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Original article

Adrenomedullin as a potential biomarker involved in patients with hereditary hemorrhagic telangiectasia

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ABSTRACT

Background: Adrenomedullin (AM) is a vasoactive peptide mostly secreted by endothelial cells with an important role in preserving endothelial integrity. The relationship between AM and hereditary hemorrhagic telangiectasia (HHT) is unknown. We aimed to compare the serum levels and tissue expression of AM between HHT patients and controls.

Methods: Serum AM levels were measured by radioimmunoassay and compared between control and HHT groups. AM levels were also compared among HHT subgroups according to clinical characteristics. The single nucleotide polymorphism (SNP) rs4910118 was assessed by restriction analysis and sequencing. AM immunohistochemistry was performed on biopsies of cutaneous telangiectasia from eight HHT patients and on the healthy skin from five patients in the control group.

Results: Forty-five HHT patients and 50 healthy controls were included, mean age (SD) was 50.7 (14.9) years and 46.4 (9.9) years ($p = 0.102$), respectively. HHT patients were mostly female (60% vs 38%, $p = 0.032$). Median [Q1-Q3] serum AM levels were 68.3 [58.1–80.6] pg/mL in the HHT group and 47.7 [43.2–53.8] pg/mL in controls ($p<0.001$), with an optimal AM cut-off according to Youden's J statistic of 55.32 pg/mL (J:0.729). Serum AM levels were similar in the HHT subgroups. No patient with HHT had the SNP rs4910118. AM immunoreactivity was found with high intensity in the abnormal blood vessels of HHT biopsies.

Abbreviations: ACVRL1, Activin A receptor type II-like 1 gene; ALK1, Activin receptor-like kinase 1; AM, adrenomedullin; AVM, arteriovenous malformation; BMP9, Bone morphogenetic protein 9; CLR, Calcitonin receptor-like receptor; CT, Computed tomography; EC, Endothelial cell; *ENG*, Endoglin gene; ESS, Epistaxis severity score; FAK, Focal adhesion kinase; GI, Gastrointestinal; HHT, Hereditary hemorrhagic telangiectasia; MAPK, Mitogen-activated protein kinase; PI3K, Phosphatidylinositol 3 kinase; PBS, phosphate-buffered saline; RAMP, receptor activity-modifying protein; SD, Standard deviation; SNP, Single nucleotide polymorphism; VMs, Vascular malformations.

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Conclusions: We detected higher AM serum levels and tissue expression in patients with HHT than in healthy controls. The role of AM in HHT, and whether AM may constitute a novel biomarker and therapeutic target, needs further investigation.

1. Introduction

Hereditary hemorrhagic telangiectasia (HHT) or Rendu–Osler–Weber syndrome (ORPHA774) is a rare autosomal dominant vascular disease characterized by telangiectases and larger vascular malformations (VMs) of the pulmonary, cerebral, or hepatic vasculature [1]. The hallmark of HHT is telangiectasis, which is an abnormal communication between an arteriole and a dilated and tortuous venule in the capillary bed. HHT can be diagnosed either through molecular genetic test or using the Curaçao clinical criteria (recurrent epistaxis, cutaneous/mucosal telangiectasia, visceral VMs, and a first-degree family member with HHT) [2,3]. Mutations in the endoglin (*ENG*) and activin A receptor type II-like 1 (*ACVRL1*) genes are detected in approximately 90% of cases submitted for molecular diagnosis and cause HHT1 and HHT2, respectively [4,5].

Endoglin (encoded by *ENG*) is an auxiliary co-receptor at the endothelial cell surface that promotes BMP9 (Bone morphogenetic protein 9) signaling through the activin receptor-like kinase 1 (ALK1; encoded by *ACVRL1*). Both proteins contribute to the signaling hub formed by BMP9–Endoglin–ALK1–Smad with high impact in endothelial cell (EC) proliferation, migration, and survival during angiogenesis. Loss of endoglin and ALK1 proteins function provoke anomalous vascular overgrowth due to the overactivation of phosphatidylinositol 3-kinase (PI3K) signaling [6,7]. Although endoglin and ALK1 are components of the same BMP9 receptor complex, pathogenic variants in their genes are related to different clinical phenotypes [4,8]. Pulmonary arteriovenous malformations (AVMs) and brain VMs are more common in patients with HHT1, while hepatic VMs are more common in HHT2 [9]. HHT exhibits age-related penetrance as epistaxis is usually the earliest clinical manifestation and most patients develop larger VMs before 40 years [4, 10]. Moreover, there is high inter- and intra-familial variability in vascular involvement and clinical manifestations [3]. Overall, the low prevalence and high clinical variability make understanding HHT a challenge, especially in uncommon situations [11].

Adrenomedullin (AM) is a 52 amino acid peptide hormone that belongs to the amylin/calcitonin gene-related peptide family and was first discovered in human pheochromocytoma cells [12]. AM is synthesized as part of a larger precursor molecule, named pro-AM, and is secreted by many cells/tissues, but mostly by vascular endothelial and smooth muscle cells [13]. This hormone has multiple actions that are exerted through combinations of the calcitonin receptor-like receptor (CLR), and either receptor activity-modifying protein 2 (RAMP2) or RAMP3, also known as AM1 and AM2 receptors, respectively [14]. AM plays critical roles in blood vessels, with vasodilatory properties and helps to regulate vascular stability and permeability by modulating the endothelial barrier [14-17]. Moreover, it participates in regulating circulatory homeostasis and in the pathogenesis of certain cardiovascular diseases [18]. It is important to note that carriers of the rs4910118 single nucleotide polymorphism (SNP), close to the AM gene, possess lower circulating levels of AM than carriers of the major allele [19]. This genetic variant may be relevant to life-long risk for different diseases, such as cancer [20].

Several knockout studies have shown that embryos that lack AM signaling (AM-/-) die in utero due to leaky and unstable blood and lymphatic vessels [21,22]. VMs have also been found among the vascular abnormalities of mice lacking AM [23]. Therefore, AM is required for the development and/or maintenance of the vasculature during embryogenesis and also appears to be essential for adult angiogenesis [24]. In addition, AM also has an integral role in linking blood flow with nitric oxide production and vasodilatation [25]. We hypothesized that AM signaling may be involved in the pathophysiology of HHT. Therefore, the aim of this study was to compare serum levels and tissue expression of AM between patients with HHT and healthy controls.

2. Material and methods

2.1. Study design and patients

In this exploratory prospective study, consecutive HHT patients were selected from the referral HHT Unit at the Hospital Universitari de Bellvitge (Barcelona, Spain). This HHT Unit caters for adult patients from all over Catalonia (Spain), which has about 7.5 million inhabitants. Consecutive patients from our database were considered eligible for enrolment if they were over 18 years old and had a positive genetic study for *ENG* or *ACVRL1*. A group of healthy volunteers were recruited from the regular donors of the Blood Bank at Hospital San Pedro (Logroño, Spain), and served as a control group. All participants provided written informed consent and all data were anonymized. Personal and clinical data collection for the study were performed in line with the Spanish Data Protection Act (*Ley Orgánica 3/2018 de 5 de diciembre de Protección de Datos Personales*). The study was approved by the Clinical Research Ethics Committee of the Hospital Universitari de Bellvitge (Barcelona, Spain; ethic approval number PR344/18).

The primary objective of the study was to compare serum AM levels between patients with HHT and healthy controls. As a secondary aim, we performed immunohistochemical studies for AM in biopsies of cutaneous telangiectasia from patients with HHT and compared these with healthy skin.

2.2. Baseline clinical variables and tests

Clinical baseline characteristics and complementary test results were collected. A “definitive” diagnosis of HHT was made if three or more of the Curaçao criteria were present [2]. The severity of nosebleeds was measured according to the epistaxis severity score (ESS), with epistaxis considered moderate or severe for scores of >4 or >7 points, respectively [26]. Anemia was defined as hemoglobin levels <13 g/dL for men and <12 g/dL for women. Because of known HHT age-related penetrance, we divided HHT patients in two distinguishable subgroups according to disease evolution (patients <40 years and >50 years) [4,10].

Contrast transthoracic echocardiography was performed to screen for pulmonary AVMs, followed by thoracic computed tomography (CT) angiography to confirm their presence, if appropriate [3]. Abdominal CT angiography was performed to study hepatic and/or abdominal VMs [27]. Gastrointestinal (GI) endoscopy was performed if either anemia was disproportionate to the degree of epistaxis or there was objectively confirmed overt GI bleeding [28]. Genetic tests were performed by Health in Code, S.L. (A Coruña, Spain), using next-generation sequencing.

2.3. Adrenomedullin serum levels

Blood samples from patients with HHT were collected at Hospital Universitari de Bellvitge (Barcelona, Spain) and from healthy donors at Hospital San Pedro (Logroño, Spain). Serum and buffy coat fractions were separated. Subsequently, the samples were anonymized and sent to the Center for Biomedical Research of La Rioja (CIBIR) for analysis.

Blood serum concentrations of AM were determined using a commercially available RIA kit (Phoenix Pharmaceuticals, Inc.,

Karlsruhe, Germany). Samples (1 mL) were initially diluted in an equal volume of 0.1% alkali-treated casein in phosphate-buffered saline (PBS), pH 7.4, and applied to pre-washed reverse-phase Sep-Pak C-18 cartridges (Waters Corp., Milford, MA). The peptide fraction was eluted from the C18 matrix with 3 mL 80% isopropanol containing 0.125 N HCl and freeze-dried overnight, as previously described [29]. AM levels in lyophilized extracts were then determined by RIA following manufacturer's instructions.

We then compared the serum AM levels of HHT patients and those of healthy volunteers. Subsequently, the serum AM concentrations of patients with HHT were compared by different subgroups: sex, age (<40 years and >50 years), genetic test result (*ENG* and *ACVRL1*), and clinical findings (ESS ≤4 and >4 points or the presence/absence of GI involvement, pulmonary AVMs, and hepatic VMs).

2.4. Single nucleotide polymorphism detection

Due to lower circulating AM levels in carriers of the SNP rs4910118, we also evaluate the presence of this SNP in HHT patients compared to its prevalence in the general population [19,20]. Genomic DNA was isolated from the buffy coat fraction of the blood samples with the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA). SNP rs4910118 (*C*>*T*) was genotyped by the polymerase chain reaction and restriction fragment length polymorphism method, as previously described, with slight modifications [30]. The following primers were used: 5′-CTACGTGGGAGTCAGCACAC-3′ and 5′-ACAATGAAGGTCTCAGGCC G-3′. PCR products were digested for 1 h at 37 °C with Cfr42I/SacII (New England Biolabs, Ipswich, MA), followed by detection on 2% agarose gels. Confirmation of the PCR genotyping was accomplished by direct sequencing of 12 randomly chosen samples. As normal controls, we used a group of 500 DNA samples from healthy donors of advanced age, from Spain, as published previously [20].

2.5. Cutaneous telangiectasia biopsies

Cutaneous telangiectasia biopsies and control samples from healthy skin in resection borders from melanoma patients were restained from two of our previously reported studies [6,7]. Briefly, a punch biopsy (3 mm) from a cutaneous telangiectasia on the fingertip of each patient was obtained by a senior dermatologist under sterile conditions. Biopsy samples were encrypted according to a code assigned to each patient and fixed in buffered formalin, dehydrated, and embedded in paraffin. All patients gave their signed informed consent for telangiectasia biopsy in accordance with local ethics committee requirements.

2.6. Immunohistochemistry studies

For AM immunohistochemistry studies, tissue samples of digital cutaneous telangiectasia were taken from four patients with HHT1 and four patients with HHT2, and five healthy skin fragments were obtained from the resection borders of melanomas. The tissue sections were dewaxed in xylene and the endogenous peroxidase was blocked with 3% H_2O_2 in methanol for 15 min. Samples were rehydrated and subjected to antigen retrieval (10 mM Sodium Citrate, 0.5% Tween 20, pH 6.0, 20 min at 95ºC). Non-specific binding was blocked by exposure to Protein block buffer (Novocastra Leica Biosystems, Newcastle, UK) for 30 min. Then tissue sections were incubated with rabbit polyclonal antibody against human AM (ab69117, Abcam, Cambridge, UK), at 1:200 dilution and 4ºC overnight. The following day, sections were incubated with Novolink Polymer (Novocastra Leica Biosystems) followed by exposure to 3,3′-diaminobenzidine (Dako, Carpinteria, CA). Slides were lightly counterstained with hematoxylin and analyzed with an Eclipse 50i microscope (Nikon, Tokyo, Japan) equipped with a DXM 1200c digital camera (Nikon). Substitution of the primary antibody by PBS in serial sections was used as a negative control.

2.7. Statistical analysis

We calculated sample size for the comparison of AM blood levels between the HHT and control groups. Assuming that the HHT group would present a minimum difference of 12 units and a variance (S^2) of 260, we needed a minimum of 40 individuals per study arm (HHT vs healthy controls) when using a bilateral Student's t-test with a confidence level of 95%, a statistical power of 80%, and an estimated 5% loss of samples during the study.

All categorical variables are expressed as frequencies and proportions, and continuous variables as means and standard deviations (SD) or medians and interquartile range [Q1-Q3]. Normality of the distribution was assessed using the one-sample Kolmogorov–Smirnov test. For those variables that were not normally distributed, results are presented as medians with interquartile range. We used the chi-square or Fisher's exact tests to compare categorical data between groups. Two-tailed unpaired Student *t*-tests were used to compare normally distributed continuous data, and the Mann-Whitney *U* test for non-normally distributed continuous data comparisons. The optimal AM cut-off point to differentiate between HHT and healthy controls was determined by Youden's *J* statistic [31]. A two-sided *p*-value lower than 0.05 was statistically significant. Analyses were performed using IBM SPSS Statistics, version 19.0 for the PC (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Baseline characteristics

We included 45 HHT patients and 50 healthy donors in the control group. Mean ages were 50.7 (14.9) and 46.4 (9.9) years, respectively ($p = 0.102$). Most patients with HHT were female (60% vs 38%, $p = 0.032$) (Table 1). In the HHT group, the *ENG* and *ACVRL1* genetic variants were present in 23 (51%) and 22 (49%) patients, respectively. Pulmonary AVMs were detected in 21 (46.7%), hepatic VMs in 28 (62.2%), and GI involvement in 17 (37.8%), but no patient with HHT had cerebral VMs.

3.2. Adrenomedullin blood levels

Serum AM levels were measured in 45 HHT patients and 50 healthy controls by RIA. The RIA assay showed that patients with HHT had significantly higher serum AM concentrations than the healthy control group. Median [Q1-Q3] serum AM levels were 68.3 [58.1–80.6] pg/mL in HHT patients and 47.7 [43.2–53.8] pg/mL in the control group ($p < 0.001$) (Table 1; Fig. 1). The optimal cut-off for AM to differentiate between HHT and healthy controls, according to Youden's J statistic, was 55.32 pg/mL ($J = 0.729$).

Serum AM concentrations in the HHT group were then compared according to previously defined subgroups. No significant differences were found by sex ($p = 0.484$), age ($p = 0.371$), or *ENG / ACVRL1* mutations ($p = 0.489$) (Table 2; Fig. 2). There were also no significant differences between serum AM concentration and the presence of the following clinical characteristics: nosebleed severity according to the ESS ($p = 0.198$), history of anemia ($p = 0.644$), and the presence of either pulmonary AVMs ($p = 0.061$), hepatic VMs ($p = 0.771$), or GI bleeding ($p = 0.246$) (Table 2).

Table 1
Characteristics of patients with HHT.

	HHT	Controls	p-value
Patients, n	45	50	
Age, years; mean (SD)	50.7 (14.9)	46.4 (9.9)	0.102
Sex (females), (%)	27 (60%)	19 (38%)	0.032
AM pg/mL, median [Q1–Q3]	68.3 [58.1–80.6]	47.7 [43.2–53.8]	<0.001

AM: Adrenomedullin; HHT: Hereditary hemorrhagic telangiectasia.

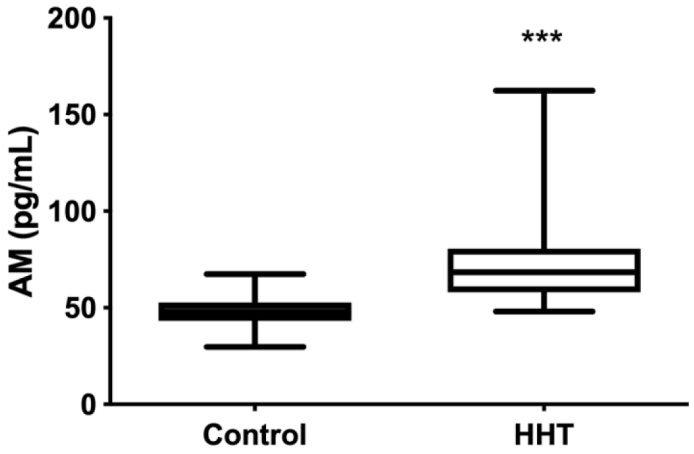

Fig. 1. AM serum levels in the 45 patients with HHT and the 50 healthy controls. The levels of AM were measured by RIA in the serum of healthy controls ($n = 50$) and HHT patients ($n = 45$). Box plots represent the interquartile range with the median as a horizontal line. Whiskers encompass the maximum and minimum values of the population. ***: $p<0.001$ compared to Control.

Table 2
AM levels in patients with HHT by clinical and genetic differences.

Sex	**Female**	**Male**	**p-value**
N, (%)	27 (60%)	18 (40%)	
AM pg/mL, mean (SD)	75.05 (23.70)	70.34 (18.88)	0.484
Age, years (yrs)	**<40 yrs**	**>50 yrs**	**p-value**
N, (%)	11 (24.4%)	24 (53.3%)	
AM pg/mL, mean (SD)	68.22 (15.55)	76.07 (26.55)	0.371
Genetic test	***ENG***	***ACVRL1***	**p-value**
N, (%)	23 (51.1%)	22 (48.9%)	
AM pg/mL, mean (SD)	75.39 (25.62)	70.83 (17.22)	0.489
ESS	**≤4 points**	**>4 points**	**p-value**
N, (%)	31 (68.9%)	14 (31.1%)	
AM pg/mL, mean (SD)	69.54 (15.56)	81.20 (30.80)	0.198
Anemia	**No**	**Yes**	**p-value**
N, (%)	21 (46.7%)	24 (53.3%)	
AM pg/mL, mean (SD)	71.53 (27.38)	74.6 (15.9)	0.644
Visceral involvement	**No**	**Yes**	**p-value**
Pulmonary AVM, n (%)	24 (53.3%)	21 (46.7%)	
AM pg/mL, mean (SD)	67.14 (17.28)	80.04 (27.91)	0.061
Hepatic AVM, n (%)	17 (37.8%)	28 (62.2%)	
AM pg/mL, mean (SD)	74.4 (23.13)	72.42 (SD 21.35)	0.771
Gastrointestinal, n (%)	5 (11.1%)	17 (37.8%)	
AM pg/mL, mean (SD)	92.69 (28.87)	76.81 (25.37)	0.246

ACVRL1: Activin A receptor type II-like 1; AM: Adrenomedullin; AVM: Arteriovenous malformation; *ENG*: Endoglin; ESS: Epistaxis Severity Score.

3.3. Analysis of SNP rs4910118

All patients with HHT were evaluated for the presence of SNP rs4910118, but none had the minor allele (either heterozygous or homozygous). In addition, only eight out of 500 healthy donors had the minor allele (7 heterozygous and 1 homozygous), but no significant differences were found between both populations ($p = 0.80$).

3.4. Morphological and immunohistochemical studies

HHT biopsies were compared with normal skin sections. In HHT, the skin epithelium covering the telangiectases was thicker than the epithelium of normal skin and contained a very thick layer of keratin. This difference can be attributed to the different biopsy sites: from the fingertips in HHT patients and from non-acral zones in controls. HHT samples were characterized by the presence of vascular abnormalities, including tortuous and very dilated blood vessels in the dermis. No morphological differences were observed between samples from the HHT1 and HHT2 patients (Fig. 3).

Concerning AM immunoreactivity, it was detected in the epithelium and associated structures, including hair follicles, sweat and sebaceous glands, in normal skin samples. By contrast, while the epithelium and glands were also positive for AM in HHT samples, the abnormal blood vessels (telangiectases) expressed high levels of AM immunoreactivity

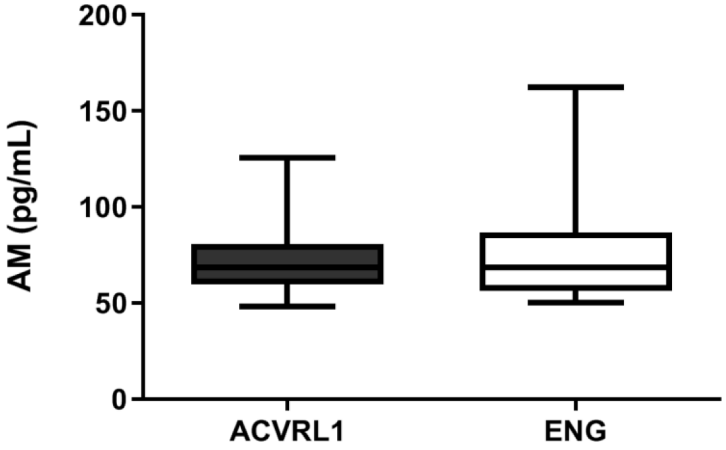

Fig. 2. AM serum levels among patients with HHT by *ENG* ($n = 23$) and *ACVRL1* ($n = 22$) mutations. The levels of AM were measured by RIA in the serum of HHT patients bearing the *ACVRL1* ($n = 22$) or the *ENG* ($n = 23$) mutations. Box plots represent the interquartile range with the median as a horizontal line. Whiskers encompass the maximum and minimum values of the population. No statistically significant differences were found between both groups.

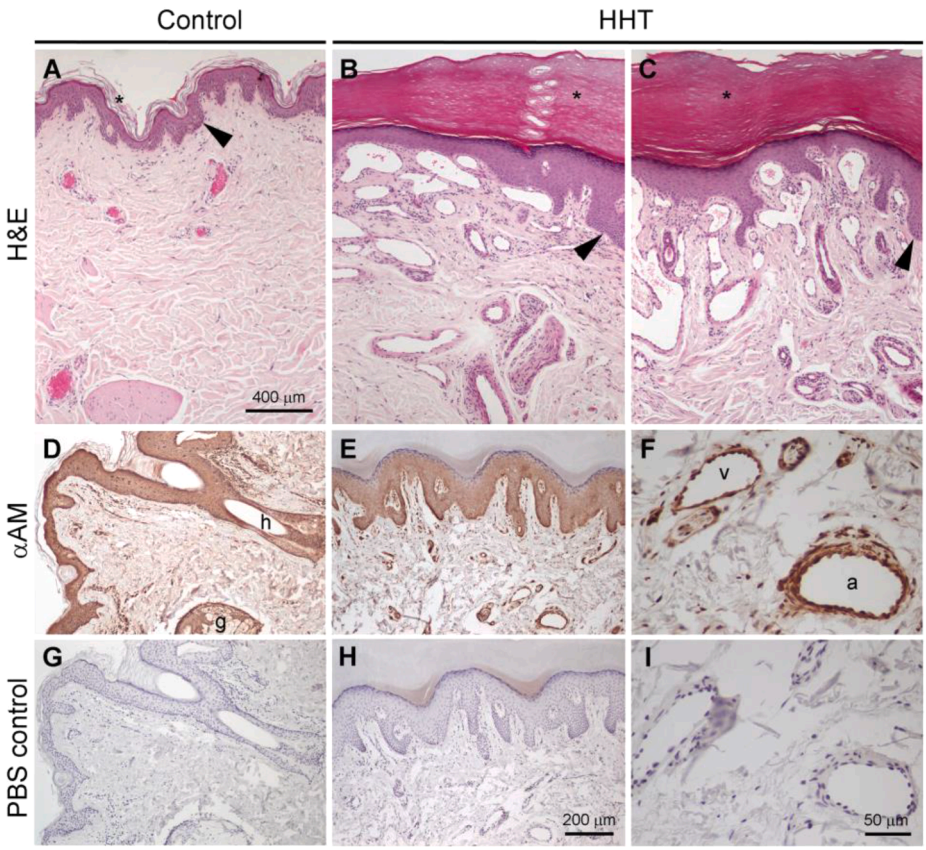

Fig. 3. Morphological and immunohistochemical characterization of HHT samples. Normal skin (A, D, G) and HHT biopsies (B, C, E, F, H, I) were stained with hematoxylin & eosin (A-C), with an antibody against AM (D-F), or with PBS as a specificity control (G-I). Samples from HHT1 (B, E, F, H, I) and HHT2 (C) patients were used. The skin epithelium is pointed out by arrowheads. The keratin layer is indicated by asterisks. A sebaceous gland (g) and a hair follicle (h) are shown in D. Panels F and I are higher magnifications of the dilated arteriole (a) and venule (v) seen in E. Scale bar for A-C: 400 μm; for D,E,G,H: 200 μm; for F,I: 50 μm.

involving the endothelium and surrounding smooth muscle cells. Negative controls, substituting the primary antibody by PBS, corroborated staining specificity (Fig.3).

4. Discussion

To our knowledge, this is the first study to investigate a possible relationship between AM and HHT. We found significantly higher AM serum levels and stronger tissue expression in patients with HHT than in healthy controls. In fact, AM directly stimulates angiogenesis and its inhibition reduces angiogenesis in animal models and endothelial cells [23,24,32]. However, how exactly AM interacts with endothelial cell biology in patients with HHT remains unknown.

Our results are consistent with previous studies where mouse and human EC with compromised BMP9 signaling, through either genetic deletion or inhibition, led to higher AM levels [33]. The elevated levels of AM found in our HHT patients may result from this faulty BMP9 signaling, caused by mutations in endoglin or ALK1 proteins, causing the known dysregulation of the signaling hub formed by BMP9–Endoglin–ALK1–Smad [8,34,35]. A study of transforming growth factor beta (TGF-β) in mice found that AM levels were lower prenatally but became significantly elevated postnatally in knockout mice compared with the wild-type mice. It was suggested that these elevated AM levels in adults that follow dysregulation of receptor signaling may be characteristic of the whole TGF-β family, including BMP9 [36].

In addition, it is known that loss of the BMP9/ALK1/Endoglin pathway causes overstimulation of the PI3K and ERK/mitogen-activated protein kinase (MAPK) signaling hubs, which in turn, promotes endothelial overgrowth [34]. Our group has previously shown that PI3K/AKT/mTOR1 pathway overstimulation is present in the telangiectases of patients with both HHT1 and HHT2 [6,7]. Other research has also shown that AM-induced angiogenesis in EC is mediated by activation of PI3K/AKT, MAPK and focal adhesion kinase (p125FAK) [24,37]. Khalfaoui-Bendriss et al. stated that using anti-AM and anti-AM receptor antibodies can induce regression in tumor neovessels. They suggested that the mechanism by which AM blockers exert their antiangiogenic effect was by inhibiting the VE-cadherin/β-catenin complex, but also by inactivating AKT through interference with EC proliferation and gene expression [38]. Therefore, signaling in the angiogenesis and vascular stability through PI3K/AKT dependent pathway, could be mediated by AM.

We demonstrated that patients with HHT have a similar frequency of SNP rs4910118 to the general population, indicating that this SNP is not involved in its pathogenesis. Given that the minor allele is associated with lower levels of circulating AM and given that we reported higher AM levels in HHT patients, the lack of correlation was to be expected [19]. Although HHT is an autosomal dominant vascular disease, significant inter- and intra-familial variability is observed [3,4,39]. However, this phenotypic variability cannot be explained exclusively by the predominant type of mutation and may be influenced by additional factors such as modifying genes, hormones, or a second hit [40,41]. Though not statistically significant, patients aged >50 years and those with an ESS >4 showed a tendency to having higher AM levels. This is consistent with the clinical worsening of epistaxis by age, and it supports the idea that higher AM levels result from BMP9/ALK1/Endoglin haploinsufficiency rather than being the primary driver of a patient's phenotype [4]. In a previous study assessing gender-related alterations in plasma AM levels in 346 japanese residents, women showed lower AM levels than men [42]. So, the higher female distribution in our HHT patients compared with controls, highlights the robustness of our results showing higher AM serum levels in HHT patients than in controls.

AM also plays an important role in regulating the growth of epidermal epithelium and in maintaining the barrier function of the skin through its antimicrobial properties [43-45]. Accordingly, we demonstrated AM immunoreactivity in the epithelium and associated glands of both normal skin and that of patients with HHT. However, a more

intense AM immunoreactivity was specifically found in the endothelial and smooth muscle cells of patients with HHT telangiectases compared with controls. This finding strengthens the concept that endoglin or ALK1 mutations in EC can lead to dysregulation of the BMP9–Endoglin–ALK1–Smad pathway, and therefore, higher AM levels [8,34,35]. Given the vasodilating properties of AM, its enhanced local expression in endothelial and smooth muscle cells may cause variations in blood flow and contribute to development of abnormal vessel growth and VMs [46]. Besides vasodilatation, AM plays an important role in preservation of endothelial integrity, and it is known that increased plasma AM levels correlate with excessive fluid volume [47]. This stabilizing of endothelial barrier function could be a protective response of AM to limit fluid overload [14].

Several pharmacological modulators of AM have been described and could be used to intervene in HHT patients. These modulators include monoclonal antibodies [48], polyclonal antibodies against either the peptide [49] or the receptors [50], the peptide fragment AM_{22-52} [51], and small interfering RNAs [52]. Adrecizumab is a humanized, monoclonal, non-neutralizing AM binding antibody that has been shown to restore the impaired vascular barrier function that causes hemodynamic instability in sepsis, by long-lasting increase of plasma AM levels [48]. This is consistent with our results and also supports the idea that the higher AM levels observed in HHT patients aims to restore the endothelial barrier function and tone of blood vessels. In addition, several small molecules have been identified that can either increase or decrease AM functions [53].

Our study has some limitations that must be mentioned. First, there was no established reference cut-off for AM for the purpose of this study. Second, AM was measured in different clinical situations of HHT, which could have influenced the levels. Third, the small number of available paraffin-embedded biopsies prevented us from performing a quantitative analysis of AM immunoreactivity. However, the consistency of higher serum AM levels and high tissue expression in patients with HHT suggests that this peptide has a role in the pathogenesis of HHT.

In conclusion, we demonstrated higher serum levels and tissue expression of AM in patients with HHT compared with healthy controls. Future studies will need to investigate whether AM could be used as a novel biomarker and therapeutic target for patients with HHT.

Ethics approval and consent to participate

The study was approved by the Clinical Research Ethics Committee of the Hospital Universitari de Bellvitge (Barcelona, Spain; ethic approval number PR344/18).

Consent for publication

This manuscript does not contain any individual's data in any form. Each patient was identified by a unique alphanumeric identification code and all data were anonymized and analyzed as aggregates.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

AI, AM, and AR-M conceptualized and designed the study; AI, LO-C, JG-S, PC, PG, JÑ-I, JMM-L, FV and AJ acquired the data; AI, LO-C, PC, JG-S, FV, AM, and AR-M analyzed the data and interpreted the findings; AI and PC performed statistical analysis; LO-C, JG-S, PG, JÑ-I, and AM performed the adrenomedullin serum tests, single nucleotide polymorphism tests, and immunohistochemistry studies; all authors contributed to drafting the manuscript and approved the final version; AR-M obtained Grant Supports.

Code availability

Not applicable

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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