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Expression patterns of aquaporin 1 in vascular tumours

Background: Aquaporins (AQPs) are a family of water channels expressed in various body tissues. Beyond osmotic balance, AQPs have recently been confirmed to be involved in processes related to cancer (tumour proliferation, angiogenesis, *etc*.). *Objectives:* To analyse the presence of these proteins in the endothelium of several vascular tumours, both benign and malignant, in order to establish whether AQPs may be used as a marker or future therapeutic target. *Materials and Methods:* We studied AQP1 expression in 39 patients with vascular tumours, classified into six groups according to ISSVA classification: haemangiomas, benign vascular tumours different from infantile haemangiomas, angiosarcomas, classic Kaposi's sarcoma (KS), and epidemic KS. *Results:* AQP1 expression was present in 28 of 39 patients, representing 92.9% benign lesions, whereas no expression was found in 72% of malignant lesions. AQP1 expression was associated with benign lesions with an OR of 34.5 (95% CI: 5-250); $p<0.0005$, and was most frequently identified with a focal endothelial pattern (38%). A kappa index of 0.823 (95% CI: 0.678-0.971) was determined regarding the patterns of expression overall. *Conclusion:* The expression of AQP1 was greater in benign lesions than malignant lesions and this difference was statistically significant, thus AQP1 expression could serve as a marker for benignity of vascular tumours. In addition, the expression pattern of AQP1 was different according to the type of vascular tumour.

Key words: angiosarcoma, aquaporins, infantile haemangioma, Kaposi's sarcoma, pyogenic granuloma, vascular tumour

Vascular anomalies are a heterogeneous group of diseases that affect a large number of patients, from paediatric age to senescence. Clinical and histological expression of these diseases, as well as their course and prognosis, are very variable. According to the ISSVA (International Society for Study of Vascular Anomalies), they are classified into two major types: vascular malformations and tumours [1]. Likewise, vascular tumours can be divided into benign lesions (such as infantile haemangioma [IH] or pyogenic granuloma), and malignant tumours (angiosarcoma or Kaposi's sarcoma [KS]).

On the other hand, aquaporins (AQPs) are a family of transmembrane water channel proteins, which are widely distributed in biological systems. Thirteen isoforms of these proteins have been described, which play a major role in transcellular water movement and regulation of osmotic balance [2]. AQPs are expressed in permeable tissues (such as kidney tubules and glandular epithelia), but also in non-permeable tissues (such as adipose tissue and astroglia) [3]. Recent evidence shows that beyond water transport, AQPs may play a key role in several tumour-related processes, including tumour oedema, tumour cell migration, tumour proliferation, and angiogenesis [4-8].

In this study, we analysed the expression of AQP1 in proliferating vascular endothelial cell membranes. Since AQP1 is linked to cell proliferation, this protein is expected to be expressed at a high level in vascular tumours. Moreover, we investigated whether AQP1 is expressed in the cells of malignant tumours or whether, given the genetic and phenotypic changes, expression is lost. The expression patterns of AQP1 were also investigated in order to establish whether it is possible to use this protein as a marker for vascular lesions. Finally, we examined whether a high level of AQP1 expression is associated with neovascularization that accompanies these tumours, as reported in other studies, which could imply that this protein is useful as a therapeutic target in the future.

Patients and methods

This was a cross-sectional observational study. Data from the Department of Dermatology and from the Multidisciplinary Committee of Vascular Anomalies of Hospital Virgen del Rocío of Seville were used for analysis. We included 39 samples of anomalies and vascular tumours collected from January 2005 to June 2011. The necessary criteria to be included in our study were: a clinical diagnosis by a dermatologist following ISSVA classification and confirmation by a dermatopathologist. Lesions were classified into six groups according to ISSVA classification: haeman-

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giomas, benign vascular tumours different from infantile haemangiomas, angiosarcomas, classic KS, and epidemic KS. The controls were healthy skin samples from five volunteers, aged from 16 to 40 years. All participants signed an informed consent form, and the Ethical Committee of Hospital Universitario Virgen del Rocío approved the study. Immunohistochemical labelling for AQP1 was used on all samples. To corroborate positive or negative expression of AQP1, the vascular marker CD34 was used as a control for all samples.

Variables studied were the type of vascular anomaly, type of haemangioma according to proliferation (superficial proliferative, superficial involuting, mixed proliferative, mixed involuting), age, sex, and AQP1 expression pattern based on immunohistochemistry. The AQP1 expression patterns observed were negative, diffuse endothelial (appearing uniformly within the lesion), focal endothelial (appearing as a membrane reinforcement in isolated or patchy areas of the lesion), focal inverse (on the periphery in a patched form), and diffuse inverse (on the periphery in a diffuse form).

For statistical analysis, percentages or absolute frequencies were used to describe the distribution of subgroups of vascular anomalies, sex, and AQP1 expression and expression pattern. Age distribution was described with median and interquartile range, since this was a non-normal sample (calculated using the Shapiro Wilk test).

Bivariate analysis was performed to study whether there was a statistically significant association between AQP1 expression and the type of vascular tumour, using the chi-square test or Fisher's exact test. The Mann-Whitney U test was performed to establish whether there was association between AQP1 expression and age. All confidence intervals were calculated at 95% and the *p* value was considered statistically significant below 0.05.

To check the reproducibility of the immunohistochemical analysis of AQP1, each sample was randomly assigned by two independent observers. The kappa concordance index was calculated for AQP1 positivity and the expression pattern (kappa values: <0.4=non-concordance, 0.4-0.6=moderate concordance, and > 0.6=good concordance).

Statistical analyses were conducted using SSPS, version 17.0.

Results

The demographic distribution according to sex and age of the patients included in the study is shown in *table 1* and *figure 1*. The controls of healthy skin showed positive AQP1 expression in the endothelium of the dermis as well as in the reticular fibres of the superficial dermis, with no expression in the epidermis. CD34 was positive in all vessels with a diffuse endothelial pattern that was present in haemangiomas, vascular malformations, pyogenic granulomas, and the tumour component of angiosarcomas and KS.

In our sample, 74.2% were lesions with a benign behaviour, whereas the remaining 25.8% were malignant (*table 1*).

AQP1 expression was identified in 28 of 39 patients, representing 92.9% of benign lesions, whereas no expression was found in 72% of malignant lesions (8/10). AQP1 expression was associated with benign lesions with an OR of 34.5 (95% CI: 5-250); $p<0.0005$ (*table 2*). The distribution of expression is shown in *table 3* and *figure 2*. The most frequent pattern was focal endothelial (38%), followed by negative (28.2%) and diffuse endothelial (23.1%). All haemangiomas were positive for AQP1. Distribution according to histological type and proliferative stage of haemangiomas is presented in *table 3*; a focal pattern was most frequently found in both the superficial and mixed forms. A

Table 1. Demographic distribution of patients included in the study.

Vascular anomaly	*n*	%	Men	Age
Infantile haemangioma	15	38.4%	5 (33%)	2.5
Superficial	8	20.5%	3 (20%)	2.65
Mixed	7	17.9%	2 (13%)	1.42
Pyogenic granuloma	7	17.9%	6 (86%)	26.7
Angiosarcoma	4	10.3%	2 (50%)	67.8
Kaposi's sarcoma	6	15.4%	5 (83%)	56.7
Classic	3	7.7%	2 (33%)	79.3
Epidemic	3	7.7%	3 (50%)	32.3
Vascular malformations	7	17.9%	4 (57%)	20.6
Capillary	4	10.3%	3 (43%)	32
Venous	2	5.1%	1 (14%)	7.5
Lymphatic	1	2.6%	-	1.5
Total	39	100%	21 (54%)	9

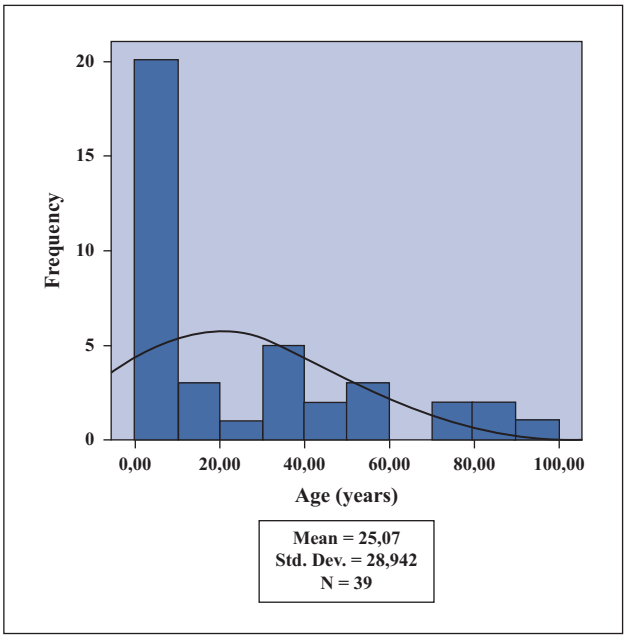

Figure 1. Frequency histogram showing the distribution of patient age in the study.

Table 2. AQP1 expression in benign and malignant lesions.

	Positive	Negative
Benign	92.9%	7.1%
Malignant	28%	72%

OR = 34; 95% CI: 5-250; p<0.0005.

Table 3. Distribution of the different AQP1 expression patterns.

Vascular anomaly	Diffuse	Focal	Negative	Inverse
Proliferative infantile haemangioma	-	4 (26%)	-	2 (14%)
Superficial	-	3 (20%)	-	1 (7%)
Mixed	-	1 (7%)	-	1 (7%)
Involuting infantile haemangioma	2 (14%)	5 (33%)	-	2 (14%)
Superficial	1 (7%)	3 (20%)	-	-
Mixed	1 (7%)	2 (13%)	-	2 (14%)
Pyogenic granuloma	4 (57%)	1 (14%)	2 (29%)	-
Angiosarcoma	-	-	4 (100%)	-
Classic Kaposi's sarcoma	-	-	3 (100%)	-
Epidemic Kaposi's sarcoma	-	2 (66%)	1 (33%)	Vacuolar
Capillary vascular malformation	4 (100%)	-	-	-
Venous vascular malformations	1 (50%)	1 (50%)	-	-
Lymphatic	-	-	1 (100%)	.

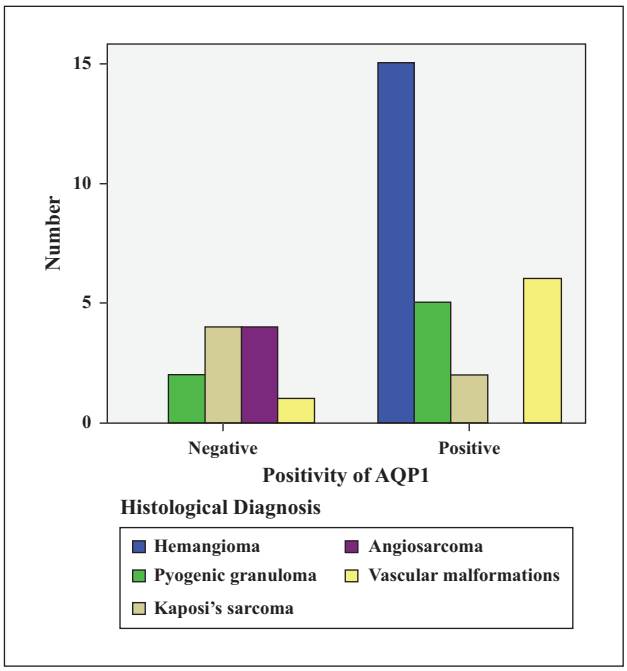

Figure 2. Frequency histogram showing the distribution of AQP1 expression.

diffuse pattern was consistently demonstrated in capillary vascular malformations, and was present in venous vascular malformations but not in lymphatic malformations. Two KS showed focal positivity, the remainder were negative, with an OR of 4 (p=0.83). We found no statistically significant relationship between expression pattern and histological stage or type of haemangioma. A kappa index of 0.823 (95% CI: 0.678-0.971) was determined regarding the expression patterns overall.

Discussion

In our study, we found a higher level of expression of AQP1 in benign lesions versus malignant lesions, and this difference was statistically significant, thus AQP1 expression could serve as a marker for benignity of vascular tumours. This result is congruent with previous studies carried out on cerebral haemangioendothelioma, in which AQP1 was shown to be a marker of benignity for another type of tumour with similar histopathology [9–11].

AQPs are transmembrane proteins that delimit a hydrophobic channel, which can only be traversed by water molecules [2]. The ions, including hydroxide ion and protons, cannot pass through this channel [12]. Thirteen types of AQPs have been described, with AQP1 being the most abundant in membranes and present in distal convoluted tubules, red blood cells, and the endothelium throughout the body, except in the brain. Several studies have shown an increase in AQP1 expression in various tumours, including reactive astrocytes and endothelial neovascularization of brain tumours [5], central nervous system hemangioblastomas [11], multiple myeloma [13], glial tumours [14], lung adenocarcinomas, and bronchioalveolar carcinomas [15]. Increased AQP3 expression was also found in colorectal cancer cell lines [16]. The mechanism by which AQP1 is involved in tumour genesis is not yet well established and recent evidence points to an important role in angiogenesis, cell proliferation, and migration [4-8]. In our study, we could not find statistically significant differences in AQP1 expression in progressing tumours versus regressing tumours, probably due to the small sample size. However, we were able to show that benign lesions present a higher level of AQP1 expression than malignant lesions.

Among benign lesions, infantile haemangioma is the most common benign tumour of childhood (affecting up to 10% before the first year of life) and is more frequent in girls and premature infants [17, 18]. Although the aetiopathogenesis is poorly understood, recent studies have implicated hypoxia as one of the major stimuli responsible for benign tumours such as IH [19, 20]. In this regard, Kleinman *et al*. found an increase in VEGF, MMP-9, and HIFα in blood and biopsies of patients with proliferating childhood haemangiomas, suggesting that a hypoxic stimulus might be involved in the aetiopathogenesis of these lesions [21]. Due to ischemia, the increment and stabilization of HIFα promotes the local production of SDF-1 (transcription factor derived from stromal cells) and VEGF-A, and these

mediators could increase the recruitment of endothelial progenitor cells. Other studies suggest that this process could be modulated by the action of oestrogens [22].

In our study, two lesions with evident clinical and histological signs of ulceration (a pyogenic granuloma and proliferative IH) were found to have much more intense AQP1 expression than non-ulcerated samples. Ulceration clinically translates into tissue hypoxia, which could explain the increased expression of AQP1 as a possible oxygen transporter. As a consequence, both higher expression of AQP1 and ulceration would probably be subsequent to hypoxia of tumour tissues. These findings are congruent with previous studies, in which hypoxia has been shown to stimulate AQP1 overexpression, as well as being a stimulus for neoangiogenesis and tumour proliferation [4-6, 23, 24].

In relation to malignant tumours, we did not find differences between angiosarcomas and KS. Nevertheless, it is noteworthy that two of the samples of the HIV-associated epidemic types showed focal positivity without compromising the spindle-cell component of the tumour. It would be interesting to study a greater number of samples of patients with KS in order to establish whether positive AQP1 expression could be used as a prognostic marker, especially for common forms. On the other hand, analysis of a superior cohort of patients with malignant tumours, such as kaposiform haemangioendothelioma (which was not included in our study), would allow us to establish the possible utility of AQP1 as a differential marker of malignancy for vascular tumours during paediatric age.

In addition, we found intense expression of AQP1 within neovascularization that accompanied two of the resected angiosarcomas in comparison with normal endothelia. This reinforces the findings from previous studies in which AQP1 appears to play a crucial role in tumour migration and proliferation, despite its absence in tumours of vascular origin, in which other aetiopathogenic alterations are likely to occur. This finding would open new future lines of research regarding the therapeutic arsenal for cancer, with the target being inhibition of neovascularization through annulment of AQP1 selectively in these cells. In a recent study, Wang *et al*. made progress in this direction, demonstrating that knocking down AQP1 suppressed cell viability, migration, and invasion, and promoted apoptosis of ovarian cancers cells [25].

We found no difference in AQP1 expression between males and females, although haemangiomas have been shown to be more frequent in women [22]. Although AQP1 expression decreased with age in our study, this is probably a confounding factor because malignant neoplasms would be more frequent in elderly patients.

Our study presents some limitations. The incidence of the pathologies described is low, for example, cutaneous angiosarcoma is diagnosed on average in one case per year in our centre. As a consequence, the number of patients included in some cases was low. Another limitation is the fact that the excised lesions are those with worst clinical evolution, either because they present an ulcerated form, because of their anatomical location, or because of a lack of response to treatment. Pathologies with therapeutic alternatives that do not require surgical excision or histological confirmation for diagnosis were not included, however, it is difficult to assemble a large cohort of such patients if no histological samples are taken in daily clinical practice.

Conclusions

We present a descriptive study of AQP1 expression in vascular tumours in which we found a greater level of expression in benign lesions versus malignant lesions, and this difference was statistically significant. Moreover, we identified a qualitatively different level of expression between benign tumours and vascular malformations, although this was not statistically significant. To sum up, we propose AQP1 as a possible marker to establish the degree of malignancy of vascular lesions as well as a possible future therapeutic target. ■

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