
	Total	12 (100)
Crural	2	8 (66.7)
	3	3 (25.0)
	4	1 (8.3)
	Total	12 (100)

branch accounted for 16.7%; the crural branch as the origin of the primary posterolateral branch accounted for 61.5%; and the main trunk as the origin of the primary posterolateral branch accounted for 23.1%. The results indicated that the probability of the absence of the anterolateral branch was higher than that of the posterolateral branch.

Spearmen rank correlation analysis was used to evaluate the relationship among the main trunk, primary branch, secondary branch and anastomoses of the phrenic nerve in the diaphragms. If the main trunk was large, the diameter of the primary crural branch was also large ($r = 0.82$; $P < 0.01$). This result is in agreement with the microscopic findings. If the diameter of the primary anterolateral branch was large, the diameter of the secondary sternal branch was small ($r = -0.61$; $P < 0.05$). On the contrary, when the diameter of the primary anterolateral branch was large, the diameter of the secondary anterolateral branch was also large ($r = 0.86$; $P < 0.01$). In cases with large diameter of the primary posterolateral branches, the diameter of the secondary posterolateral branches was also large ($r = 0.85$; $P < 0.01$).

In the presence of anastomoses, the diameter of the main trunk was large ($P < 0.05$). However, the primary and secondary branches were not statistically significant with respect to anastomoses ($P > 0.05$).

Discussion

The phrenic nerve–diaphragm relationship in rats comprises a muscle and a simple system of 220 motor neurones. The primary branching pattern of rat diaphragm is composed of the phrenic nerve branching as two secondary branches, rostral and caudal. In addition to rostral and caudal primary branches of the phrenic nerve, a third primary branch innervating the diaphragmatic crus was shown (Laskowski and Sanes, 1987). On the other hand, Noakes et al. (1983) have claimed that both the left and right main trunks of the phrenic nerve branch off into three primary branches: the crural, sternal and posterolateral branches. Botha (1956) studied the diaphragms of various species and found that the phrenic nerve divided into two to seven branches in the diaphragm. In our study, the phrenic nerve had three or four branches: sternal, anterolateral, posterolateral and crural. The sternal and crural branches were observed in all cases.

According to Skandalakis (2004), each phrenic nerve divided into two or three branches just before entering the hemidiaphragm from above. These branches course together in the muscle. The motor branches give off three or four major branches: sternal, anterolateral, posterolateral and crural. However, there is no detailed information about the origin, peripheral course and/or branching model of any one of the phrenic nerve branches. Botha (1956) reported the posteromedial (crural) branch to be the biggest branch, always present and coursing in always the same direction. On the other hand, they detected that the posterolateral branches were different for either side: on the right-hand side, constantly short, thick and always advancing behind the inferior vena cava, and producing very few or no branches anteriorly. In our study, the evaluation of the distribution of the phrenic nerve in diaphragms revealed that the sternal and crural branches existed, while the anterolateral and posterolateral branches considerably varied as to their presence and points of origin.

Skandalakis (2004) reported the probability of anastomoses between the sternal branches of the phrenic nerve behind the sternum in humans. Nevertheless, in the present study, evaluations of the diaphragm showed that each phrenic nerve innervated its own hemidiaphragm and did not anastomose with the phrenic nerve of the opposite side by transversing the midline, except in one case. There were, however, anastomoses between the primary and secondary branches of the phrenic nerve. These anastomoses were evident between the anterolateral branches. There were also anastomoses between the anterolateral and sternal branches in some cases.

The literature reveals few studies on the intramuscular nerve branching pattern and morphological changes during this

course by using Sihler's staining method (Liem and Van Willigen, 1988; Wu and Sanders, 1992; Mu et al., 1994; Liu et al., 1997; Peker et al., 2001). Peker et al. (2000) reported that the nerves innervating the tongue had a smooth course in the root but followed a tortuous course towards the apex. In contractile organs such as the tongue, the nerve branches follow a tortuous course to adjust the movement. In the present study, the evaluation of the course of the phrenic nerve in the diaphragm was not tortuous despite the contractile structure of the diaphragm.

In conclusion, the number, the point of origin, and the diameters of the primary and secondary branches of the rabbit diaphragms may provide essential information about the microanatomy and innervation, and help comprehend mortal diaphragm pathologies. However, we believe further comprehensive studies are needed.

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