INTRODUCTION

Hemangiomas are very common tumors, characterized by increased numbers of normal or abnormal vessels filled with blood. They may be difficult to distinguish from vascular malformations.\(^1\) Vascular malformations are abnormal clusters of blood vessels that occur during fetal development. The exact cause of these malformations remains unknown. Hemangioma and vascular malformation are both vascular anomalies with different outcomes. International Society for the Study of Vascular Anomalies has further classified vascular lesions into vascular neoplasm and vascular malformation. Vascular neoplasms include infantile hemangioma, congenital hemangioma, Kaposiform hemangioendothelioma, spindle cell hemangioendothelioma, epithelioid hemangioendothelioma, angiosarcoma and acquired benign vascular tumor like pyogenic granuloma. Vascular malformations are further classified into slow flow malformations, which include capillary, venous and lymphatic malformations and the fast flow ones which include arterial, arteriovenous, and combined malformations.\(^2\) Infantile hemangiomas...
usually occur few weeks after birth, mainly on the head and neck region with female predominance (M:F = 1:3). Histologically they are characterized by rapid proliferation followed by involution. Surgery may be required in up to 40% cases due to various reasons such as incomplete involution, cosmetic reasons, obstruction to vision, bleeding, infection, pain and rarely cardiovascular complications. Vascular malformation is a rare vascular anomaly characterized by malformed vascular channels. It occurs since birth, increases in size and grows proportionate to the body growth. They become symptomatic at any age due to complications and/or accelerated growth triggered by trauma and hormonal influence, which require surgical and medical intervention. Skin, brain, spinal cord and visceral organs are the common sites affected. Managing vascular malformation is a challenge to a surgeon. Incomplete resection or treatment failure can even lead to recurrence. Thus, it is of utmost importance to diagnose vascular malformations and hemangiomas correctly and early in the course of the disease.

Histopathologically, hemangiomas show capillaries with endothelial proliferations, while vascular malformations show mature but dysplastic vessels which infiltrate into the deeper tissues. The histopathological diagnosis of malformation is supported by directly communicating arteries and veins, with presence of abnormal vessel wall with disrupted internal elastic lamina and eccentric intimal fibrous thickening in artery, arterialization of veins in arteriovenous malformation (AVM) and presence of varying size and thickness of abnormal venous channels with disrupted or abnormal vessel wall in venous malformation. AVMs can be confirmed by demonstrating elastic tissue of arteries and arterioles using Verhoeff van-Gieson (VVG) and orcein stains. Recent studies have shown the presence of increased intralesional nerve bundles in vascular malformations as an additional tool in diagnosing vascular malformation. This prompted us to study and review the cases of vascular malformations and hemangiomas diagnosed in our department. The aim of this study was to differentiate hemangioma and vascular malformation based on histopathological features and to assess the utility of various histochemical stains such as VVG, Masson’s trichrome (MT) and toluidine blue in differentiating these lesions.

**MATERIALS AND METHODS**

A total of fifty cases were retrieved from surgical pathology records. The lesions diagnosed as vascular malformation \( n = 18 \) and hemangioma \( n = 32 \) on hematoxylin and eosin (H and E) stained sections were included in the study. Vascular malformations included AVM, \( n = 17 \) and lymphatic malformation \( n = 1 \). Hemangiomas included infantile hemangiomas \( n = 4 \), capillary hemangiomas \( n = 14 \) and pyogenic granuloma \( n = 14 \). Vascular tumors diagnosed as hemangioendotheliomas and angiosarcomas were excluded from the study. Institute Ethics Committee clearance was taken for the study. Relevant clinical history including age, sex, and site of lesion were collected. However, follow-up was not available. One case of AVM had a history of recurrence. Formalin-fixed, paraffin-embedded tissue blocks were made and tissue sections were cut at 4 \( \mu \) for special stains. Routine H and E stain was performed and special histochemical stains VVG, MT and toluidine blue were done in all cases as per standard guidelines. VVG stain was used for identification of arteries and arterioles and MT stain for identification of various soft tissue components such as nerves, smooth muscles and vessels. The presence of intra-lesional nerve bundles was scored as follows: score 0 if there was no increase in nerves, score 1 if there was focal and slight increase in nerves and score 2 if there was multifocal or diffuse increase in nerve. The mast cell density was counted in toluidine blue-stained sections using Olympus CX 21 model microscope in all the cases using oil immersion objective and x10 eyepiece. With these specifications, the magnification is x1000 and field view diameter is 0.20 mm. Therefore, area of the field view using the formula for the area of a circle = \( \pi r^2 \) (where \( r \) is the radius of a circle) is 0.0314 mm\(^2\). Thus, for counting in 1 mm\(^2\) area, we have to do the cell count in 1/0.0314 = 31.84 fields, or rounded up to 32 fields. Therefore, 32 non-overlapping fields were counted to obtain mast cell count per mm\(^2\) area. In each representative section, 32 fields were counted to obtain the number of cells per mm\(^2\).

**RESULTS**

As per the surgical record diagnosis, there were 32 cases of hemangioma [Figure 1a] and 19 cases of vascular malformation (AVM \( n = 17 \) [Figure 1b], lymphatic malformation \( n = 1 \)) diagnosed on H and E. Of 32 cases diagnosed as hemangiomas, two cases were reclassified as AVM as they revealed arteries and/or arterioles and increased nerve bundles on VVG and MT stains [Figure 2a-c]. Myelinated fibers appeared red with green endoneurial sheath [Figure 2d]. One case of hemangioma was also reclassified as venous
malformation as it revealed malformed venous channels without arteries/arterioles. It also showed increase in small nerve bundles. Reclassified AVMs and venous malformation also revealed increase in nerve fibers. All other originally diagnosed cases of vascular malformation were also reconfirmed on VVG and MT. After this reclassification, there were a total 29 cases of hemangiomas, 19 of AVM and one case each of venous and lymphatic malformation. Sex ratio in hemangioma and vascular malformation were 1:0.75 (M:F) and 1:1 (M:F), respectively. Hemangiomas were seen in all age groups ranging from 12 months to 60 years (mean = 25 years). The age in vascular malformation ranged from 9 months to 75 years (mean age = 21 years). Increased intra-lesional nerves/bundles were present in 18 of 21 (85.66%) cases of vascular malformation (16/19 AVM, 1/1 lymphatic malformation, and 1/1 venous malformation), whereas these were not increased in any case of hemangioma. The increase in nerves was further scored as shown in Table 1. The salient histochemical findings to differentiate hemangioma from vascular malformation are highlighted in Table 2. Mean mast cell density calculated with toluidine blue stained sections was 42.23/mm² and 29.56/mm² in hemangiomas and vascular malformations, respectively [Figure 3a and b].

**DISCUSSION**

Hemangiomas are benign vascular tumors, characterized by endothelial hyperplasia and rapid growth. They respond to corticosteroids. Vascular malformations are characterized by vascular dysmorphogenesis and normal endothelial turnover. They never regress and require medical and surgical treatment. If vascular malformation is misdiagnosed as hemangioma, it will lead to treatment failure and a possible recurrence. Therefore, correct diagnosis of both these lesions is essential. We found that hemangiomas were more common in females than males; however, there was no sex predilection in vascular malformations. The sites of vascular malformations and hemangiomas in our study were similar to those described in literature, although age varied significantly in hemangiomas as compared to vascular malformations. This variation could be explained by the inclusion of cases of lobular capillary hemangiomas (pyogenic granuloma) which are acquired lesions occurring in adults.

Various studies in the past have evaluated the use of elastic stains for identification of arteries/arterioles. Koutras and Jessurun used orcein stain to study the presence and distribution of elastic fibers in the vascular components of arteriovenous hemangiomas. Adegboyega and Qui used Movat pentachrome stain to identify various tissue components in hemangiomas and AVMs. Pawane et al. used VVG stain in 44 cases of AVMs. In our study, VVG and MT helped to re-classify two cases of hemangioma as AVM and one case of hemangioma as venous malformation.

Routine H and E stain can identify nerves as wavy bundles. We used MT stain to identify and evaluate nerves. We found nerve bundles in 85.66% (18 out of 21) cases of vascular malformation (16/19 AVMs, 1/1 lymphatic malformation and 1/1 venous malformation). Adegboyega and Qui used immunohistochemical stain S-100 to assess the presence of nerves and nerve fibers in hemangiomas and vascular malformations. Intra-lesional nerves were identified in 91% (69 out of 76) cases of AVMs. In contrast, nerve bundles were not found to be increased in any of
the 91 cases of hemangioma. Pawane et al. identified intra-lesional nerves with H and E in 81.8% (36 cases) of AVM, whereas they were seen in only 6% cases (three cases) of hemangioma. Our findings are in agreement with Adegboyega and Qui’s study and Pawane et al. and various other studies. It has been proposed that peripheral blood vessels and nerves share patterning mechanism during development. This could explain increase in nerve bundles in vascular malformations.

We also support Meijer-Jorna et al. observation that AVMs on head and neck and upper extremities show relatively more nerves than trunk and lower extremities Table 1. It is postulated that the congruency of the nervous system and the vascular system differs according to the location as seen in experimental studies.

We found that on H and E stain, it may be difficult to differentiate between small arterioles and large capillaries with fibro-lamellar hyperplasia. Even elastic stain may not be helpful to differentiate between them as internal elastic lamina may be inconspicuous or absent in small arterioles. Presence of continuous wreath of concentric smooth muscle layer in arteries and arterioles as compared to discontinuous wreath of smooth muscle bundles in veins can be used as a feature to differentiate between them. This can be done easily by MT and/or VVG. We could easily identify dysplastic vessels and study their characteristic features such as leaky vessels, eccentric intimal fibrosis of artery, arterialization of vein, and disrupted internal elastic lamina in these vascular malformations Figures 4 and 5a. Thus, we found that histochemical stains play an important role in identifying different type of vessels with characteristic histological features.

Table 1: Vascular malformation site and scoring of nerve fibers/bundles

| Site of lesion   | Number of cases of vascular malformation (n=21) AVM (n=19), venous malformation (n=1), lymphatic malformation (n=1) | No increase in nerves Score 0 | Focal/slight increase in nerves Score 1 | Diffuse increase in nerves Score 2 |
|------------------|-------------------------------------------------------------------------------------------------------------|-------------------------------|--------------------------------------|----------------------------------|
| Head and neck    | 6                                                                                                           | 0                             | 0                                     | 6                                |
| Trunk            | 2                                                                                                           | 1                             | 1                                     | 0                                |
| Upper extremities| 8                                                                                                           | 2                             | 1                                     | 5                                |
| Lower extremities| 5                                                                                                           | 2                             | 2                                     | 1                                |

AVM: Arteriovenous malformation

Table 2: Salient histochemical features of hemangioma and vascular malformation

| Vascular lesion type | H and E | VVG | MT | Toluidine blue |
|----------------------|---------|-----|----|----------------|
| Hemangioma           | Capillaries with plump endothelial proliferations | No elastic tissue or smooth muscle in vessel wall | No increase in nerves | Increased mast cells (purple) more than vascular malformation |
| Arteriovenous malformation | Elastic lamina (pink refractile) Collagen (pink) Smooth muscle (dark pink) | Presence of internal elastic lamina of artery/arterioles (black) which may be disrupted | No smooth muscles in vessel wall RBCs (red) | Increased nerves (perineural and endoneurial sheath-(green), myelinated axon (red), Schwann cell nuclei - (bluish black)) Fibrosis of artery/arterioles wall (green) Concentric Smooth muscle (red) arrangement in artery/arteriole wall Discontinuous smooth muscle in bundles (red) in venous wall RBCs (red) | Increased mast cells (purple) less than hemangioma |
| Venous malformation  | Absence of well-formed internal elastic lamina Thin layer of discontinuous smooth muscle in bundles (dark pink) | Absence of arteries/arterioles (lack of well-developed internal elastic lamina in vessel wall) | Increased nerves Discontinuous thin layer of smooth muscle bundles in vessel wall | Increased mast cells (purple) |
| Lymphatic malformation | Only endothelial lining without smooth muscles | Absence of arteries/arterioles/vein | Increased nerves Thin-walled vessels with lack of smooth muscles in vessel wall | Increased mast cells (purple) |

VVG: Verhoeff–van Gieson, MT: Masson’s trichrome, H and E: Hematoxylin and Eosin, RBCs: Red blood cells
Various authors have utilized toluidine blue stain to study mast cells in hemangiomas and AVM and found that mast cell numbers are increased in hemangiomas and AVMs.[13,17] We found mean mast cell density of 42.23/mm² in hemangiomas and 29.56/mm² in vascular malformations which ranged from 6 to 90/mm² in hemangiomas and 4–60/mm² in vascular malformations. Mast cells were found concentrated more near vessels than normal surrounding area. Various studies have shown that mast cells are decreased in involuting hemangiomas as compared to proliferating hemangiomas which suggest the role of mast cells in maintaining hemangiomas in proliferating stage.[13] In our study, there were no involuting hemangiomas. Although mast cells have a role in the pathogenesis of hemangiomas, mast cell density has not been found to be a useful marker to differentiate hemangiomas from AVM.[13] We also found increased mast cell density in both hemangiomas and vascular malformations (hemangioma > vascular malformations), but this increase was not statistically significant.

Non-involuting congenital hemangioma (NICH) is a rare, congenital cutaneous vascular anomaly which is fully developed at birth and histologically characterized by small, thin-walled vessels having hobnailing of the endothelial cells with a large and often stellate central vessel. They also contain interlobular veins and arteries which may be dysplastic. Differentiating NICH from AVM/venous malformation would certainly be difficult as dysplastic arteries and veins are seen even in NICH,[18] although, we did not have any case of NICH in this study. However we believe that presence of increased nerve bundles may help in differentiating even NICH from AVM on histological ground. It has been found that NICH is GLUT-1 negative and may show increase in mast cell density.[18] In addition, absence of direct venous filling in NICH on radiological investigation (ultrasonography [USG]) would help differentiating it from AVM/vascular malformation.[18] Thus, clinicopathological and radiological correlation is a must in such cases for definitive diagnosis.

We reclassified two cases of hemangioma in to AVM, based on large number of nerve bundles and vascular dysmorphogenesis observed on MT and VVG. The third case diagnosed as hemangioma was reclassified as venous malformation as MT stain revealed the presence of dysplastic venous channels with discontinuous smooth muscles in their walls in addition to the presence of capillary proliferations and increased nerves [Figure 5b]. Endothelial and capillary-like proliferations may also be seen in vascular malformations as seen in this case. These capillary-like endothelial proliferations in vascular malformations are due to ischemia and vascular remodeling.[19,20] GLUT-1 is a new immunohistochemical marker which has been recently utilized by various authors in differentiating hemangiomas and AVMs as it is found mainly positive in endothelial proliferation of infantile hemangiomas.[19,21] Expression of Wilms tumor-1 gene distinguishes vascular malformation from proliferating endothelial lesions,[22] but availability and cost are limiting its utility in routine practice. Mast cells have a role in endothelial proliferation.

Although various authors have used these histochemical stains, these characteristic features of dysplastic vessels have not been highlighted as demonstrated in our study. We could even identify small nerve bundles with MT stain, which were easily missed on routine H and E stain. This definitely increased the sensitivity of nerve detection and helped further in nerve bundle scoring.

Radiological investigations, especially, USG plays an important role in classifying these lesions based on
the flow of the lesion. Although clinical diagnosis and radiological correlation are sufficient for diagnosis in most of the cases, confirmation of all resected specimens is must because many times vascular malformations can simulate hemangiomas clinically and radiologically. A few cases in our study were clinically misdiagnosed a lipoma or cyst. Few radiologically diagnosed cases of hemangioma were confirmed as AVM on histopathology. Hence, multidisciplinary approach and careful evaluation of each case of vascular lesion is essential for proper management.

Limitations
This was a retrospective study. In few cases, clinical and radiological correlation was not possible as they were not subjected to radiological investigation. These cases were diagnosed solely based on HPE. Although MT stain was used for identification of nerve bundles, S-100 immunostaining was not used for confirmation.

CONCLUSION
Hemangioma and vascular malformation, especially AVM, should be clearly differentiated to reduce the risk of treatment failure and recurrence. With the use of histochemical stains such as VVG and MT, the diagnostic difficulty can be reduced and certain characteristic features can be easily studied and evaluated.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Mitchell RN, Schoen FJ. Blood vessels. In: Kumar V, Abbas AK, Fausto N, et al., editors. Robbins and Cotran Pathologic basis of disease. 8th ed. Philadelphia, PA: Elsevier; 2009. p. 520.
2. Lowe LH, Marchant TC, Rivard DC, Scherbel AJ. Vascular malformations: Classification and terminology the radiologist needs to know. Semin Roentgenol 2012; 47:106‑17.
3. Ronchese F. The spontaneous involution of cutaneous vascular tumors. Am J Surg 1953; 86:376‑86.
4. Haggstrom AN, Drolet BA, Baselaa E, Chamlin SL, Garzon MC, Horii KA, et al. Prospective study of infantile hemangiomas: Clinical characteristics predicting complications and treatment. Pediatrics 2006; 118:882‑7.
5. Breugem CC, van Der Horst CM, Hennelam RC. Progress toward understanding vascular malformations. Plast Reconstr Surg 2001; 107:1509‑23.
6. Richter GT, Friedman AB. Hemangiomas and vascular malformations: Current theory and management. Int J Pediatr 2012; 2012:645678.
7. Gallagher PJ, van der Wal AC. Blood vessels. In: Mills SE, editor. Histology for Pathologists. 3rd ed. Philadelphia, PA, USA: Lippincott Williams and Wilkins; 2007. p. 217‑38.
8. Meijer‑Jorna LB, Breugem CC, de Boer OJ, Ploegmakers JP, van der Horst CM, van der Wal AC. Presence of a distinct neural component in congenital vascular malformations relates to the histological type and location of the lesion. Hum Pathol 2009; 40:1467‑73.
9. Bancroft JD, Gamble M. Theory & Practice of Histological Technique. 5th ed. New York: Churchill Livingstone; 2002.
10. Rachappa MM, Trivene MN. Capillary hemangioma or pyogenic granuloma: A diagnostic dilemma. Contemp Clin Dent 2010; 1:119‑22.
11. Koutlas IG, Jessurun J. Arteriovenous hemangioma: A clinicopathological and immunohistochemical study. J Cutan Pathol 1994; 21:343‑9.
12. Adegboyega PA, Qiu S. Hemangioma versus vascular malformation: Presence of nerve bundle is a diagnostic clue for vascular malformation. Arch Pathol Lab Med 2005; 129:772‑5.
13. Pawane P, Anshu, Gangane N. Hemangiomas versus arterio‑venous malformations: Role of elastic stains and mast cell density. Indian J Pathol Microbiol 2014; 57:191‑5.
14. Bates D, Taylor GI, Neugreen DF. The pattern of neurovascular development in the forelimb of the quail embryo. Dev Biol 2002; 249:300‑20.
15. Mukouyama YS, Gerber HP, Ferrara N, Gu C, Anderson DJ. Peripheral nerve‑derived VEGF promotes arterial differentiation via neuropilin 1‑mediated positive feedback. Development 2005; 132:941‑52.
16. Dalton SR, Fillman EP, Ferringer T, Tyler W, Elston DM. Smooth muscle pattern is more reliable than the presence or absence of an internal elastic lamina in distinguishing an artery from a vein. J Cutan Pathol 2006; 33:216‑9.
17. Glowacki J, Mulliken JB. Mast cells in hemangiomas and vascular malformations. Pediatrics 1982; 70:48‑51.
18. Enjolras O, Mulliken JB, Boon LM, Wassef M, Koazekwich HP, Burrows PE. Noninvoluting congenital hemangioma: A rare cutaneous vascular anomaly. Plast Reconstr Surg 2001; 107:1647‑54.
19. Mo JQ, Dimashkieh HH, Bove KE. GLUT1 endothelial reactivity distinguishes hepatic infantile hemangioma from congenital hepatic vascular malformation with associated capillary proliferation. Hum Pathol 2004; 35:200‑9.
20. Hashimoto T, Mesa‑Tejada R, Quick CM, Bollen AW, Joshi S, Pile‑Spelman J, et al. Evidence of increased endothelial cell turnover in brain arteriovenous malformations. Neurosurgery 2001; 49:124‑31.
21. Meijer‑Jorna LB, van der Loos CM, de Boer OJ, van der Horst CM, van der Wal AC. Microvascular proliferation in congenital vascular malformations of skin and soft tissue. J Clin Pathol 2007; 60:798‑803.
22. Lawley LP, Cerimele F, Weiss SW, North P, Cohen C, Koazekwich HP, et al. Expression of Wilms tumor 1 gene distinguishes vascular malformations from proliferative endothelial lesions. Arch Dermatol 2005; 141:1297‑300.
23. Jakubowski LA, Chun RH, Drolet BA, Jensen JN, North PE. Misdiagnosed as infantile hemangioma: Early presentation of small vessel‑rich AVM. Int J Pediatr Otorhinolaryngol Extra 2013; 8:71‑4.