

Morphometry of the Lymph Nodes Excluding the Thoracic and Abdominal Cavity in Wistar Rats

İlknur DABANOĞLU

Department of Anatomy, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın - TURKEY

H. Erbay BARDAKCIOĞLU

Department of Animal Science, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın - TURKEY

Figen SEVİL, Didem ALP

5th Grade Students, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın - TURKEY

Received: 06.06.2003

Abstract: Ten male adult Wistar rats were used to obtain some measurements of the lymph nodes located in the head, neck, forelimb and hindlimb. The lymph nodes were marked by the injection of Evans Blue and were then dissected. In order to provide sufficient data for the morphometric properties of lymph nodes, the lymph nodes on each side were counted, and the longest and widest diameters of the lymph nodes were also measured.

The mandibular lymph nodes were found to be higher in number in comparison with the other lymph nodes investigated. The largest was found in the axillary accessory lymph nodes. The differences between the longest and the widest diameters of ischiadic lymph nodes on the right and left sides were statistically significant ($P < 0.05$). In addition, it was observed that both cranial and caudal profound cervical nodes were inconstant.

Key Words: Lymph node, rat, morphometry

Wistar Irkı Ratlarda Göğüs ve Karın Boşluğu Dışındaki Lenf Yumrularının Morfometrisi

Özet: Çalışmada ratların baş, boyun, ön ve arka bacaklarında bulunan lenf yumruları incelendi. Bunun için on adet erkek Wistar ırkı rat kullanıldı. Ratlara Evans Blue enjekte edilip lenf yumruları belirgin hale getirildikten sonra anatomik diseksiyonu yapıldı. Wistar ırkı ratlarda lenf yumruları ile ilgili morfometrik bilgi elde etmek için, lenf yumrularının sayıları ile en uzun ve en geniş çaplarının ölçümleri alınarak değerlendirildi.

Wistar ırkı ratlarda İn. mandibularis'lerin en fazla sayıda, İn. axillary accessori'lerinin ise en büyük çaplara sahip olduğu belirlendi. İn. ischiadicum'un inkonstant olduğu ve gerek en uzun, gerekse en geniş çapları bakımından sağ ve sol ortalamaları arasındaki farkın istatistiksel olarak önemli olduğu belirlenmiştir ($P < 0,05$). Ayrıca İn. cervicalis profundus cranialis ve caudalis'in de inkonstant olduğu gözlemlendi.

Anahtar Sözcükler: Lenf yumrusu, rat, morfometri

Introduction

The lymph nodes comprise the main components of the immune system. The functions of lymph nodes include filtering the lymphatic substance, phagocytosing particles and producing antibodies. The lymph nodes also contribute to the proliferation and recirculation of lymphocytes. The morphology of the lymph nodes has always attracted attention due to their important role in the diagnosis of many diseases and some cancer metastases (1-3). Therefore, many investigations have been carried out into the lymphatic system in animals such as goats (4), dogs (5), and gray kangaroos (6), as

well as laboratory animals such as hamsters (7,8), mice and gerbils (7).

Rats have frequently been used as experimental animals, and the lymph nodes of rats are important subjects for researchers (9-12). Studies of the lymph nodes in Wistar rats are limited to the lung and abdominal region and are also focused on histological information (13,14). On the other hand, a number of studies (15-20) have been carried out on the morphometry of the lymph nodes without indicating the rat strain. Therefore, our aim was to provide some useful data on the lymph nodes in Wistar rats for use by researchers.

Materials and Methods

Ten male Wistar rats with a mean weight of 210.79 ± 14.95 g were used. The rats were 6 months old and were obtained from the Laboratory Animal Unit of the Veterinary Physiology Department of Adnan Menderes University. They were anesthetized with ether inhalation, and Evans Blue (2%, w/v) (6) was injected subcutaneously into the intermandibular area, the ventral aspect of the tail, the plantar and palmar surface of each limb, and both sides of the thoracic, abdominal and cervical regions. After 24 h, the rats were lethally anesthetized with an overdose of ether inhalation.

The rats were dissected and the lymph nodes were counted under a stereomicroscope. The longest and widest diameters of the each lymph node were measured with the aid of digital calipers. Data were analyzed statistically by Student's t test.

Results

The measurements and numbers of the lymph nodes are shown in Tables 1 and 2, respectively. In the region of the head and neck, the most prominent nodes in terms of number and size were the mandibular nodes. However, both the cranial and caudal profound cervical nodes were inconstant in this region. The cranial profound cervical nodes numbered 3 on the right and 4 on the left. The caudal profound cervical nodes numbered 5 on the right and 6 on the left.

In the forelimb, the axillary nodes consisted of the axillary nodes proper and accessory nodes. There were more axillary accessory nodes than axillary nodes proper on each side. Moreover, the longest and widest diameters of the axillary accessory nodes were greater than those measured for the axillary nodes proper. In the hindlimb, the number of inguofemoral nodes on each side was

Table 1. The numbers, longest (LD) and widest (WD) diameters of the lymph nodes on the right and left sides.

Lymph nodes	N Right		$\bar{X} \pm S\bar{x}$ Right	N Left	$\bar{X} \pm S\bar{x}$ Left	t
Ln. mandibularis	32	Number	3.20 ± 0.51	29	2.90 ± 0.23	0.74 ^{NS}
		WD	2.28 ± 0.01		2.37 ± 0.10	0.74 ^{NS}
		LD	3.64 ± 0.19		3.70 ± 0.22	0.21 ^{NS}
Ln. cervicalis superficialis	12	Number	1.20 ± 0.13	13	1.30 ± 0.15	1.26 ^{NS}
		WD	1.95 ± 0.12		1.95 ± 0.11	0.44 ^{NS}
		LD	3.70 ± 0.27		3.27 ± 0.18	1.39 ^{NS}
Ln. cervicalis profundus cranialis	3	Number	0.30 ± 0.15	4	0.40 ± 0.16	0.55 ^{NS}
		WD	1.20 ± 0.40		1.51 ± 0.18	0.80 ^{NS}
		LD	2.77 ± 0.81		2.71 ± 0.72	0.05 ^{NS}
Ln. cervicalis profundus caudalis	5	Number	0.50 ± 0.17	6	0.60 ± 0.16	0.47 ^{NS}
		WD	1.45 ± 0.35		1.53 ± 0.23	0.20 ^{NS}
		LD	1.95 ± 0.38		3.10 ± 0.52	1.72 ^{NS}
Ln. axillaris proprii	18	Number	1.80 ± 0.36	13	1.30 ± 0.21	1.43 ^{NS}
		WD	2.27 ± 0.12		2.53 ± 0.19	1.23 ^{NS}
		LD	3.87 ± 0.31		4.29 ± 0.39	0.85 ^{NS}
Ln. axillaris accessorii	17	Number	1.70 ± 0.15	15	1.70 ± 0.26	1.12 ^{NS}
		WD	2.39 ± 0.17		2.50 ± 0.23	0.40 ^{NS}
		LD	4.39 ± 0.52		4.64 ± 0.61	0.02 ^{NS}
Ln. inguofemoralis	16	Number	1.60 ± 0.34	19	1.60 ± 0.34	0.84 ^{NS}
		WD	2.22 ± 0.18		1.89 ± 0.13	1.52 ^{NS}
		LD	4.10 ± 0.45		3.65 ± 0.54	1.60 ^{NS}
Ln. popliteum	10	Number	1.00 ± 0.00	11	1.10 ± 0.01	0.90 ^{NS}
		WD	1.45 ± 0.10		1.87 ± 0.23	1.67 ^{NS}
		LD	2.46 ± 0.29		2.91 ± 0.28	1.12 ^{NS}
Ln. ischiadicum	4	Number	0.40 ± 0.16	6	0.60 ± 0.31	0.92 ^{NS}
		WD	1.96 ± 0.23		1.25 ± 0.14	2.80*
		LD	2.90 ± 0.17		1.94 ± 0.23	3.07*

• $P < 0.05$, NS - not significant.

Table 2. The numbers of lymph nodes in the head, neck, forelimb and hindlimb on the right left sides.

Lymph nodes	Number of lymph nodes											
	1		2		3		4		5		6	
	Right n	Left n	Right n	Left n	Right n	Left n	Right n	Left n	Right n	Left n	Right n	Left n
Ln. mandibularis			5	3	2	5	1	2				2
Ln. cervicalis superficialis	8	7	2	3								
Ln. cervicalis profundus cranialis	3	4										
Ln. cervicalis profundus caudalis	5	6										
Ln. axillaris proprii	3	5	4	4	1		1					
Ln. axillaris accessorius	3	5	7	3		2						
Ln. inguinofemoralis	2	2	4	1	2	5						
Ln. popliteum	10	9		1								
Ln. ischiadicum	4	3				1						

higher than those of the ischiadic and popliteal nodes. The longest and widest diameters were observed on the right inguinofemoral lymph nodes of the hindlimb. However, ischiadic nodes were inconstant and were only found in 4 of the 10 rats. The differences between the longest and the widest diameters of ischiadic lymph nodes on the right and left sides were statistically significant ($P < 0.05$).

Discussion

Some authors have reported that the lymph nodes can easily be distinguished from the adjacent tissues using black India ink (18,19). However, in our experience the lymph nodes cannot be successfully colored using this method. Therefore, we preferred to use Evans Blue (2%, w/v) as reported by Hopwood (6). The weight of the lymph nodes could not be determined, because it is difficult to separate the associated fat tissue completely. There is no nomenclature of laboratory animals and therefore the lymph nodes are named differently by different researchers. In this study, the terminology of Hebel and Stromberg (15) was used, because the lymph nodes reported by these authors were similar in many respects, to those reported here.

The number of lymph nodes observed in the current study was similar to those reported previously (15-19), with the exception of the superficial cervical and axillary nodes proper. Our results are in agreement with those of Hebel and Stromberg (15) and of Miotti (16) with regard

to the number of superficial cervical nodes. Similar results, although only on the right side, with regard to the number of superficial cervical nodes were also reported by Sarsilmaz and Gezici (18). In this study, 1 to 2 superficial cervical nodes were observed on each side in the 10 rats. According to Sarsilmaz and Gezici (18), the axillary nodes proper on the left side numbered 3 in 12 rats, 2 in 5, 6 in 5 and 4 in 3 rats, representing higher levels than in our results. The lengths and the longest diameters of the mandibular, superficial cervical, cranial profound cervical, inguinofemoral and popliteal nodes measured by Sarsilmaz and Gezici (18) were generally greater than those in our study. In this study, the presence of ischiadic nodes was inconstant in Wistar rats. Similar results were also obtained by Hebel and Stromberg (15) and Miotti (16).

When the results of this study were compared with those of Hebel and Stromberg (15), Miotti (16), Sarsilmaz and Gezici (18) and Sarsilmaz et al. (19), a number of differences were observed. These researchers did not record the rat species, and so the differences may depend on species, sex, age and individual variations. It is suggested that similar studies should be performed on other species of rat.

Acknowledgments

The research was supported by The Scientific and Technical Research Council of Turkey (TÜBİTAK-BAYG).

References

1. Ropolo, A., Moron, V.G., Maletto, B., Pistoresi-Palencia, M.C.: Diminished percentage of antigen bearing cells in the lymph nodes of immune aged rats. *Exp. Gerontol.*, 2001; 36: 519-535.
2. Van der Planken, H.J., Hermens, A.F.: Lymphatic metastasis from tumors transplanted into the pre-irradiated footpad of the rat. *Strahlenther Onkol.*, 1999; 175: 32-38.
3. Yamagata, K., Kumyai, K., Shimizu, K., Masuo, K., Nishida, Y., Yasui, A.: Gastrointestinal cancer metastasis and lymphogenous spread: viewpoint of animal models of lymphatic obstruction. *Jpn. J. Clin. Oncol.*, 1998; 28: 104-106.
4. Rahim, S.E.A., Bland, K.P.: The lymphatic drainage of the cranial part of the sheep's uterus and its possible functional significance. *J. Anat.*, 1985; 140: 705-709.
5. de Freitas, V., Zorzetto, N.L., Prates, J.C., Seullner, G.: Experimental study of lymphatico-venous communications after thoracic-duct ligation in dogs. *Anat. Anz.*, 1979; 146: 27-38.
6. Hopwood, P.R.: An investigation of the grey kangaroo (*Macropus giganteus*) 1. The superficial lymphatic system. *J. Anat.*, 1988; 157: 181-195.
7. Kawashima, Y.: The lymph system in rodents. *Jap. J. Vet. Res.*, 1972; 20: 35-43.
8. Miotti, V.R.: Die lymphknoten und lymphgefasse des syrischen Gold hamsters. *Acta Anat.*, 1961; 46: 192-216.
9. Chernyshenko, L.V., Syrtsov, V.K., Chernokulskii, S.T.: The morphofunctional changes in the perivascular and lymphoepithelial nodes of the respiratory organs following antigenic stimulation. *Vrach Delo.*, 1991; 2: 70-73.
10. Dolgova, M.A., Nadiarnia, T.N.: Changes in the lymph nodes of vaccinated rats after prenatal exposure. *Arkh. Anat. Gistol. Embriol.*, 1991; 100: 31-37.
11. Fernandez-Lopez, A., Revilla, V., Condela, M.A., Aller, M.I., Soria, C., Pazos, A.: Identification of beta-adrenoceptors in rat lymph nodes and spleen: An autoradiographic Study. *Eur. J. Pharmacol.*, 1994; 262: 283-286.
12. Rety, F., Clement, O., Siauve, N., Cuenod, C.A., Carnot, F., Sich, M., Buisine, A., Frija, G.: MR lymphography using iron oxide nanoparticles in rats: Pharmacokinetics in the lymphatic system after intravenous injection. *J. Magn. Reson. Imaging*, 2000; 12: 734-739.
13. Marniok, B., Mikusek, J., Rudnichi, P.: Morphology of the abdominal hemolymph nodes in Wistar rats. *Folia Morphol.*, 1997; 56: 237-247.
14. Rosenbruch, M.: Inhalation of amorphous silica: Morphological and morphometric evolution of lung associated lymph nodes in rats. *Exp. Toxicol. Pathol.*, 1992; 44: 10-14.
15. Hebel, R., Stromberg, M.W.: *Anatomy of the Laboratory Rat. The Williams and Wilkins Comp.*, Baltimore, 1976; 112-116.
16. Miotti, V.R.: Die lymphknoten und lymphgefasse der weissen Ratte (*Rattus Norvegicus* Berkenhout, *Epimys norvegicus*). *Acta Anat.*, 1965; 62: 489-527.
17. Sainte-Marie, G., Peng, F.S., Guay, G.: Ectasias of the subcapsular sinus in lymph nodes of athymic and euthymic rats: A relation to immunodeficiency. *Histol. Histopathol.*, 1997; 12: 637-643.
18. Sarsılmaz, M., Gezici, M.: Göğüs ve karın dışındaki rat lenf nodüllerinin sınıflandırılması. *Yeni Tıp Dergisi*, 1992; 9: 7-12.
19. Sarsılmaz, M., Gümüşalan, Y., Çelik, H.H., Gezici, M., Akşit, M.D.: Classification of the thoracic and abdominal lymph nodes of the rat. *Turk. J. Med. Res.*, 1994; 12: 185-190.
20. Ward, J.M., Uno, H., Frith, C.H.: Immunohistochemistry and morphology of reactive lesions in lymph nodes and spleen from rats and mice. *Toxicol. Pathol.*, 1993; 21: 199-205.