Pathological Features of Salivary Gland Cysts in a Shiba Dog with GM1 Gangliosidosis: A Possible Misdiagnosis as Malignancy

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ABSTRACT. Salivary gland cysts are often concurrent with GM1 gangliosidosis in Shiba dogs. Although the etiology is unknown, these cysts may be misdiagnosed as malignant due to the accumulation of foamy cells. The present study investigated the cytological, histopathological, immunohistochemical and electron microscopic characteristics of salivary gland cysts in a Shiba dog affected with GM1 gangliosidosis. The salivary gland masses were surgically enucleated and examined clinicopathologically and pathologically in a 7-month-old Shiba dog with GM1 gangliosidosis. Many large cells with rich cytoplasm including vacuoles of various sizes, i.e., foamy cells, were observed in stamp smears from the cut-surface of the masses and histopathologically in major parts of the cyst wall. Some of these foamy cells presented features similar to a spider-web appearance. The foamy cells were confirmed to have originated from macrophages based on marked immunohistochemical expression of vimentin, HLA-DR, lysozyme and Iba1. An ultrastructural study demonstrated electron-dense vesicular structures in the vacuolated cells. Therefore, the masses were diagnosed pathologically as benign salivary gland cysts with accumulation of foamy cells. In conclusion, the histopathological features of the salivary gland cysts in this Shiba dog were similar to those of lipoma and/or liposarcoma. In such cases, immunohistochemical and ultrastructural examinations were useful in the differential diagnosis. Practitioners, clinical pathologists and pathologists should take GM1 gangliosidosis into consideration when they encounter salivary gland cysts in Shiba dogs.

KEY WORDS: canine GM1 gangliosidosis, foamy cell, ranula, salivary gland cyst, Shiba Inu.


GM1 gangliosidosis, a lysosomal storage disease that affects the brain and multiple systemic organs, is due to an autosomal recessively inherited deficiency of acid β-galactosidase encoded by the GLB1 gene [19]. GM1 gangliosidosis in Shiba dogs was first reported in 2000 [27]. The homozygous recessive mutation causing GM1 gangliosidosis in Shiba dogs has been identified as a deletion of C nucleotide 1647 (c.1647delC) in the putative coding region for the canine GLB1 gene [21], allowing diagnosis with polymerase chain reaction (PCR)-based DNA tests [2, 24], as well as an enzyme assay using leukocyte or tissue specimens [23, 25]. Affected Shiba dogs manifest neurological symptoms of progressive motor dysfunction starting from 5 to 6 months of age and finally die by approximately 15 months of age after a clearly defined clinical course [9, 20, 26, 27], which is associated with the progressive accumulation of GM1 ganglioside in the central nervous system [16, 27] and cerebrospinal fluid [15–17, 27]. The carrier frequency of the disease was estimated to be approximately 3% based on a preliminary genotyping survey carried out in northern Japan [22]. To date, the disease has been diagnosed definitively in at least 17 Shiba dogs as widely distributed sporadic cases in Japan [10].

Salivary gland cysts (SGCs) are often concurrent with GM1 gangliosidosis in Shiba dogs but the etiology remains unknown. Although the incidence has not been accurately determined, about half of Shiba dogs affected with GM1 gangliosidosis seem to develop SGCs bilaterally beneath their tongues, and in some cases, SGCs arose even before the onset of neurological signs related to GM1 gangliosidosis. In such cases, immunohistochemical and ultrastructural examinations were useful in the differential diagnosis. Practitioners, clinical pathologists and pathologists should take GM1 gangliosidosis into consideration when they encounter salivary gland cysts in Shiba dogs.

SGC is a collection of saliva that has leaked from a damaged salivary gland or duct and follows the path of least resistance [6, 13]. The most frequent collection sites of extravasated saliva are sublingual tissues and the intermandibular or cranial cervical area. A less common site is the pharyngeal wall. The sublingual SGC is also called ranula (rana meaning frog and ula meaning little) because the swelling resembles the vocal or air sac of the frog. In general, differentiation between benign SGCs and malignant tumors is difficult in clinical practice. Therefore, histopathological examinations are required for the differential diagnosis [1, 6].
The present study was conducted to investigate the cytological, histopathological, immunohistochemical and electron microscopic characteristics on the SGCs in a Shiba dog affected with GM1 gangliosidosis to find an approach for the accurate diagnosis.

A male Shiba dog that developed bilateral salivary gland masses at 3 months of age, started to exhibit neurological signs such as ataxic gait and tremor at 5 months of age and was referred to an animal hospital for evaluation of neurological disorders. Since GM1 gangliosidosis was strongly suspected based on the signalment and clinical signs, PCR-based genotype test [2] was carried out resulting in the establishment of a diagnosis of GM1 gangliosidosis. At 7 months of age, these salivary gland masses were surgically enucleated since they had become an obstacle to eating (Fig. 1). Stamp smear specimens were obtained from the cut-surface of the masses and stained with Giemsa. The masses were fixed in 10% phosphate-buffered formalin (PBF) at room temperature, embedded in paraffin, sectioned, and examined histopathologically. At 12 months of age, the dog died spontaneously due to progressive central nervous system disorders but necropsy was not performed. Sections of the SGCs were routinely stained with hematoxylin and eosin (HE), and histochemically by alcian blue-periodic acid-Schiff (AB-PAS). A part of the masses fixed in 10% PBF, was embedded in OCT compound (Sakura Finetek Japan, Tokyo, Japan) and frozen at −80°C, then sectioned at 10 µm, stained histochemically by Sudan III and counterstained with hematoxylin.

For immunohistochemistry, sections were deparaffinized in xylene and rehydrated through a graded alcohol series in accordance with a reported procedure [7]. As primary antibodies, anti-vimentin monoclonal antibody (clone Vim 3B4, 1:200) (Dako Cytomation Japan, Kyoto, Japan), anti-HLA-DR α monoclonal antibody (clone TAL.1B5, 1:1,000) (Dako Cytomation Japan), anti-lysozyme polyclonal antibody (1:2,000) (Dako Cytomation Japan), anti-Iba1 polyclonal antibody (1:250) (Wako Pure Chemical Industries Ltd., Osaka, Japan), anti-cytokeratin monoclonal antibody (clone AE1/AE3, 1:50) (Dako Cytomation Japan), and anti-S100 polyclonal antibody (1:1,000) (Dako Cytomation Japan), were used. The sections treated with each primary antibody were incubated with the EnVision polymer reagent (Dako Cytomation Japan). Immunoreactivity was visualized with 0.075% 3,3’-diaminobenzidine tetrachloride. The sections were then washed, counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted.

For electron microscopy, tissues fixed in 10% PBF were cut into small pieces and thoroughly washed in 0.1 M cacodylate buffer (CB, pH 7.4), then fixed in 2.5% glutaraldehyde in CB. After being washed in CB, specimens were post-fixed in 1% osmium tetroxide in CB, then dehydrated through a graded ethanol series. After being routinely embedded in Quetol 812 (Nissin EM, Tokyo, Japan), ultrathin sections stained with uranyl acetate and modified Sato’s lead were observed using a transmission electron microscope (H-7000KU, Hitachi, Tokyo, Japan).

Grossly, the cysts appeared as bilateral masses (left: approximately 3 × 2 cm; right: approximately 3 × 3 cm), were pale red in color and contained a little translucent mucus (Fig. 1). The thickness of wall measured 1–5 mm. Stamp smear of the enucleated mass showed many large cells with rich cytoplasm containing vacuoles (Fig. 2).

As shown in Fig. 3, histopathologically, many large cells with rich cytoplasm including a single clear vacuole (like lipocyte) or various sized vacuoles (like foamy cell) were observed in major parts of the cyst wall. Some of the large foamy cells presented the features of spider-web appearance similar to those in liposarcoma cells and lipoblasts. The nuclei showed slight atypism such as irregular in size while there were no mitotic figures in the tissue. The nuclei were also irregular or oval shape and there were no
multinucleated giant cell such as Langhans cell suggesting that these cells were different from histiocytosis. Spindle cell proliferation was also recognized around the large cell accumulation. Infiltrations of a few inflammatory cells including neutrophils were also observed in a part of the wall. No secretory epithelial linings were found in the cyst wall.

The immunohistochemical and histochemical results are summarized in Table 1. The developed foamy cells were strongly positive for vimentin, HLA-DR (Fig. 4a), lysozyme (Fig. 4b) and Iba1 (Fig. 4c), but negative for cytokeratin and S-100. Sudan III was slightly positive (Fig. 4d) in a part of the vacuoles of foamy cell cytoplasm suggesting that some vacuoles contained triglyceride. AB-PAS staining was negative in the vacuoles, suggesting that the vacuoles containing materials were neither glycogen nor mucus. The spindle cells around the large cell accumulation were strongly positive for vimentin, HLA-DR and lysozyme, but negative for Iba1, cytokeratin and S-100. The immunohistochemical and histochemical features of the wall of the salivary gland cyst in a Shiba dog affected with GM1 gangliosidosis. (a–c) Immunohistochemical characteristics of the salivary gland cyst wall with antibodies against (a) HLA-DR, (b) Lysozyme and (c) Iba1. (d) Histochemical features of the salivary gland cyst wall. Note that Sudan III was slightly positive in the vacuoles of foamy cell cytoplasm. Bars=20 μm.
rounded to oval electron-dense vesicles and some of these cells observed by light microscopy. These structures contain membrane bound electron-lucent structures in the foamy findings remain to be resolved. Inflammation although the precise mechanisms of these suggests that the spindle cells may be fibroblasts in chronic tochemical result together with the cellular morphology suggests that the spindle cells may be fibroblasts in chronic inflammation although the precise mechanisms of these findings remain to be resolved.

Transmission electron microscopic examination showed membrane bound electron-lucent structures in the foamy cells observed by light microscopy. These structures contain rounded to oval electron-dense vesicles and some of these structures fused each others’ (Fig. 5).

To the authors’ knowledge, there are no reports describing the relationship between SGCs and neurodegenerative diseases such as GM1 gangliosidosis in either humans or animals. In general, the categorical causes of SGCs are not identified in veterinary medicine, but blunt trauma, foreign body and sialolith are suspected to be major causes [1]. There is one canine case in which dirofilariasis was suspected as the cause [14]. In the present study, although an underlying etiology of the concurrent development of SGCs with GM1 gangliosidosis was not identified, self-biting due to the neurologically impaired function affecting eating movement can cause blunt trauma around the salivary gland ducts. The cells of the salivary gland duct swollen by lysosomal storage materials may narrow and block the ducts of the salivary glands. Another hypothesis is that this type of SGC may be based on an unknown gene that is present on the same allele as the pathogenic mutation of GM1 gangliosidosis in Shiba dogs. In previously reported cases, SGCs developed more frequently in dogs than in cats and there is a slight breed predisposition [11]. All dog breeds are susceptible to SGCs but it seems to be more common in Poodles, German shepherds, Dachshunds and Australian silky terriers [6]. These reports suggest the contribution of genetic background in SGC development.

Diagnosis of SGCs is mainly based on clinical signs, history and results of paracentesis [18]. Sialography is available to confirm the diagnosis [4, 5]. Histopathologically, the diagnosis of SGCs can be confirmed using a mucus-specific stain method such as PAS [1, 4]. For the differential diagnosis of salivary gland masses including benign cysts and malignant tumors, histopathological examination is required [1, 4, 6, 12]. In the present case, benign SGCs were suspected clinically, but the large foamy cells in the stamp smear and pathological sections are so similar to liposarcoma cells and lipoblasts that it might mislead practitioners, clinical pathologists and pathologists.

Immunohistochemically, the foamy cells were confirmed to have originated from macrophages based on the strongly positive expression of vimentin, HLA-DR, lysozyme and Iba1 [8] and negative expression of S-100 [3]. Lysosome-like appearance and a small amount of triglyceride were confirmed in a part of the cytoplasmic vacuoles of the foamy cells. Ultrastructurally, electron dense vesicular structures were evident in the cytoplasmic vacuoles of the foamy cells. These electron dense vesicular structures are thought to be complex lipids such as glycolipids rather than simple triglyceride or cholesterol since the complex lipids were insoluble in organic solvents used in this study. Therefore, it seems likely that the abnormally accumulated glycolipid activate macrophages to induce inflammatory response. The macrophages later turned into foamy cells by the phagocytosis of abnormal lipids induced by GLBI gene mutation. In addition, saliva leak from the salivary glands and ducts accumulate in the adjacent tissues. Consequently, the accumulated saliva may also participate to the inflammatory reactions. Accordingly, salivary gland masses in the dog were diagnosed as benign SGCs with accumulation of foamy cells in association with GM1 gangliosidosis.

In conclusion, the results of this study indicate that the histopathological features of SGCs in canine GM1 gangliosidosis were similar to those of lipoma and/or liposarcoma. In such cases, immunohistochemical and ultrastructural examinations were useful in the differential diagnosis. In addition, practitioners, clinical pathologists and pathologists should take GM1 gangliosidosis into consideration when they encounter this type of SGC in Shiba dogs.

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