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ORIGINAL ARTICLE

GLUT-1 in oral benign vascular lesions

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AIM: To investigate the accuracy of histological diagnosis of oral hemangioma, oral vascular malformation and oral pyogenic granuloma according to immunohistochemical evaluation of the human erythrocyte-type glucose transporter protein (GLUT-1), and to observe the immunoexpression of this protein in oral varix.

MATERIALS AND METHODS: Immunohistochemistry for GLUT-1 was performed in 93 histologically diagnosed cases of oral benign vascular lesions: 17 vascular malformations, 19 hemangiomas, nine varix, and 48 pyogenic granulomas. Descriptive analyses were performed.

RESULTS: None of the cases of the oral benign vascular lesions evaluated were immunopositive to GLUT-1. The 19 cases histologically diagnosed as oral hemangioma that showed negative staining to GLUT-1 were reclassified as oral pyogenic granuloma or oral vascular malformations. The histological evaluation itself is not enough to obtain the correct diagnosis of oral HEM as none of the sample cases were true hemangioma. All sample cases with initial vascular malformation or pyogenic granulomas classification were negative to GLUT-1, demonstrating the accuracy of histological diagnosis of these lesions itself. Oral varix showed negative staining to GLUT-1 in blood vessels.

CONCLUSIONS: GLUT-1 is an useful, effective and important auxiliary marker for the diagnosis of oral benign vascular lesions.

CLINICAL RELEVANCE: This study showed that histological diagnosis alone is not sufficient to correct diagnoses of oral hemangioma. Moreover, immunohistochemistry to GLUT-1 is a useful and easy diagnostic method that may be used to avoid such misdiagnosis. Accurate diagnosis of these oral lesions has an important clinical relevance allowing: (1) correct management, (2) adequate communication among the multidisciplinary team (dentist, dermatologist, pediatrist, radiologist, pathologist, and surgeon), (3) understanding of the biological behavior of the lesions, and (4) facilitate the development of new therapeutic modalities. Thus, supporting the use of this marker in medical and dentistry communities is warranted.

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Introduction

Benign vascular lesions are widely studied in the medical literature, but their classification and nomenclature are divergent. In 1996, the International Society for the Study of Vascular Anomalies approved a classification system modified from Mulliken and Glowacki (1982). Vascular diseases were subdivided into (1) tumors: hemangioma (HEM), pyogenic granuloma (PG), rapidly involuting congenital hemangioma (RICH), noninvoluting congenital hemangioma (NICH), hemangiopericytoma, tufted angioma and kaposiform hemangioendothelioma; and (2) vascular malformation (VM) (Enjolras and Mulliken, 1997). HEM, VM, PG and varix (VAR) are benign lesions with a vascular component and are common in the head and neck regions (Southam and Ettinger, 1974; Finn *et al*, 1983; Epivatianos *et al*, 2005). HEM, VM and PG have distinct clinical evolution; however, histological similarities may be found as well (North *et al*, 2000; Leon-Villapalos *et al*, 2005).

Hemangioma is a red macula, mass or swelling that develops during late fetal stages or in infancy, which grows quickly and generally presents spontaneous regression. HEM has three different phases: proliferating, involution, and involuted. The proliferating phase (0–1 year of age) corresponds to increased activity and proliferation of endothelial cells with organization of masses showing vascular lumens or not. The involution phase (1–7 years of age) is characterized by an initial maturation of blood vessels with dilatation of the vascular lumen and decreased cellular activity. The involuted phase is a final maturation of the lesion with a few tiny capillary-like feeding vessels and draining veins

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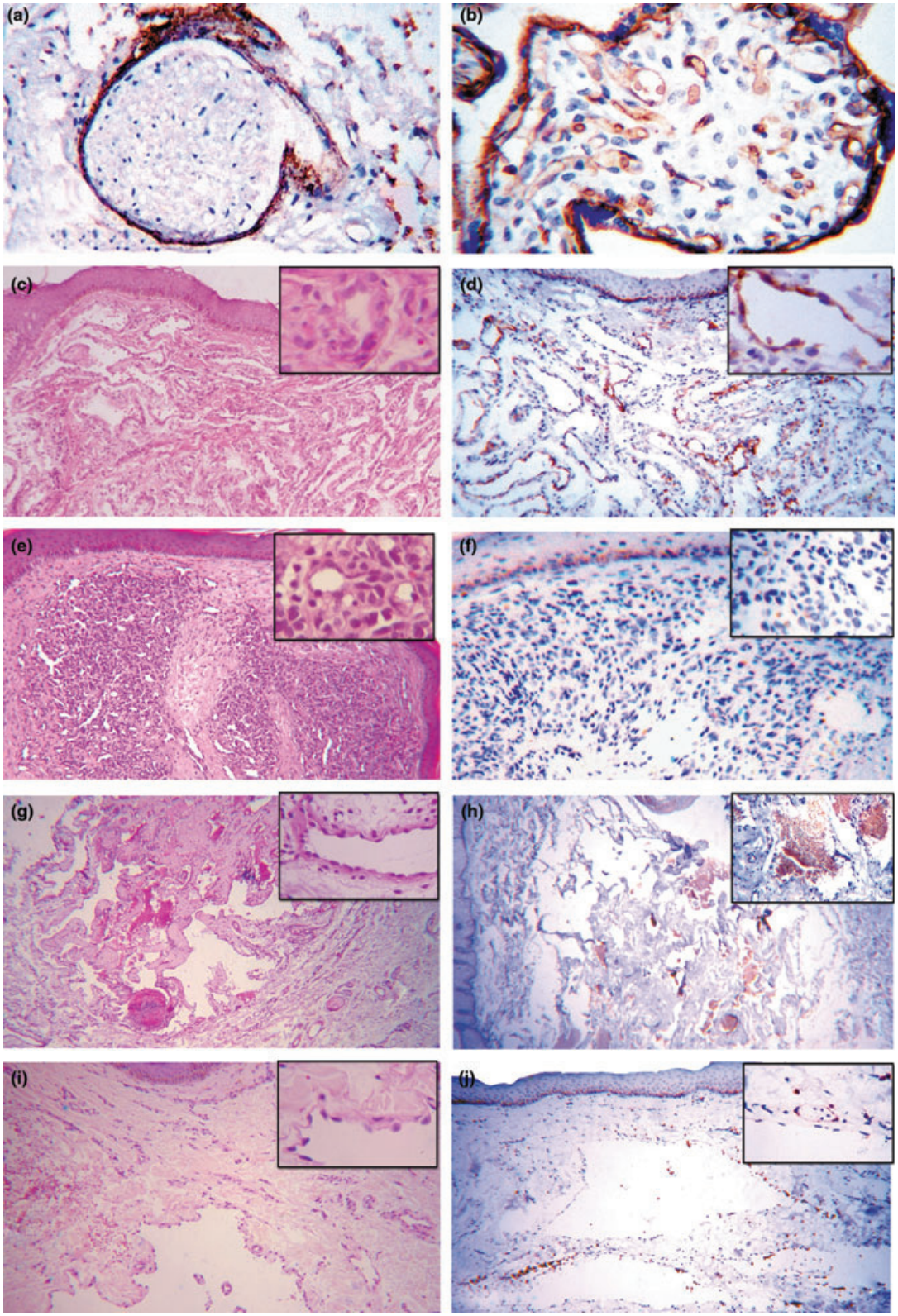

lined with flat mature endothelium (Mulliken and Young, 1988; Enjolras and Mulliken, 1997).

Vascular malformation is an abnormality that occurs during embryonic development, probably caused by disturbance of the signal factors that regulate vascular embryogenesis. VM may be composed of capillaries, veins, arteries, or a combination of these. Mutated genes have been identified as a cause of specific inherited forms

of venous, arteriovenous and capillary-venous malformations (Vikkula *et al*, 2001). VM appears at birth and grows in proportion to the development of the patient. VM consists of tortuous blood vessels lined by a continuous endothelium. HEM, in the involuted phase, can present histological similarities with VM (North *et al*, 2000; Leon-Villapalos *et al*, 2005).

Varix is an acquired benign lesion which is related to alteration of the supportive tissue of the vessel or consequence of an alteration of blood flow, and it is constituted by an extensive and tortuous abnormal vein (Southam and Ettinger, 1974). VAR is characterized as a red to purple papule or nodule, commonly found on the tongue, lip or cheek, mainly in the seventh decade of life (Neville *et al*, 2002).

Pyogenic granuloma is an inflammatory proliferation of capillary blood vessels. It is characterized as a pink to purple, smooth or lobulated, sessile or pedunculated mass, which does not regress spontaneously and bleeds easily (Epivatianos *et al*, 2005). HEM with inflammation present histological similarity with PG (Leon-Villapalos *et al*, 2005).

The histological differential diagnosis among HEM and VM or PG may be difficult. HEM in the involuted phase can present histological similarities with VM, and HEM with inflammation can present histological similarities with PG (North *et al*, 2000; Leon-Villapalos *et al*, 2005). Thus, this nosologic difficulty has promoted the necessity of finding an auxillary marker for HEM: the human erythrocyte-type glucose transporter (GLUT-1).

GLUT-1 is a member of one of 14 glucose-transport type proteins: GLUT-1 to GLUT-12, HMIT–H+– coupled with the *myo*-inositol transporter and GLUT-14 (Wu and Freeze, 2002). GLUT-1 is a protein constitutively found in the perineurium, microvessels of the brain (blood–brain barrier), germinal centers of lymphoid tissues, eyes, placenta, erythrocytes, fetal membranes, and renal tubules (Younes *et al*, 1997). North *et al* (2000, 2001a) identified GLUT-1 as being a specific and sensible immunohistochemical marker of skin HEM in all phases. Others studies verified positive GLUT-1 immunostaining on the HEM of the skin, chorion, liver, mammary and submaxillary glands, and genitalia, as well as in isolated cases of oral mucosa (two cases in lips and two in cheek). GLUT-1 is considered as a tool for differential diagnosis with HEM and is undetectable in blood vessels of normal skin and in VM and PG (North *et al*, 2000, 2001a,b; Drut and Drut, 2004; Mo *et al*, 2004; Nguyen *et al*, 2004; Hernández *et al*, 2005; Leon-Villapalos *et al*, 2005). Two subtypes of congenital HEM (completely formed at birth) are GLUT-1 negative: (1) NICH that do not grow or regress in postnatal life, and RICH that rapidly regress during early infancy (North *et al*, 2001b; Berenguer *et al*, 2003; Mulliken and Enjolras, 2004).

In this study, GLUT-1 was selected for evaluating the oral benign vascular lesions for being: (1) widely studied in benign vascular lesions, (2) sensible and specific in the HEM diagnosis, and (3) easily applicable. This antibody identifies GLUT-1 expression in the HEM with a strong stain in the membrane of endothelial cells. To date, no large study on GLUT-1 immunolocalization in oral benign vascular lesions has been carried out. Nevertheless, there are no reports on immunoexpression of GLUT-1 in VAR.

This study approaches three issues: (1) to verify the accuracy of histological diagnosis of oral HEM, oral VM and oral PG based in immunohistochemistry to GLUT-1; (2) to reclassify the lesions according to GLUT-1 expression; and (3) to investigate the immunoexpression of this glucose transporter in oral VAR.

Materials and methods

Institutional ethical board

The protocol of the study was approved by the Committee of Bioethics in Research at the Federal University of Minas Gerais, UFMG (COEP – 467/04).

Specimens

Specimens with previous histological diagnosis of oral VM (17 cases), oral HEM (19 cases), oral VAR (9 cases) and oral PG (48 cases) were obtained from the files of the Oral Pathology Service of UFMG (Belo Horizonte, Brazil) from 1997 to 2004. The lesions with previous histological diagnosis of oral HEM were not congenital lesions. This information was obtained from biopsy records. Immunohistochemistry was performed in all cases.

Immunohistochemistry

Immunohistochemistry was performed using streptavidin–biotin standard protocol. Sections of 4 μ from routinely processed paraffin-embedded blocks were deparaffinized and dehydrated. Specimens were

Figure 1 **(a)** Control to GLUT-1: positive erythrocytes and perineurium (streptavidin–biotin, ×400 magnification). **(b)** Control to GLUT-1-positive vessels and trophoblastic cells of placenta tissue (streptavidin–biotin, ×200 magnification). **(c)** HEM control was characterized by a great number of vessels (covered by cells with rounded nuclei) exhibiting an initial maturation with the dilatation of vascular lumen (hematoxylin–eosin, ×200 magnification). **(d)** GLUT-1 was positive in vascular endothelial cells, erythrocytes, and perineurium of HEM control (streptavidin–biotin, ×200 magnification). **(e)** Oral PG organized in lobular arrangement of capillaries covered by cells with rounded nuclei and scarce cytoplasm (hematoxylin–eosin, ×200 magnification). **(f)** GLUT-1 was negative in oral PG vessels but positive in erythrocytes. It was initially diagnosed as proliferating phase HEM but was reclassified as PG after immunohistochemistry (streptavidin–biotin, ×200 magnification). **(g)** Oral VNM was characterized by tortuous blood vessels lined by a flat mature endothelium (hematoxylin–eosin, ×200 magnification). **(h)** GLUT-1 was negative in oral VNM vessels but positive in erythrocytes. It was initially diagnosed as involuted phase HEM but was reclassified as VNM after immunohistochemistry (streptavidin–biotin, ×200 magnification). **(i)** Oral VAR was characterized by an extensive and tortuous abnormal vein lined with a flat mature endothelium (hematoxylin–eosin, ×200 magnification). **(j)** GLUT-1 was negative in oral VAR vessels but positive in erythrocytes (streptavidin–biotin, ×200 magnification).

immersed in a 10 mM citrate buffer (pH = 6.0, 30 min at 98°C) for antigen retrieval. Avidin/biotin was blocked as determined by Miller *et al* (1999). Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide. Sections were incubated with primary antibody GLUT-1 at a 1:200 dilution (Dako, Carpinteria, CA, USA) for 18 h at 4°C. Primary antibody was detected using a LSAB® + system, HRP Peroxidase Kit (Dako Corporation) and 3,3′-diaminobenzidine tetrahydrochloride chromogen (DAB; Sigma Chemical, St Louis, MO, USA). Erythrocytes and perineurium (Fig. 1a) on the oral benign vascular lesion slices were considered internal positive control. Two other positive controls were used: (1) placenta-positive immumoreactivity in erythrocytes, throphoblast and microvascular endothelial cells (Fig. 1b); and (2) a case of HEM in involution phase localized on the lip of a 2-year-old patient who was GLUT-1 positive in vascular endothelial cells and erythrocytes (Fig. 1c,d).

Analysis and reclassification of diseases

The diseases were re-evaluated and submitted to histological analyses for reclassification. Considering that the lesions of our sample were not congenital and that GLUT-1 is sensible and specific to HEM, those lesions with negative stain to GLUT-1 and that presented initial diagnosis for oral HEM must be reclassified.

The lesion was classified as oral VM when it was morphologically composed by tortuous blood vessels lined with flat mature endothelium and was immunonegative to GLUT-1 (Mulliken and Young, 1988; Enjolras and Mulliken, 1997; North *et al*, 2000, 2001a; Mo *et al*, 2004; Hernández *et al*, 2005; Leon-Villapalos *et al*, 2005). The lesion was classified as oral PG when it was morphologically composed of proliferating endothelial cells organized in capillaries lined by flattened endothelial cells and an acute and chronic inflammatory infiltrate and being immunonegative to GLUT-1 (North *et al*, 2001a; Epivatianos *et al*, 2005). The lesion was classified as oral HEM when it showed proliferation of plump endothelial cells organized in masses, forming dilated blood vessels, or when tiny capillary-like feeding and draining vessels lined by flat mature endothelium were seen (Mulliken and Young, 1988; Enjolras and Mulliken, 1997). Immunopositivity to GLUT-1 should also be observed (North *et al*, 2000, 2001a; Drut and Drut, 2004; Mo *et al*, 2004; Nguyen *et al*, 2004; Hernández *et al*, 2005; Leon-Villapalos *et al*, 2005). The disease was classified as oral VAR when it was morphologically composed by one to three extensive and tortuous blood vessels lined by flat mature endothelium (Southam and Ettinger, 1974).

Results

None of the cases of the oral benign vascular lesions evaluated were immunopositive to GLUT-1.

Oral HEM were reclassified as oral PG (nine cases; Fig. 1e,f) or oral VM (10 cases; Fig. 1g,h). The histological evaluation itself is not sufficient to render the correct diagnosis of oral HEM as none of the sample cases were true HEM.

All sample cases with an initial VM or PG classification were negative for GLUT-1, which corroborates the accuracy of their histological diagnoses.

Oral VAR showed negative staining to GLUT-1 in blood vessels (Fig. 1i,j).

Discussion

The histological differential diagnosis among oral HEM and VM or PG may be difficult. HEM in the involuted phase can present histological similarities with VM, and HEM with inflammation can present histological similarities with PG (North *et al*, 2000; Leon-Villapalos *et al*, 2005). Because of this difficulty, there arose the necessity of finding an auxiliary marker of HEM: GLUT-1.

Hemangioma immunopositive to GLUT-1 was observed in the studies of North *et al* (2000, 2001a,b), Drut and Drut (2004), Mo *et al* (2004), Nguyen *et al* (2004), Hernández *et al* (2005) and Leon-Villapalos *et al* (2005).

North *et al* (2000, 2001a,b) found intense GLUT-1 immumoreactivity in endothelial cells in 100% of skin HEM; however, no lesional GLUT-1 expression was found in any VM or PG of skin. Mo *et al* (2004) identified intense GLUT-1 immumoreactivity in endothelial cells in 100% of liver HEM, and lack of staining in liver VM. Drut and Drut (2004) found intense GLUT-1 immumoreactivity in endothelial cells in 100% of HEM in chorion, skin, liver, and in mammary and submaxillary glands. Nguyen *et al* (2004) showed GLUT-1 immumoreactivity in endothelial cells in 100% of HEM in head (including oral mucosa: two cases in lip and two in cheek), trunk, and genitalia. Hernández *et al* (2005) classified 11 cases of liver vascular tumors based upon GLUT-1 expression despite their histological diagnosis. A comparison of the results of GLUT-1 expression to the histological diagnosis showed that the histology of GLUT-1-positive tumors corresponded to HEM.

Leon-Villapalos *et al* (2005) observed intense GLUT-1 immumoreactivity in endothelial cells in 95% of HEM and lack of stain in NICH, VM, and PG. The HEM that did not present GLUT-1 expression may be due to evolution. However, this postulation contradicts the findings of North *et al* (2000, 2001a) and Mo *et al* (2004) who found GLUT-1-positive staining in all phases of HEM development.

The immunohistochemical study of GLUT-1 is a discriminant and easy diagnostic method (North *et al*, 2000, 2001a; Leon-Villapalos *et al*, 2005). Accurate diagnosis of these oral lesions has important clinical relevance for it allows: (1) correct management, (2) adequate communication among the multidisciplinary team (dentist, dermatologist, pediatrist, radiologist, pathologist, and surgeon), (3) understanding the biologic behavior of these lesions, and (4) development of new therapeutic modalities. Hence, it is very important to advocate the use of this marker in medical and dental communities.

In our study, oral VAR were negative to GLUT-1. It is in accordance with the hypothesis that these lesions result from structural alterations (Southam and Ettinger, 1974).

North *et al* (2001a) proposed that the presence of GLUT-1 in HEM and in placental microvessels suggests two possible pathogenic mechanisms of HEM: (1) the aberrant differentiation of angioblasts toward the placental microvascular phenotypes within fetal tissues, or (2) an origin from embolized placental cells that reach fetal tissues through right-to-left shunts observed in normal fetal circulation.

Considering that HEM has spontaneous involution and VM and PG do not, the distinction among these lesions is important for management, prognosis and assessment in response to therapies. The decision of treatment of HEM may be based on the evolution of the lesion. The clinician must evaluate if it is possible to follow up the patient's lesion throughout the whole involution stage, or determine if intervention is necessary due to possible complications (bleeding, pain, and esthetic problems). Moreover, VM does not present spontaneous involution. Thus, the clinician must evaluate if the lesion may cause problems to the patient and decide upon the most appropriate approach (Mo *et al*, 2004; Leon-Villapalos *et al*, 2005). As PG is a reactive lesion, the treatment comprises both the ablation of the lesion and controlling the inducing trauma (Al-Khateeb and Ababneh, 2003).

In conclusion, GLUT-1 is a useful, easy and important auxiliary marker for the diagnosis of oral benign vascular lesions.

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