HIF-1α and VEGF expression correlates with thrombus remodeling in cases of intravascular papillary endothelial hyperplasia

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Abstract: Intravascular papillary endothelial hyperplasia (IPEH) is histopathologically characterized by endothelium-lined papillary structures encircling an acellular fibrin core. The process of IPEH pathogenesis is unclear. The purpose of our study was to identify histopathological and immunohistochemical characteristics of IPEH to better understand the pathogenesis of this disease. After reviewing microscopic and medical records from Kyungpook National University Hospital, we selected 16 cases of IPEH. Masson’s trichrome and immunohistochemical staining as well as hematoxylin-eosin staining for 16 cases of IPH were performed. Immunohistochemical studies included CD31, CD68, mast cell tryptase, hypoxia-inducible factor-1 (HIF-1α), and vascular endothelial growth factor (VEGF). Sections from all our cases showed three distinct histological regions including a papillary portion with hyalinized fibrous or fibroblastic cores, an area containing an unorganized thrombus, and organization area with an ingrowth of endothelial cells, myofibroblasts, and fibroblasts. In the organization area, HIF-1α-positive cells were identified in the loose connective tissue. Endothelial cells forming vascular channels were negative for HIF-1α while VEGF was highly expressed in both interstitial mononuclear and endothelial cells. In the papillary portion, the cellular cores were strongly positive for both HIF-1α and VEGF, but the acellular cores were negative. Our investigation confirmed that IPEH is a reactive lesion that incidentally arises during the organization process of older thrombi. It was also found that HIF-1α and VEGF expression was dependent on the thrombus remodeling stage in cases of IPEH.

Keywords: HIF-1α, VEGF, hypoxia, thrombus, intravascular papillary endothelial hyperplasia

Introduction

Intravascular papillary endothelial hyperplasia (IPEH) is microscopically characterized by endothelium-lined papillary structures encircling an acellular fibrin core. In 1923, Pierre Masson [1] first described a peculiar papillary endothelial hyperplasia in the lumen of hemorrhoid veins. This disorder was known as “hemangioendotheliome vegetant intravasculaire” and was considered a vascular neoplasm. IPEH is now regarded as not as a neoplasm but a reactive lesion associated with thrombus organization within a vein or vascular abnormalities including arteriovenous malformations, cavernous hemangiomas, lymphangiomas, and pyogenic granulomas [1]. IPEH is pathologically classified into the following categories [2, 3]: a “pure” form that occurs within a dilated vascular space, a “mixed” form that appears as focal changes superimposed on preexisting vascular lesions (e.g., hemangioma, venous lake, aneurysm, arteriovenous malformation, lymphangioma, or pyogenic granuloma), and a third form that is an unclassifiable lesion which does not appear as either form. This third type is known as an extra-vascular form that occurs during hematoma organization. Aside from this information, the underlying the pathogenesis of this disorder are not well known.

The purpose of this study was to identify histopathological and immunohistochemical characteristics of IPEH occurring in various vascular lesions. In particular, we focused on cases of thrombus organization in the hypoxic environment created by the thrombus itself.
**Materials and methods**

**Materials**

Surgery and biopsy records of cases diagnosed as benign vascular lesions in the skin and soft tissue between 1999 and 2009 were reviewed. These data were recovered from the dermatopathology files of Kyungpook National University Hospital (Daegu, South Korea). After reviewing microscopic slides, we selected 16 cases of IPEH.

**Methods**

A diagnosis of vascular lesions was assigned based on the consensus of specialists from the dermatology, plastic surgery, and orthopedic surgery, radiology, and pathology departments of the hospital. The medical and microscopical records of the 16 cases were retrospectively reviewed. These records included information about patient gender, age, and symptoms along with the site, color, and size of the lesions, and previous imaging findings. All tissues obtained from 16 cases of IPEH were 10% phosphate-bufed formalin-fixed and paraffin-embedded. The tissues were processed, cut at a thickness of 4 μm, stained with hematoxylin-eosin and Masson’s trichrome, and evaluated by light microscopy.

In addition, immunohistochemical staining was performed to evaluate cellular composition and molecular pathogenesis for all cases. Tissue sections were cut at a thickness of 4 μm and placed on Probe on Plus microscope slides (Fisher Scientific). Using the Benchmark XT automated immunohistochemistry stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA), slides were stained as follow procedure. Detection was done using the Ventana Ultraview DAB Kit (Ventana Medical Systems). Sections were deparaffinized using EZ Prep solution. CC1 standard (pH8.4 buffer contained Tris/Borate/EDTA) was used for antigen retrieval for 60 min at 100°C. DAB inhibitor (3% H₂O₂, Endogenous peroxidase) was blocked for 4 min at 37°C temperature. Slides were incubated with antibodies CD31 (DAKO, Glostrup, Denmark, diluted 1:100), CD68 (DAKO, Glostrup, Denmark, diluted 1:100), mast cell tryptase (MCT; NeoMarkers, Fremont, USA, diluted 1:100), hypoxia-inducible factor-1α (HIF-1α; NOVUS, Littleton, USA, diluted 1:1000), and vascular endothelial growth factor (VEGF; BD Biosciences, Bedford, USA, diluted 1:100) for 32 min at 37°C, and a secondary antibody of Universal HRP Multimer for 8 min at 37°C. And then DAB+ H₂O₂ substrate for 8 min followed by hematoxylin and bluing reagent counter stain at 37°C. Reaction buffer (pH7.6 Tris buffer) was used as washing solution. After staining, the slides were mounted and evaluated by light microscopy.

**Results**

The 16 cases evaluated in the present could be classified as “pure” (six lesions) or “mixed” (10 lesions) forms. The mixed form was observed in three venous malformations, three venous lakes, two lymphatic malformations, one case of phlebitis, and one case of angiodermatitis. No lesions were identified as the unclassifiable “third” form.

**Clinical features (Table 1)**

Patient age at the time of sampling ranged from 2 to 59 years with an average of 34.3±16.1 years. There were slightly more male IPEH patients (56%, nine cases). The most common location of the lesion was the lip (five cases) followed by the palm, finger, and scalp (two cases each). The lesion usually presented as a localized swelling or nodules. Bluish or reddish discoloration of the overlying skin was observed in 15 patients while the remaining patient had no significant discoloration. Seven patients suffered from tenderness or pain and nine were asymptomatic. One patient had a history of trauma. The lesion diameter varied from 0.2 to 2.5 cm with a median of 1.1 cm.

**Histopathological findings**

Three distinct histological zones were identified in the histological sections from all cases within and around the thrombus: a thrombosis area (Figure 1A, inset), an organization area (Figure 2A, inset), and an IPEH area (Figure 3A, inset). Three zones were clearly identified by Masson’s trichrome staining. The thrombosis area was stained bright red, the papillary core of the IPEH lesion was vivid blue, and a subtle blue tint was observed in the organization area.

The thrombosis area was defined as a region containing an unorganized thrombus composed of a tangled mesh of fibrin, platelets, red blood cells, and degenerating leukocytes (Figure 1A). CD31-positive cells were not found.
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Table 1. Clinical features of the patients presenting IPEH

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Case</th>
<th>Gender/Age (years)</th>
<th>Lesion site</th>
<th>Pain/Tenderness</th>
<th>Color</th>
<th>Lesion size (cm)</th>
<th>Imaging history</th>
<th>Etiology</th>
</tr>
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<tbody>
<tr>
<td>Pure</td>
<td>1</td>
<td>M/45</td>
<td>scalp</td>
<td>+/-</td>
<td>red</td>
<td>0.5 x 0.2 x 0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F/17</td>
<td>scalp</td>
<td>+/+</td>
<td>skin</td>
<td>2.5 x 1.5 x 1.0</td>
<td>USG</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>F/22</td>
<td>palm</td>
<td>+/-</td>
<td>blue</td>
<td>0.8 x 0.3 x 0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M/37</td>
<td>palm</td>
<td>+/-</td>
<td>red</td>
<td>0.8 x 0.7 x 0.5</td>
<td>USG</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>M/28</td>
<td>lip</td>
<td>+/-</td>
<td>blue</td>
<td>0.4 x 0.4 x 0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>M/32</td>
<td>finger</td>
<td>+/-</td>
<td>blue</td>
<td>1.0 x 0.5 x 0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mixed</td>
<td>7</td>
<td>M/14</td>
<td>sole</td>
<td>+/-</td>
<td>blue</td>
<td>1.7 x 1.4 x 0.7</td>
<td>USG</td>
<td>VM</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>F/25</td>
<td>axilla</td>
<td>+/-</td>
<td>blue</td>
<td>1.8 x 1.6 x 0.7</td>
<td>MRI</td>
<td>VM</td>
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<tr>
<td></td>
<td>9</td>
<td>F/12</td>
<td>calf</td>
<td>+/-</td>
<td>blue</td>
<td>2.4 x 2.0 x 0.7</td>
<td>USG, CT</td>
<td>VM</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>M/55</td>
<td>lip</td>
<td>+/-</td>
<td>blue</td>
<td>0.5 x 0.3 x 0.2</td>
<td>-</td>
<td>VL</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>M/59</td>
<td>lip</td>
<td>+/-</td>
<td>blue</td>
<td>0.7 x 0.3 x 0.2</td>
<td>-</td>
<td>VL</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>F/51</td>
<td>lip</td>
<td>+/-</td>
<td>blue</td>
<td>0.5 x 0.5 x 0.3</td>
<td>MRI</td>
<td>VL</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>F/57</td>
<td>lip</td>
<td>+/-</td>
<td>blue</td>
<td>0.3 x 0.3 x 0.3</td>
<td>-</td>
<td>LM</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>M/15</td>
<td>chin</td>
<td>+/-</td>
<td>blue</td>
<td>2.0 x 2.0 x 1.5</td>
<td>-</td>
<td>LM</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>F/39</td>
<td>finger</td>
<td>+/-</td>
<td>red</td>
<td>0.7 x 0.5 x 0.4</td>
<td>-</td>
<td>phlebitis</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>M/40</td>
<td>scrotum</td>
<td>+/-</td>
<td>red</td>
<td>0.2 x 0.1 x 0.1</td>
<td>-</td>
<td>AK</td>
</tr>
</tbody>
</table>

USG, ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging; IPEH, intravascular papillary endothelial hyperplasia; VL, venous lake; VM, venous malformation; LM, lymphatic malformation; AK, angiokeratoma.

in this area even though extracellular CD31 staining was observed in the loose fibrin meshwork (Figure 1B). The majority of mononuclear cells were positive for CD68 or VEGF (Figure 1C, 1F) while MCT- or HIF-1α-positive cells were rarely seen (Figure 1D, 1E).

The organization area was defined as a region containing an organized or recanalized thrombus with an ingrowth of endothelial cells, myofibroblasts, and fibroblasts (Figure 2A). CD31 was expressed in the endothelial cells that formed vascular channels (Figure 2B) and many CD68-positive cells were observed in the loose connective tissue between the channels (Figure 2C). MCT-positive extracellular staining and MCT-immunoreactive cells were frequently found in the loose connective tissue (Figure 2D). Cells expressing high levels of HIF-1α or VEGF were diffusely dispersed but more plentiful than those in the thrombosis area. HIF-1α-positive cells were observed in the loose connective tissue while endothelial cells forming vascular channels were negative for HIF-1α. On the other hand, high levels of VEGF were seen not only in interstitial mononuclear cells but also endothelial cells that formed vascular channels (Figure 2E, 2F).

The IPEH area was defined as the location containing papillary structures with hyalinized fibrous or fibroblastic cores lined with single layer of plump endothelial cells positive for CD31. Based on the presence of cellular components within the papillae core, the IPEH area was divided into cellular and acellular areas (Figure 3A). Cores of the cellular papilla (Figure 3A) were composed of fibroblastic stroma containing a few mononuclear and endothelial cells. The acellular hyalinized cores (Figure 3A) that were covered by a flattened endothelium were completely devoid of mononuclear and endothelial cells. Impending development of an acellular papilla detached from the cellular papilla was observed in several specimens (Figure 3B, in the green circle). Both endothelial cells surrounding the acellular or cellular papillae and cells forming irregular channels reminiscent of vessels within the cellular papillae were CD31-positive while mononuclear cells within the core were negative (Figure 3B). Additionally, CD68- or MCT-positive cells were found within the cellular papillae cores but not the acellular cores (Figure 3C, 3D). Both HIF-1α and VEGF were highly expressed within the cellular papillary cores but not in the acellular papillary cores (Figure 3E, 3F). VEGF expression...
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Figure 1. Thrombosis area; (A) Degenerating red blood cells and aggregated platelets embedded in a fibrin mesh (H & E, 400 x magnification). (B) Extracellular CD31 staining in the loose fibrin meshwork (400 x magnification). (C) A few cells stained with an anti-CD68 antibody (400 x magnification). (D, E) A few cells positive for (D) MCT and (E) HIF-1α (400 x magnification). (F) VEGF was expressed in most mononuclear cells (400 x magnification).

Figure 2. Organizing area; A: Many inflammatory cells infiltrated the perivascular area (H & E, 400 x magnification). B: Endothelial cells forming vascular structure were positive for CD31 (400 x magnification). C: Several CD68-positive cells were observed (400 x magnification). D: A few cells positive for MCT, many of which showed degranulation (400 x magnification). E, F: Both HIF-1α and VEGF were highly expressed. HIF-1α was expressed in the majority of mononuclear cells but not in the endothelial cells. On the other hand, VEGF was expressed in both the mononuclear and endothelial cells (400 x magnification).
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was occasionally observed in endothelial cells encircling the papilla.

Discussion

Our investigation confirmed the clinical findings of IPEH [1]. The lesions we examined occurred at all ages (ranged from 2 to 59 years) and usually developed spontaneously as a single mass with bluish or reddish discoloration of the overlying skin. Seven patients suffered from tenderness or pain. We determined that these symptoms more associated with the “pure” form (50%) compared to the “mixed” form (40%).

Initially, it was debated whether or not IPEH lesions are a sarcoma. After it was decided that IPEH is not a sarcoma, questions about the etiology or pathogenesis of IPEH arose. To date, IPEH characteristics and mechanisms underlying the pathogenesis of this disorder are not well known. IPEH is generally thought to develop during the organization and recanalization of thrombi because IPEH lesions contain variable numbers of fresh and organizing thrombi. Potential outcomes of thrombi include the following: 1) propagation, 2) embolization, 3) dissolution, and 4) organization with recanalization [4]. Among these events, fibrinolytic dissolution and organization are processes of thrombus resolution. Rapid shrinkage or total lysis of recent thrombi can be achieved by activating fibrinolytic cascades. Older thrombi tend to be organized because those that are composed of extensively polymerized fibrin are more resistant to proteolysis (or fibrinolysis). Capillary channels may be formed during the organization process with the ingrowth of endothelial cells and fibroblasts into the thrombus and anastomose that creates conduits in the thrombus, thereby re-establishing the continuity of the original obstructed vessel lumen. Such recanalization may convert the thrombus into a mass composed of highly vascularized connective tissue that may be incorporated as a subendothelial swelling of the vascular wall. All stages of such organization and recanalization were simultaneously observed in our specimens, indicating that IPEH might be a reactive lesion associated with thrombus organization.
Histological sections from all our cases showed spatial and temporal variability. Organization areas composed of highly vascular connective tissue were juxtaposed next to thrombus areas. The organization areas were mixed with IPEH areas composed of cellular and acellular areas. Cellular areas associated with active, ongoing organization coexisted with acellular areas that lacked active collagen deposition. Impending acellular papillae that were detached from cellular papillae in the IPEH areas were also noted in our specimens.

Immunohistochemical staining revealed that HIF-1α and VEGF were highly expressed within the cellular cores in IPEH areas similar to the expression levels found in conventional organized areas. Hypoxic cells expressing HIF-1α or VEGF in the papillary cellular cores might respond to accumulative hypoxic insult by continuously activating the remodeling process and angiogenic cascade via HIF-1α. This could result in further fragmentation or remodeling of the papillae and vasculature restoration to a limited extent. On the other hand, the acellular cores consisting of fibrin or collagenous connective tissue with hyalinization were negative for both HIF-1α and VEGF, indicating the final stage of organization. Consequently, the acellular papillae lacking cells responding to hypoxia would not undergo the remodeling process and continue to present IPEH characteristics. Based on our findings, we believe that IPEH is associated with thrombosis, and is actually a variant of organization and recanalization (or remodeling). Unfortunately, we did not identify a factor responsible for the development of papillary growth during the organization process. Further investigation is essential to understand how papillary growth can progress during the organization process.

Hypoxia is generally correlated with the presence of thrombi, macrophages, angiogenesis, and the expression of HIF and VEGF [5]. Thrombi usually induced hypoxia and are initially penetrated by various inflammatory cells [6, 7]. Macrophages, as facilitators of healing processes like thrombus resolution, not only promote remodeling of the extracellular matrix [8] but also angiogenesis through the production of multiple cytokines including VEGF [9, 10], epidermal growth factor (EGF) [11], and platelet-derived growth factor (PDGF) [12]. This escalates the inflammatory reaction [13]. VEGF, Transforming Growth Factor-β (TGF-β), PDGF, and Transforming Growth Factor-α (TGF-α) promote angiogenesis, increase vascular permeability, and stimulate endothelial cell proliferation [4].

A recent study has shown that human macrophages responded to hypoxia by up-regulating the expression of transcription factors such as HIF-1α [14]. HIF-1α is actively degraded [15] under normoxic conditions and then the downstream of HIF-1α vanishes. On the other hand, HIF-1α degradation is blocked under hypoxic conditions, subsequently resulting in the initiation of VEGF cascades [16, 17] and induction of endothelial cell proliferation. In addition to VEGF cascade activation, HIF-1α might also regulate the expression of other target factors, including vascular endothelial growth factor-1 (VEGFR-1), endothelin-1, inducible nitric oxide synthase, and monocyte chemotactic protein-1, which have been confirmed as stimulators of matrix remodeling and angiogenesis [18-20]. This factor also regulates multiple cellular and physiologic processes including glucose/energy metabolism, apoptosis, and proliferation [21].

In our investigation, VEGF and HIF-1α expression was typically higher and more diffuse in the organization area and cellular papillae of the IPEH area than in the thrombosis area or acellular papillae of the IPEH area. Hypoxia-responsive cells positive for HIF-1α or VEGF were observed in the organization area and cellular papillae of the IPEH area with high levels of vascular proliferation in contrast to the thrombus area or acellular papillae. Combined with findings from previous studies [5, 14-17], our results indicate that hypoxia induced by thrombi might correlate with vascular proliferation and the presence of hypoxia-responsive cells expressing HIF-1α or VEGF. Furthermore, we determined that HIF-1α or VEGF expression was dependent on the thrombus remodeling stage in cases of IPEH.

In conclusion, we confirmed that IPEH incidentally arises during a variant of the organization process under the hypoxic conditions rather than as an isolated pathological entity. It was also determined that HIF-1α and VEGF expression was correlated with the thrombus remodeling stage in cases of IPEH. Taken together, our data suggested that thrombus-induced hypoxia might initiate the thrombus organization process, and is associated with vascular
proliferation as well as the expression of HIF-1α and VEGF.

Disclosure of conflict of interest
None.

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