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Immunophenotypic characterization of mixed type gingival vascular hamartoma in a calf - a case report

Amir Amniattalab[1]*, Seifollah N. Dehghani[2], Alireza Najafpour[3], Abdollah Araghi-Sureh[3], and Morteza Kalbkhani[3]

[1]*Department of Pathology, Veterinary Faculty, Urmia Branch, Islamic Azad University, Urmia, Iran*

[2]*Department of Surgery, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

[3]*Department of Clinical Sciences, Veterinary Faculty, Urmia Branch, Islamic Azad University, Urmia, Iran*

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ABSTRACT

A one month-old heifer calf affected by gingival vascular hamartoma, of measured mass 3×2.5×2 cm is presented. This gingival vascular hamartoma was a mixture of redundant vessels ranging from capillaries or capillary buds and large arteries separated by edematous and loose fibrostroma. The results of Immunohistochemistry showed strong expression of the alpha smooth muscle Actin, weak reaction of the Von Willebrand factor and negative reaction of Vimentin, CD31, Ki67 and PCNA in endothelial cells, suggesting vascular hamartoma.

Key words: calf, gingival vascular hamartoma, immunohistochemistry

Introduction

Hamartomas are disorganized in structure but mature mesenchymal or epithelial tissues found in their normal anatomic location (KUSEWITT and RUSH, 2007). There are a focal malformation that resembles a neoplasm but does not show any particular tendency towards neoplastic evolution (SHARMA, 1987). Congenital lesions of vascular tissue are rare in animals (WILSON, 1990). Vascular hamartoma is considered to be a congenital, non-neoplastic tumor-like anomaly of vascular origin that is present at birth and grows until puberty. It is characterized by an excessive focal over growth of mature endothelial

*Corresponding author:

Amir Amniattalab, DVM, Ph.D, Assistant Professor, Department of Veterinary Pathology, Islamic Azad University, Urmia, P.O. Box 969, Iran, Phone: +98 91 444 14613; Fax: +98 441 336 7494; E-mail: a.amniattalab@iaurmia.ac.ir

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cells with disorderly arrangement and imperfect differentiation (BENOIT et al., 2005). It is very hard to differentiate haemangioma from vascular hamartoma. The only way to clarify these two impairments (haemangioma vessels from hamartoma) is to classify the pathological features. In haemangioma the pathologic cross sections present the detrimental endothelial cells and absence of muscular structure in the connective tissue of the vascular wall. Meanwhile, in vascular hamartoma, the endothelial cells of vessels are intact and they are well-differentiated with irregular size and proliferation. Moreover, pericytes locate in primitive capillaries environments (GUALTIERI et al., 2009; BILDFELL et al., 2002). One of the differences between haemangioma and vascular hamartoma is the greater cellularity and enlargement in haemangioma than in vascular hamartoma. Also, unlike haemangioma, hamartomatous growth is harmonic with the host growth (JOHNSON et al., 1996). A gingival vascular hamartoma in a calf has already been reported previously. Since it is difficult to distinguish the difference between vascular hamartoma and haemangioma, in this report Histopathology and Immunohistochemistry have been used together to confirm the diagnosis.

Case presentation

A 1-month-old female cross-bred Holstein calf was referred to the Veterinary Clinic of Urmia Islamic Azad University. Gross examination of the oral cavity revealed a reddish pink, firm lobulated mass that was granular on palpation, located in the vestibular surface of the mandibular central incisor teeth on the gingiva and measuring 2×2×1 cm in dimension on the 1st day of hospitalization. The surface of the mass was ulcerated in a few spots. The mass was growing so that its dimensions changed to 3×2.5×2cm on the 5th day of hospitalization (Fig.1). The mass was removed surgically under general anesthesia and the calf recovered without any complication or recurrence of the growth of the mass. The tissue sample was placed in 10% buffer formalin for fixation. Histopathologic sections were prepared after trimming and processing the tissue. The sections were cut into 5µm thickness and were stained with Haematoxylin and Eosin (H&E). However, the sections were also stained by 1% Toluidine blue for evaluation of the mast cell infiltration rate in the vascular mass stroma. The procedure of immunohistochemical staining was performed on the basis of the standard protocol of the manufacturer using the EnVision^{+} Dual Link System-HRP (horseradish peroxidase) staining technique. The tissue sections were placed in Tris-EDTA (ethylenediaminetetraacetic acid) buffer (TBS, pH 9.0) and immersed by 3% H_2O_2 in methanol for 10 minutes for quenching the endogenous peroxidase. Then the slides were transferred into a microwave for antigen retrieval. In order to block the sections, they were incubated in TBS containing 5% bovine serum albumin (BSA) for an hour (ALKAFAFY et al., 2010). The primary antibodies used included: polyclonal rabbit anti-human von Willebrand factor (vWF) (Dako, USA), monoclonal mouse anti-human CD31 (Clone: JC70A, Dako,USA), monoclonal mouse anti-cow vimentin (Clone: Vim

3B4, Dako, USA), mouse anti-human alpha smooth muscle actin 1A4 (Dako, USA), monoclonal mouse anti-human ki67 antigen (Clone: MIB-1, Dako, USA), monoclonal mouse anti-proliferating cell nuclear antigen (PCNA) (Clone PC10, dilution 1 : 200, Dako, Denmark), as the marker panel. All the primary antibodies were ready-to-use (RTU) and did not require dilution, except PCNA. After washing by TBS for 5 minutes and incubating in a humid room, the sections were coated with labeled polymer using peroxidase (secondary antibody). For staining of the tissue surfaces, they were coated with chromogen plus 3, 3-diamino benzidine (DAB plus) substrate solution (1 drop chromogen in 1 mL substrate) and incubated in a humid room with a self-regulated temperature of 37 ºC for 10 minutes. Other procedures were performed routinely, the sections were counterstained with Harris haematoxylin for 1 minute and the slides mounted with mounting media.

Results and discussion

Microscopically, the mass consisted of a proliferation of multiple, variably-sized, blood-filled vessels, which were located in loose connective stroma (Fig. 1). The walls of the vessels were made of a row of spindle to ovoid shaped endothelial cells, and there were thrombi in some vessels (Fig. 1). The nuclei of the endothelial cells were uniform and no nucleus polymorphism or mitotic figures were observed. Necrosis and hemorrhage were observed in the squamous epithelium and the close-to-surface layer of lamina propria (Fig. 1). The focal aggregation of inflammatory cells was in the superficial layer of the lamina propria, diffused throughout the whole mass of the stroma and dominated by neutrophils. There was slight infiltration of mast cells in the mass stroma in the sections stained by toluidine blue (0-1) (Fig. 1).

Results of immunohistochemical markers showed: 1) Expression of vWF in cytoplasm of vascular endothelial cells was weak and expression of CD31 was negative (Fig. 2). 2) The strong expression of alpha smooth muscle actin by the endothelial cells of the vascular structures, especially in larger vessels, was normal in amount and orientation of smooth muscle bundles (Fig. 2). 3) There was a negative vimentin reaction in the cytoplasm of the endothelial cells. 4) Ki67 and PCNA showed negative reactivity in the nucleus of the vascular endothelial cells. The findings of this gingival lesion suggested its hamartomatous nature, therefore a diagnosis of vascular hamartoma was confirmed.

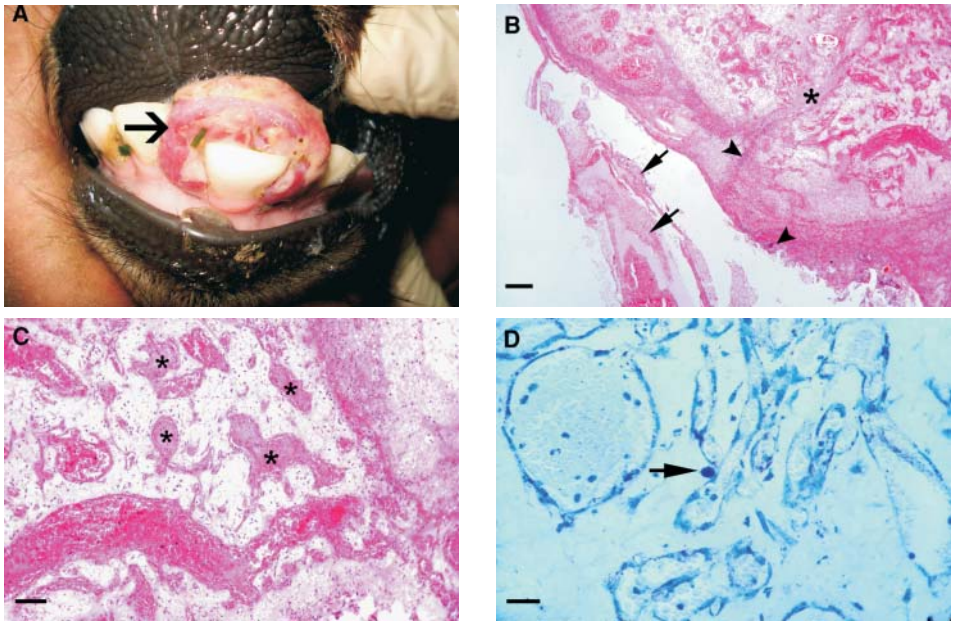

Fig. 1. (A) Macroscopic appearance of vascular mass on gingiva in a calf. Scale bar = 1 cm. (B) Redundant vessels separated by edematous (asterisk) and loose fibrostroma. Necrosis, hemorrhage (arrows) and infiltration of inflammatory cells (arrowheads). H&E, scale bar = 400 µm. (C) Thrombosis in some vessels in vascular mass (asterisks). H&E, scale bar = 200 µm. (D) Slight infiltration of mast cells in vascular mass stroma (arrow). Toluidine blue, scale bar = 50 µm.

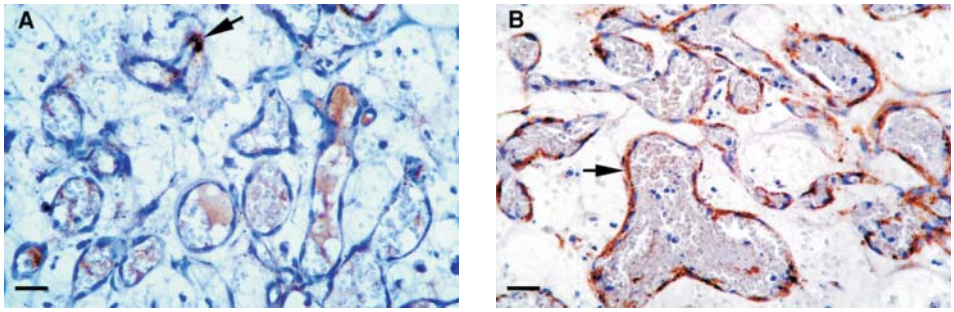

Fig. 2. Labeling of endothelial cells of the vascular mass by immunohistochemical markers. (A) Weak expression of vWF in some of the endothelial cells (arrow). (B) Strong cytoplasmic immunoreactivity for alpha smooth muscle actin (arrow). Immunohistochemistry; scale bars = 50 µm.

The use of Vimentin in soft tissue tumors has diagnostic limits, therefore it is used as a control antibody to evaluate whether the tissue preserving and processing are desirable or not. Factor VIII -related antigen or von Willebrand factor (vWF) is a very large polymeric protein that is synthesized only in endothelial cells and megakaryocytes. Packing of vWF is within the Weibel-Palade bodies (WPBs) in the endothelial cells. Therefore ultrastructural analysis revealed better synchronization of the existing organelles with its immunoreactivity. Since WPBs are poorly differentiated in the neoplasm of the blood vessels, the sensitivity of vWF is approximately 10% to 15% for diagnosis of a high-grade angiosarcoma. Obviously vWF is a marker for the diagnosis of benign and borderline endothelial tumors, such as haemangioma variants and haemangioendotheliomas (WICK and HORNICK 2010). In this calf, the immunoreactivity of the vWF was very weak in the vascular endothelial cells, and since there were negative results for Vimentin, Ki67 and PCNA, the hamartomatous nature of this vascular lesion was confirmed.

The Ki67 antigen is of major importance for the definitive diagnosis of hemangiosarcoma, since this antigen is a nuclear protein expressed in all of the active phases of the cell cycle (G_1, G_2, S, M), but not in other cells (G_0) (WEGGE et al., 2009). PCNA has a relatively long half-life of 20 h and may be immunohistochemically detected in cells that have recently left the cell cycle, and may be in G0 (LEONG, 1999). The platelet/endothelial cell adhesion molecule CD31 (PECAM-1) is a transmembrane glycoprotein, expressed in platelets, monocytes and endothelial intercellular junctions. CD31 has an extracellular domain that mediates endothelial cell to-cell contact and a cytoplasmic domain with potential sites for phosphorylation after cellular activation, and it has been proposed that it is involved in interactive events during angiogenesis. CD31 expression by tumor cells may therefore represent a selective advantage for tumor progression and invasion (WICK and HORNICK, 2010). It has been suggested that mast cells play a role in the production of angiogenic factors that regulate the growth of the vascular lesion (SANGUEZA and REQUENA, 2003). Histamine has been shown to promote vascular smooth muscle cell growth (CHESTER, 2002). The light infiltration of mast cells in this mass and also the negative reactivity of Ki67, PCNA and CD31 in this case confirmed the benign and non-proliferative characteristics of this vascular lesion (OSOFSKY et al., 2004). The strong expression of alpha smooth muscle actin by the endothelial cells from all the vascular lesions reflects the contractile ability of these cells (SABATTINI and BETTINI, 2009). Strong alpha smooth muscle reactivity was observed in the endothelial and smooth muscle cells in the walls of most of the vessels in this vascular mass. The majority of reports of hamartomas in animals are about vascular hamartoma (STAROST, 2007; SAIFZADEH et al., 2006). Vascular hamartomas have been previously reported in cattle in the testis, liver, ovary, heart and gingiva (SUGIYAMA et al., 2007). Sugiyama et al classified three types of vascular hamartomas. Gingival vascular hamartomas in a calf reported previously were type 3. Type 2 was seen in the testis of cattle, the skin of goats

and dogs and in the brain of a dog (SUGIYAMA et al., 2007). In this report, a new type of gingival vascular hamartoma was diagnosed, since it was a mixture of types 2 and 3, that can be described as redundant vessels ranging from capillaries or capillary buds to large arteries, separated by edematous and loose fibrostroma. Furthermore, this study reports the immune-labeling of this type of gingival vascular hamartoma.

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SAŽETAK

Prikazan je slučaj gingivo-vaskularnog hamartoma velikog 3×2,5×2 cm u jednomjesečnoga ženskog teleta. Hamartom se sastojao od mješavine krvožilja, od kapilara do nezreloga kapilarnog tkiva i velikih arterija odvojenih edematoznom i rahlom fibrostromom. Rezultati imunohistokemije pokazali su jaku izraženost aktina alfa-glatkih mišića, slabu reakciju Von Willebrandovog faktora i negativnu reakciju na vimentin, CD31, Ki67 i PCNA u endotelnim stanicama, što upućuje na hamartom.

Ključne riječi: tele, gingivo-vaskularni hamartom, imunohistokemija