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Title: Lipomatosis of Axillary Lymph Nodes in a Cynomolgus Monkey (*Macaca fascicularis*)

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A short running head: Lipomatosis of Axillary Lymph Nodes in a Cynomolgus Monkey

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Abstract: Lipomatosis of lymph nodes is defined as the replacement of the lymphatic parenchyma by adipose tissue which grows in the node from the hilus toward the cortical zone. In humans, it is considered as part of the normal aging process and is common in obese patients, but there are no reports in non-human primates. In this report, we describe the first case of lymph node lipomatosis in the bilateral axillary lymph nodes of a young adult cynomolgus monkey. Macroscopically, there were no apparent abnormalities in the axillary lymph nodes on either side, and their volumes were unchanged. At the cut surface, pale yellow fat-like tissue was observed in the medullary area. Histopathologically, well differentiated adipocytes replaced a large part of the lymphatic parenchyma in the area from the hilus to the medulla without any malignant findings. Based on these findings, the patient was diagnosed with lipomatosis of the lymph nodes.

Key words: lipomatosis, adipocytes, axillary lymph node, cynomolgus monkey, spontaneous lesion

Lipomatosis of lymph nodes is the replacement of lymphatic parenchyma by adipose tissue which grows in the node from the hilus toward the cortical zone^{1,2} and is regarded as a non-malignant change and a part of the normal aging process in humans.^{3,4} Lipomatosis of lymph nodes is also called fatty infiltration,¹ lipomatous atrophy,^{2,5} fatty replacement,⁴ or fatty change.^{5,6} Lipomatosis has been observed in the axillary lymph nodes of aging mice,⁷ but to our knowledge, there have been no reported cases in non-human primates. Here, we describe a case of spontaneously arising lipomatosis of the axillary lymph nodes in a cynomolgus monkey.

A six year old male cynomolgus monkey (*Macaca fascicularis*) of Cambodian origin was purchased from Shin Nippon Biomedical Laboratories, Ltd. Blood was sampled without any test article treatment. No remarkable findings were observed in the clinical observations or blood tests from routine medical examinations, and the final body weight was 6.5 kg. At necropsy, only involution of the thymus was observed. All animal procedures were conducted in accordance with the Chugai Pharmaceutical Guide for the Care and Use of Laboratory Animals, and all experimental protocols were approved by the Institutional Animal Care and Use Committee.

Macroscopically, there were no apparent abnormalities in the axillary lymph nodes on either side, and their volumes were unchanged. Therefore, all organs, including one axillary lymph node from each side, were fixed in 10% neutral-buffered formalin and embedded in paraffin for specimen preparation. After fixation, pale yellow fat-like tissue was observed in the medullary area at the cut surface of both the axillary lymph nodes (Fig. 1).

All tissue sections embedded in paraffin were stained with hematoxylin and eosin (HE), and the sections of the axillary lymph nodes were stained with azan stain using standard methods. Frozen sections were used for Oil Red O staining of the axillary lymph nodes. For immunohistochemical analysis,⁸ the sections of the axillary lymph nodes were subjected to primary antibody against Ki-67 (SP6, rabbit monoclonal anti-Ki-67, Abcam, Cambridge, UK, ab16667) to analyze the proliferative activity of adipocytes. The sections were incubated with goat anti-rabbit secondary antibody (Dako, Glostrup, Denmark, E0432) followed by incubation with avidin-biotin-peroxidase complex (ABC peroxidase standard staining kit,

Thermo Fisher Scientific, Waltham, MA, USA). The reaction was visualized using 3,3-diaminobenzidine tetrachloride. Finally, the sections were counterstained with Mayer's hematoxylin.

On microscopic examination, the medullary lymphatic parenchyma was replaced by adipose tissue-like structures, and the medullary area, including the hilus, expanded in both the axillary lymph nodes (Fig. 2a, b). Adipose tissue-like structures were composed of Oil Red O positive well-differentiated adipocytes, with a single large fat droplet in the cytoplasm and peripherally located nuclei (Fig. 2c). The adipose tissue that mainly occupied the area from the hilus to the medulla was focally located adjacent to the paracortical area, but the adipocytes did not show invasive growth and cellular atypia (Fig. 2d). There were few morphological changes in the cortical area, except for the vague formation of germinal centers (Fig. 2e). The periphery of the axillary lymph node was covered with a fibrous capsule, and intranodal adipocytes did not infiltrate the adjacent adipose tissue beyond the capsule (Fig. 2f). Immunohistochemically, Ki-67 expression was detected in lymphocytes of the lymphoid follicles, but the increased number of adipocytes were negative for Ki-67 (Fig. 3).

In the present case, a large proportion of the medullary parenchyma of the bilateral axillary lymph nodes was occupied by adipose tissue without any malignant findings. This lesion was diagnosed as lipomatosis of the lymph nodes. To confirm whether lipomatosis appeared in other lymph nodes in this monkey, the mesenteric lymph node and pancreatic hilar lymph node were histopathologically examined. Lipomatosis was not observed in these lymph nodes (data not shown). There were no remarkable histopathological findings in other organs or tissues, except for some spontaneous changes.

The lesion in this case is a common finding in aged^{2,5,6} and obese patients^{5,9} and is not a malignant change.^{3,4} It is especially common in peripheral lymph nodes that usually receive little antigen stimulation, such as cubital, axillary, and popliteal nodes.² Bilateral lipomatosis has been reported in the human axillary,¹⁰ cervical, and inguinal lymph nodes.³ Generally, in humans, adipose tissue replaces normal lymphatic parenchyma; therefore, nodal volume shows no change, but in some cases, lymph nodes may increase in volume.^{3,5} The axillary lymph nodes were not enlarged in the present case, but histopathologically, the adipose tissue replaced a large part of the medullary parenchyma. In cynomolgus monkeys, there are no

previously reported lipomatosis cases; however, in a discussion among experts in experimental animal histopathology at an academic conference, a few specialists mentioned finding a similar lesion in the axillary lymph nodes of cynomolgus monkeys used in toxicity studies. They suggested that lipomatosis might be associated with aging in cynomolgus monkeys, although the incidence of similar lesions was low.

Considering the histopathological features of lymph node lipomatosis, a possible differential diagnosis can include lipomatous tumors such as well-differentiated liposarcoma¹¹ and lipoma.¹² However, to our knowledge, there are no reports of primary lipomatous tumors arising in lymph nodes, and the metastatic spread of liposarcoma to regional lymph nodes has rarely been reported in humans.^{13,14} In the present case, lipoblasts and nuclear atypia of adipocytes that are typically found in well-differentiated liposarcoma¹¹ were absent. In addition, a fibrous capsule surrounding proliferative adipocytes, which is one of the common findings in lipoma,¹² was not present. Intranodal adipocytes did not exhibit invasive growth and did not compress adjacent tissue. According to immunohistochemical analysis, the adipocytes were negative for Ki-67, suggesting that these cells lacked proliferative activity. Furthermore, there were no extra-lymphatic primary lipomatous tumors in the cynomolgus monkey. These findings indicate that this lesion is not a primary or metastatic lipomatous tumor.

The site of lesion formation in this case corresponds to the site specificity of lipomatosis of the lymph nodes in humans. This monkey was a healthy young adult¹⁵ and was slightly overweight,¹⁶ which does not completely reflect the findings normally associated with the majority of human patients, such as aging and obesity. However, in humans, lipomatosis of the axillary, popliteal, and cubital lymph nodes can be seen, to some extent, during infancy and childhood, with a comparatively higher rate in axillary lymph nodes.² Similar to humans, lipomatosis of lymph nodes may occur in cynomolgus monkeys at an early age in a site-specific manner, particularly in regions that are rarely invaded by antigens, for example, axillary lymph nodes. The origin of adipocytes observed in lipomatosis was suspected to be the reticular cells in lymph nodes,^{3,17} although the relationship between adipocytes and reticular cells is still under discussion.^{18,19} In the present case, adipocytes existed completely inside the lymph node capsule, similar to previously reported human and rodent lipomatosis cases,^{7,20} which means that the original cells were present in the lymph nodes. Although

the pathogenesis of lipomatosis of lymph nodes is poorly understood, reticular cells may be responsible for this lesion.

In conclusion, the lymphatic parenchyma of both the axillary lymph nodes was replaced by adipose tissue without any malignant findings in a young adult cynomolgus monkey, and this lesion was diagnosed as lipomatosis of the lymph nodes. This is the first reported case of lipomatosis of lymph nodes in a cynomolgus monkey, and it is unclear whether lipomatosis is associated with aging and obesity or whether it occurs in a site-specific manner in this species, as in humans. More cases must be investigated to fully understand the etiology of lipomatosis of the lymph nodes in cynomolgus monkeys.

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Figure legends

Fig. 1. The macroscopic finding of axillary lymph node on one side. On the cut surface, a pale yellow fat-like tissue was present in medullary area. The nodal volume showed no change. Bar = 2 mm

Fig. 2. The microscopic findings of axillary lymph nodes (a) The histopathological image corresponds to the cut surface of Fig. 1. The adipose tissue replaced large part of normal medullary lymphatic parenchyma. The medullary area including hilus was expanded by adipose tissue. The circles on the figure with letters “c”, “d”, and “e” indicates the display region in Fig. 2c, d, and e, respectively. The area labeled “f” is equivalent to the Azan-stained area in Fig. 2f. HE stain. Bar = 1 mm. (b) The adipose tissue was also observed in the medullary area of the contralateral axillary lymph node. HE stain. Bar = 1 mm. (c) The intranodal adipose tissue was composed of well-differentiated adipocytes. HE stain. (inner box: Positive staining for Oil Red O demonstrated that these cells were adipocytes.) Bar = 100 μ m. (d) In a part of the lesion, the adipose tissue adjacent to the paracortical area without invasive growth and cellular atypia. Arrowheads point at high endothelial vein located in paracortical area. HE stain. Bar = 100 μ m. (e) Few morphological changes were seen in cortical area, although germinal center was obscure. HE stain. Bar = 250 μ m. (f) The axillary lymph node was covered with the fibrous capsule and intranodal adipocytes did not invade adjacent adipose tissue beyond the capsule. Azan stain. Bar = 100 μ m.

Fig. 3. Intranodal adipocytes were negative for Ki-67 immunohistochemical staining. (inner box: Lymphocytes of the lymphoid follicle were positive for Ki-67.) Bar = 100 μ m.

Fig. 1

Fig. 2

Fig. 3