Original Article

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Morphological Characterisation of Capillary Hemangioma Pattern: An Indian Study

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Abstract

Context: Capillary hemangiomas are fairly common benign tumors encountered across all age groups.Even though they consist of number of clinicopathologically distinct entities, clinicians and pathologists alike have traditionally tended to lump these tumors, under overly generic terms like capillary hemangioma which do little to guide proper clinical management. *Aims:* To delineate various lesions with capillary hemangioma pattern and outline the morphological features that distinguish each of them. *Results:* We characterised a collection of 93 cases with capillary hemangioma pattern received over a period of 60 months. Mast cells were counted in 10 fields of high density and mast cell density per square millimetre area was calculated. *Statistical Analysis:* Pearson correlation coefficient was used to analyse the relationship between mast cell numbers and the age of the lesion. *Conclusions:* Pathogenesis of capillary hemangiomas remains a hot topic receiving widespread attention of researchers. For a pathologist to distinguish a lobular capillary hemangioma from an Infantile hemangioma or a congenital hemangioma based on morphology is important, pointing to a need for looking beyond using a generic term of capillary hemangioma, and study of a large cohort of cases.

Keywords: Congenital Hemangioma; Epithelioid Hemangioma; Infantile Hemangioma; Lobular Capillary Hemangioma.

Introduction

Benign vascular tumors are very common and most frequently occur in skin. At all sites, it is often difficult to determine whether benign vascular lesions are malformations, true neoplasm or, in some cases, reactive processes. The difficulties are due to the coexistence of entities, unclear pathogenesis and similar histological characteristics [1].

An absolute requisite of meaningful diagnosis and study of these lesions is precise histological description, combined with knowledgeable clinical and radiological evaluation. Modern ancillary techniques such as immunohistochemistry and molecular genetics which have been so helpful in classifying other soft tissue tumors, have done little to advance our knowledge of these lesions therefore we must often rely on morphology to correctly classify them.And as our understanding and knowledge of vascular proliferations have improved and new entities have been described, the classification and nomenclature have changed accordingly [2-6].

International society for study of vascular anomalies (ISSVA) and WHO have classified vascular tumors and have included lobular capillary hemangiomas(LCH), infantile hemangioma (IH), congenital hemangioma(CH), cherry angiomas, glomeruloid hemangioma and epithelioid hemangiomas(ALHE) as capillary hemangiomas [2,7,8].

Using morphology as the basis, this study intends to characterize and determine the features of various lesions with capillary hemangioma pattern, as we enter a new era in understanding these common lesions.

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Materials and Methods

All cases with capillary hemangioma pattern diagnosed in our institution over the period of 5 years from 2009-2013 were included in this study. All the case details available in the request forms and case records when available were collected (Table1). Sections were stained with H&E staining. Reticulin stain was utilised to better delineate the vascular nature of the lesions. Acidified Toluidine blue stain at pH 3.2 was used to identify mast cells in the hemangiomas. Mast cells were counted in 10 fields of high density at 450x magnification and mast cell density per square millimetre area was calculated. Pearson correlation coefficient was determined to find if there was any correlation between duration of lesion and mast cell density.

Results

Table 1: Comparison of clinical data: LCH, IH, CH and ALHE

	LCH (69 cases)	IH (20 cases)	CH (1 case)	ALHE (3 cases)
Age				
Mean(years)	31.1	1.34	8	24
Median(years)	23	1	---	25
Sex				
Ratio(M:F)	1.1:1	1:2	1 male	3 males
Location*	HN/LL/UL/T	HN/T/G	UL	HN
Duration				
Mean	5 months	1.34 years	8 years	3 months

*HN- head & neck, UL- Upper limb, LL- Lower limb, T- trunk, G- genitalia.

Table 2: Distinguishing morphologic features:LCH, IH, CH and ALHE

	LCH	IH	CH	ALHE
Gross size(cm)	0.98	2.25	2	1.5
Microscopy				
Epidermis/dermis/subcutis	**+/+/-**	**-/+/+**	**-/+/+**	**-/+/+**
Lobular architecture	**+**	**+**	**+**	**-**
Endothelial cells Plump/flat/epithelioid	**+/+/-**	**+/+/-**	**+/-/-**	**-/-/+**
Entrapped native tissue	**-**	**+**	**+**	**-**
Nerve infiltration	**-**	**+**	**+**	**-**
Arteriovenous microfistulae	**-**	**-**	**+**	**-**
Damaged vessel in centre of lesion	**-**	**-**	**-**	**+**
Ulceration	**+**	**+**	_	_
Other cells				
Neutrophils	**++**	**+/-**	**-**	**+**
Eosinophils	**+/-**	**-**	**-**	**++**
Mast cells(/mm²)	75	192	134	60
Lymphocytes	**+/-**	**+/-**	**-**	**+/+++**
Plasma cells	**+/-**	**+/-**	**-**	**+**
Mitoses	**+**	**+**	**-**	**+**

Lobular Capillary Hemangioma

History of duration was available in 43 cases, which ranged from 3 days to 3 years (Mean: 5 months). A diagnosis of pyogenic granuloma (PG) was rendered clinically only in 34 cases. Other clinical diagnosis offered were papilloma, dermatofibroma, angiofibroma, granulation tissue and bleeding polyp.

Average size of the lesions was 0.98 cm at its greatest dimension. Majority were exophytic masses, with stalk seen in few lesions. On microscopy, lesions were well circumscribed with an ulcerated area on the surface and an underlying area with capillaries arranged in lobules. Ulceration was present in 52/69 cases (Figure 1A &B), at the summit of the lesion. Inward growth of acanthotic epithelium was noticed towards base of the lesions in many cases, referred to as epithelial collarette.

Deeper portion of lesions showed proliferating masses of capillaries arranged in distinctive lobular pattern (Figuare 1A, Figure 2B). Some lobules extended to deeper dermis. Capillaries were in various stages of canalisation ranging from minute slit-like to rounded

to irregular congeries filled with RBCs. Lobules were separated from each other by fibrous septa of variable thickness. Draining vessels were observed in the deeper part of the lesions. Infiltrate of neutrophils, lymphocytes and plasma cells were observed in superficial regions of ulcerated lesions but were hardly present in non-ulcerated lesions. On high power magnification, capillaries were lined by plump endothelial cells.

Infantile Hemangiomas

The age range was 6 months to12 years (mean 46 months). This is the age at presentation but the lesions were present since birth in thirteen cases and appeared at various months following birth in the other eight cases. Female to male ratio was 1:1.6. Head and neck was the commonest region involved (71.4%) and most of them were recognised by clinicians as hemangioma except four cases.

On gross, average size of the lesion was 2.25 cms at its greatest dimension (median 1.5 cms). All lesions were poorly circumscribed, irregular with wrinkled appearance on the surface; ulceration was grossly identifiable in four of the cases. Cut section of the lesions showed a pale pink lobulated appearance with interspersed grey white areas.

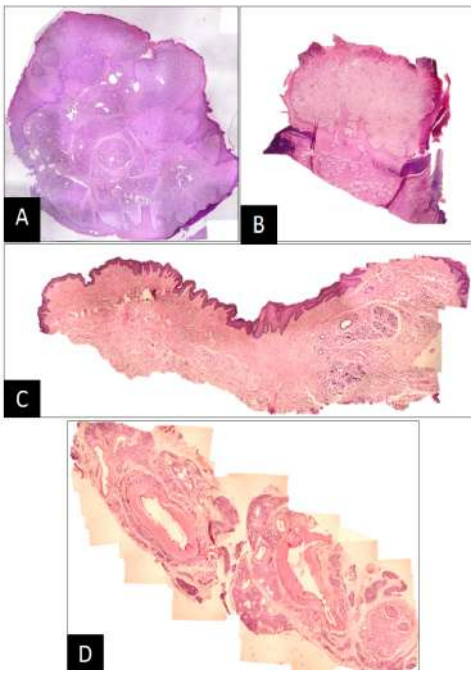

Fig. 1: A&B: Whole mounts of lobular capillary hemangiomas, showing lobular arrangement, surface ulceration and epithelial collarette, **C)** Whole mount of Infantile hemangioma showing diffuse capillary proliferation involving the appendages and nerve twigs, **D)** Whole mount of congenital hemangioma showing central artery and arterioles surrounded by garlands of capillary lobules.

Histologically, the lesions showed multilobular pattern with masses of endothelial cells occupying the dermis and sub cutis (Figure 1C).Ten lesions were in involuting stage with cases showing varying degrees of involution. Densely cellular areas were present towards centre of the lesion with hardly any lumen formation seen. The endothelial cells were seen wrapping around adnexal structures and residual acini. Towards periphery the lobular architecture was more evident and capillaries with well-formed lumina were present (Figure 2A).

The cells in these areas were flattened in comparison to the center of lesion. The basement membrane and lumina of capillaries were highlighted using reticulin stain and capillary architecture could be recognised even in the centre of the lesion which otherwise appeared just as a mass of endothelial cells on H & E sections. IHs were not circumscribed even on microscopy and showed endothelial cells wrapping around the adnexal structures, sheets of endothelial cells investing in between the mature adipocytes in the subcutis. Perineurial invasion was present in eight cases (Figure 2F).

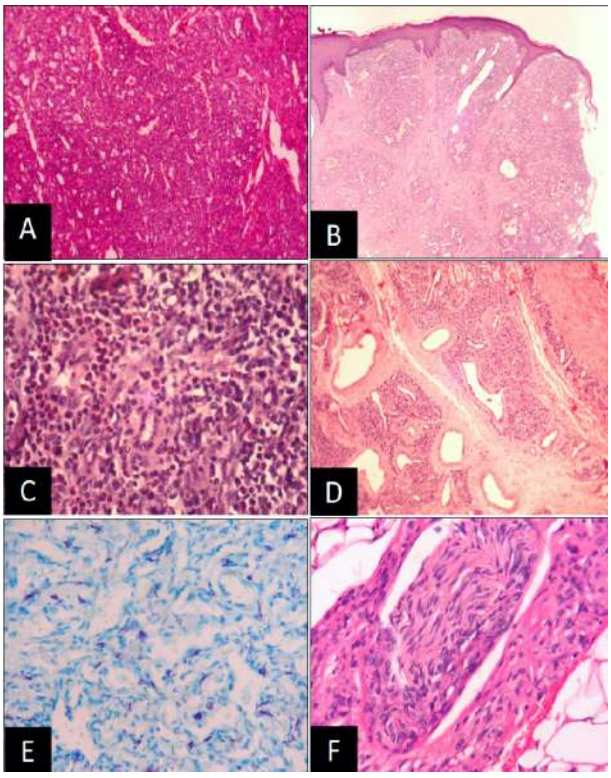

Fig. 2: A. Infantile hemangioma showing diffuse proliferation of capillary channels with inconspicuous lumina, **B.** Lobular capillary hemangioma with capillary lobules, **C.** Epithelioid hemangioma with mixed inflammatory cells and eosinophils surrounding the capillaries, **D.** Congenital hemangioma with capillary lobules surrounding the arterioles, **E.** Mast cells demonstrated by Toluidine blue stain in a LCH, F) Perineurial invasion in a case of Infantile hemangioma.

Congenital Hemangioma

A single case of non involuting congenital hemangioma(NICH) presented as a diffuse lesion on the left ring finger with a ridged surface and prominent (telangiectatic) vessels with pulsations seen on the surface. It was called as an AV malformation clinically. Microscopically capillaries with rounded lumina arranged in a back to back fashion forming lobules were seen with areas showing only masses of endothelial cells. Many lobules showed central vessels with thickened wall, groups of capillaries were seen garlanding the wall of a medium sized artery at the centre of lesion (Figure 1D, Figure 2D). Nerve infiltration by endothelial cells was also noticed. Elastic stain (Verhoeff-van Gieson) showed some of the vessels in the centre of capillary lobules to be arterioles.

Angiolymphoid Hyperplasia with Eosinophilia (Epithelioid Hemangioma)

Grossly, the first two cases were circumscribed masses while the last one was a recurrent lesion after 1 year. Only one case showed a damaged vessel (medium sized artery) in its centre. No such vessels could be made out in the other two cases. Capillary sized channels surrounded the damaged vessel and stroma was infiltrated by mixed inflammatory cells. Peripheral eosinophilia was noted in one of the cases(Figure 2D).

Mast Cells in Capillary Hemangiomas

Staining for mast cells was done in cases of LCH, IH, CH, ALHE. Mast cells were stained using acidified toluidine blue at pH 3.2 and then counted in 10 areas of high density with 45x objective(Figure 2E). Mast cell density per square millimeter area was calculated. Statistical tests were applied to find if any correlation exists between duration of the lesion and mast cell density in LCH and IH. Other two lesions did not have adequate number of cases to apply the tests.

Pearson correlation coefficient was 0.626. Even though not significant it shows a good correlation between duration and mast cell numbers, but there is a need for more cases to be studied. No positive correlation was found between duration of lesion and mast cell numbers with respect to LCH cases (Pearson correlation coefficient was – 0.068). As other two lesions did not have sufficient number of cases statistical tests could not be applied.

The average number of mast cells in IH and LCH were compared using unpaired T- test. t-value was 6.77,p- level <0.01. The values indicate that significantly more number of mast cells were seen in IH than in LCH.

Discussion

Vascular proliferations occurring in soft tissue and skin are a diverse and morphologically complex group of lesions that can cause diagnostic difficulty, even for those with years of experience and expertise.Unfortunately the modern ancillary techniques which are of so much help in classifying other soft tissue tumours are of little help to advance our knowledge of these lesions. Therefore we must rely on morphology and limited battery of IHC stains to further classify them [3].

The category of benign vascular neoplasms is by far the most numerous and controversial group. It is classified in accordance with the cell lineage of differentiation. Subsequent categories are according to size of involved vessels [9].

Lobular capillary hemangiomas, histologically have an ulcerative area and a lobular area are described, with lobular area underlying the ulcerative area. In our study ulceration was seen microscopically in 52 of 69 cases. Lobular area consists of capillaries arranged in lobular pattern separated by fibrous septa that intersect the lesion. Each lobule is composed of aggregates of capillaries and venules lined by plump endothelial cells. This description holds good for a fully evolved lesion. Early lesions are more oedematous and contain less organized capillaries resembling granulation tissue. Older lesions with evolution develop epithelial collarette, widening of fibrous struts and eventual conversion of LCH into a fibroma. Such an evolutional pattern with same lesion exhibiting different appearances has led some authors to subclassify the lesion as LCH-PG and non-LCH PG,depending on whether the lesion shows typical lobular pattern or not. The proponents of such subdivision have however also demonstrated that both lesions are similar immunohistochemically [7,10-12].

There was a marked difference in the morphology of vascular elements between lobular and ulcerative area. Morphologic features of the vascular elements in ulcerative area were similar to those in common granulation tissue. However, in the lobular area, the vascular elements exhibited a marked uniformity in both their luminal size and proportion of cell types comprising the wall. Along with an inner layer of endothelial cells, one or several outer layers of ASMA

positive mesenchymal cells were present, thus distinguishing these vessels from normal capillaries which would have a single layer of endothelial cells and a few embedded pericytes in the wall. Based on these findings, it is suggested that vascular elements composing the lobular area may not be true capillaries [10].

Mills et al proposed the lobular arrangement of capillaries as the critical identifying feature for the lesion, proposing the name lobular capillary hemangioma to replace pyogenic granuloma [13].

Ulcerated area of pyogenic granuloma consists of mixed inflammatory infiltrate of lymphocytes, plasma cells, neutrophils, histiocytes and an increased number of mast cells. Price et al however found the mast cell levels to be normal. Hence they suggest that this finding could be used as a supporting feature to distinguish LCH from proliferative and involuting infantile hemangiomas which show increased number of mast cells, with respect to pediatric population [7,13].

Infantile hemangiomas have been grouped based on their clinical course by Mulliken and Glowacki, these groups can be distinguished morphologically as well. They group IHs into proliferating phase, involuting phase and involuted phase. Proliferating lesions show a rapid growth clinically during initial 3 to 6 months of life, but may extend up to 12 months of age, Histologically, mass of plump endothelial cells, which on reticulin stain show arrangement of vascular architecture and developing lumina. Maturation commences at the periphery of the lesion gradually involving all the zones. With time there is thickening of basement membrane and progressive flattening of the endothelium [14,15].

Involution, once initiated, progresses at a consistent rate, showing complete resolution in 70% of children by 7 years of age, although involution has been noted up to 12 years. Involution is completed by a progressive diffuse interstitial fibrosis. Fibro fatty tissue interspersed with dense collagen and reticular fibres replace the previous vascular network [16].

Perineurial invasion in IHs, is an often overlooked finding. The perineurial invasion is a worrisome finding in persistent lesions that are excised surgically. Accompanied by atypical features, a differential diagnosis of angiosarcoma could be entertained, even though it is unwarranted.Eight of the twenty cases we studied showed perineurial invasion. It has been proposed that the nerves are not just 'innocent bystanders' trapped by proliferating cells. Nerves might interact actively through secretion of growth factors with surrounding tissues; thereby stimulating cells to proliferate and differentiate and in the due process becoming entrapped by the proliferating cells [15,17].

Lesions that appear fully grown at birth, having arisen in utero and are designated as congenital hemangiomas. Lumped together in the past with IHs and vascular malformation, two clinical subtypes have now been identified in congenital hemangiomas: rapidly involuting congenital hemangioma (RICH) and non involuting congenital hemangioma (NICH) [18].

RICH usually shrinks in the first year of life, a time course that is more rapid than that of IH. Both RICH and NICH do not show rapid neonatal growth, variably sized lobules are seen consisting of small vascular channels that are usually fairly regular with prominent draining channels in center. Basement membrane thickens as the lesion ages [19].

NICH is a rare variant of CH, with female preponderance and same anatomic distribution as IH. NICHs never involute or disappear. They show proportional growth with the child and appearances of increased draining veins in the periphery of the lesion.Unlike AV malformations which tend to recur on incomplete excision, NICH does not recur after excision [20,21].

Two features which have been proposed to identify NICH on microscopy are presence of large lobules of small vessels with arteriovenous or arteriolymphatic microfistulae, and/or hobnailed endothelial cells. IHs invariably show GLUT-1 positivity on immuno histochemistry, whereas CHs do not [22].

Another entity described as being distinct by its authors from IH, NICH and RICH has been congenital non progressive hemangioma. North *et al* [23] in their article contended that overlying epidermis is atrophic in CNPH. GLUT-1 and LeY expression was absent. Intraneural involvement was conspicuous by its absence, which is otherwise a common finding in IHs. Even though authors successfully distinguish CNPH from IH, no convincing argument is put forward to distinguish CNPH from more firmly established NICH and RICH. Further, all patients with CNPH were operated at less than 3 months of age, thus little is known of the natural history of those lesions. Moreover features described are all found in RICH and it is possible that these lesions might be just RICHs [23].

Epithelioid hemangioma was suggested as a synonym for ALHE by Enzinger and Weiss in 1983 and they felt that Kimura's disease and ALHE probably represented distinct entities. Since then

many studies have highlighted this distinction and now they are dealt as two separate entities [24,25].

The hallmark of ALHE are the epithelioid endothelial cells that line a majority but not necessarily all the vessels and protrude deeply into the lumen in a manner that has been likened to tombstones. The ultrastructural characteristics of these epithelioid cells are similar to endothelial cells elsewhere with a few differences. Vacuoles in the cells were not a common finding. Pleomorphism of endothelial cells was seen in all cases [24-26].

Epithelioid hemangiomas were associated with prominent inflammatory component. Eosinophils are characteristic, lymphocytes, mast cells and plasma cells are also present. Mast cells were demonstrated in only one of our cases. Lymphoid aggregates with germinal centers have been described, and were seen, in one of our cases.Their presence is said to be a feature of longstanding lesions or a peculiar host response [26].

It is important to distinguish ALHE from Kimura's disease. The lesions are smaller in EH with a shorter duration. Peripheral eosinophilia is a more common finding, while one case is our study did show peripheral eosinophilia. Increased serum immunoglobulin E, proteinuria and nephrotic syndrome are described as other features seen in Kimura's disease. No data was available regarding these findings in our cases. In contrast to ALHE, which shows small to medium sized blood vessels lined by epithelioid atypical endothelial cells, the proliferating blood vessels in Kimura's disease are mostly post-capillary venules lined by plump or flat endothelial cells, surrounded by a heavier inflammatory infiltrate including many well-formed lymphoid follicles. Interstitial collagenous fibrosis was noted only in Kimura's disease [24,26].

Mast cell numbers are increased in hemangiomas, with involuting phase IHs showing higher counts. A drastic increase in mast cells numbers is observed in hemangiomas subsequent to steroid – induced accelerated regression. These findings point to products secreted by mast cells playing an active role in regression of hemangiomas. In our study we found an indication towards a possible relation to mast cell numbers and duration of lesions (Pearson correlation coefficient 0.626) prompting a need for more cases to be studied before a positive correlation could be established. Mast cell numbers in infantile hemangiomas was significantly higher than in lobular capillary hemangiomas. The role of mast cells in progression of hemangiomas is likely to be complex involving stimulation of angiogenesis in the proliferative phase but inhibition in later phases[27,28].

Conclusion

Pathogenesis of capillary hemangiomas remains a hot topic receiving widespread attention of researchers. The exercise begins with the ability to distinguish a lobular capillary hemangioma from an infantile hemangioma or a congenital hemangioma, thus it becomes important for a pathologist to start looking beyond using a generic term of capillary hemangioma, and further our understanding by studying larger cohort of cases.

References

1. Sheth S, Lai CK, Dry S, Binder S, Fishbein MC. Benign vascular tumors and tumor like proliferations. Sem Diag Pathol. 2008; 25(1):1-16.
2. North PE, Waner M, Buckmiller L, James CA, Vascular tumors of infancy and childhood: beyond capillary hemangioma. Cardiovascular Pathol. 2006; 215:303-17.
3. O'Hara CD, Nascimento AG. Endothelial lesions of soft tissue: A review of reactive and neoplastic entities with emphasis on low–grade malignant vascular tumors. Adv Anat Pathol. 2003; 10(2):69-87.
4. Garzon MC, Enjorlas O, Frieden IJ. Vascular tumors and vascular malformations; evidence for an association.J Am Acad Dermatol. 2004; 42:275-9.
5. Enjorlas O, Wassey M, Chapot R. Color atlas of vascular tumors and vascular malformations. Cambridge university press, Cambridge;2002:1-11
6. Goh SG, Calonje E. Cutaneous vascular tumors; an update. Histopathol 2008; 52:661-73.
7. Leboit PE, Burg G, Weedon D, Sarasin A (Eds). World Health organization classification of tumors. Pathology and genetics of skin tumors. IARC Press: Lyon 2006; 229-50.
8. Fletcher CD, Unni KK, Mertens F.(Eds) World Health Organization. Classification of tumors. Pathology and genetics of tumors of soft tissue and bone. IARC Press; Lyon 2002; 135-139.
9. Sangueza OP, Requena L. Pathology of vascular skin lesion. Clinicopathologic correlation. Humana Press. Totowa (NJ), 2003
10. Toida M, Hasegawa T, Watanabe F, Kato K, Makita H, Fujitsuka H. Lobular capillary hemangioma of oral mucosa; clinicopathologic study of 43 cases with a special reference to immunohistochemical characterization of the vascular elements. Pathol Intl

2003; 53:1-7.

11. Epivantianos A, Antoniades D, Zaraboukas T, Zairi E. Pyogenic granuloma of oral cavity:comparative study of its clinicopathological and immunohistochemical features. Pathol Intl 2005; 55:391-7.
12. Puxeddu R, Berlucchi M, Ledda GP, Parodo G, Farina D. Nicolai P. Lobular capillary hemangioma of the nasal cavity:A retrospective study of 40 patients. Am J Rhinol 2006; 20:480-4.
13. Patrice SJ, Wiss K, Mulliken JB, Pyogenic granuloma (Lobular capillary hemangioma): A Clinico-pathologic study of 178 cases. Pediatr Dermatol 1991; 8: 267-76.
14. Beck DO, Gosain AK. The presentation and management of hemangiomas. Plast Reconstr Surg; 2009; 123:181e-191e.
15. Chatura KR, Nayak VJ. Morphological characterization of infantile hemangiomas: An Indian study. J Med Radiol Pathol Surg 2016; 2(2):1-6.
16. Benett HA, Metry DW, Baselga E. Lucky AW, Adams D, Cordisco M.IHs with unusually prolonged growth phase, Arch Dermatol 2008; 144:1632-7.
17. Calonje E, Mentzel T, Fletcher CD. Pseudomalignant perineurial invasion in cellular (infantile) capillary hemangiomas. Histopathology; 1995; 26:159-64
18. Drolet BA, Swanson EA, Freiden IJ. IHs:An emerging health issue linked to an increased rate of low birth weight infants. J Pediatr 2008; 153:712-5.
19. Goh SG, Calonje E. Cutaneous vascular tumors; an update. Histopathology 2008; 52:661-73.
20. Enjorlas O, Mulliken JB, Boon LM, Wassef M, Kozakwich HP, Burrows PE. Non involuting congenital hemangioma: A rare cutaneous vascular anomaly. Plast Reconstr Surg 2001; 107:1647-54.
21. Krol A, Mac Arthur CJ. Congenital hemangiomas. Rapidly involuting and non involuting congenital hemangiomas. Arch Facial PlastSurg 2005; 7:307-11.
22. Gorincour G, Kokta V, Rypens F. Garel L, Powell J, Dubois J. Imaging characteristics of two subtypes of congenital hemangiomas; rapidly involuting congenital hemangioma and non-involuting congenital hemangiomas. PediatrRadiol 2005; 35:1178-85.
23. North PE, Waner M, James CA, Mizeracki A, Frieden IJ, Mihm MC, Congenital non progressive hemangioma. Arch Dermatol 2001; 137:1607:20.
24. Ahn JH, Lee KG. A clinicopathological study of kimura's disease and epithelioid heamangioma. Yonsei MedJ 1990; 31:205-11.
25. Chun SI, Ji HG. Kimura's disease and angiolymphoid hyperplasia with eosinophilia. Clinical and histopathological differences. J Am Acad Dermatol 1992; 27:954-8.
26. Weiss SW, Goldblum JR (Eds). Chapter 22 in Enzinger and Weiss's soft tissue tumors. 5th edition. Mosby Elsevier; Philadelphia 2008; 633-679.
27. Tan ST, Wallis RA, Davis PF, He Y. Mast cells and hemangioma. Plast Reconstr Surg 2004; 113:999-1011
28. Tan ST, Velickovic M, Ruger MB, David PF, Cellular and extracellular markers of hemangioma. Plast Reconstr Surg 2000; 106:529-38.