



# The vascular characteristics of melasma

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Received 27 October 2006; received in revised form 24 January 2007; accepted 24 January 2007

## KEYWORDS

Melasma;  
Angiogenesis;  
Vascular endothelial  
growth factor

## Summary

**Background:** The pathogenesis of melasma is not yet fully understood. Previous studies indicate that dermal environment such as fibroblasts may have an important role in the development of melasma. Recently, it has been suggested that interactions between the cutaneous vasculature and melanocytes might have an influence on the development of pigmentation.

**Objectives:** We investigated the vascular characteristics in melasma lesions. The expression of vascular endothelial growth factor (VEGF), a major angiogenic factor of the skin, was also investigated in melasma.

**Methods:** Erythema intensity was quantified by the increase of the  $a^*$  parameter using a colorimeter. Skin samples were obtained from lesional and non-lesional facial skin of 50 Korean women with melasma. Immunohistochemistry was performed to determine the expression of factor VIIIa-related antigen and VEGF in melasma.

**Results:** The values of  $a^*$  was significantly higher in the melasma lesion than that of perilesional normal skin. Computer-assisted image analyses of factor VIIIa-related antigen-stained sections revealed a significant increase of both the number and the size of dermal blood vessels in the lesional skin. There was significant relationship between the number of vessels and pigmentation in melasma. The expression of VEGF was significantly increased in melasma.

**Conclusions:** These data suggest that increased vascularity is one of the major findings in melasma. VEGF may be a major angiogenic factor for altered vessels in melasma.

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## 1. Introduction

Melasma is a common acquired symmetrical hyper-melanosis on sun-exposed areas of the skin. It is very common in *oriental women*. The major etiological factors include genetic influences, exposure to

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ultraviolet (UV) radiation, and sex hormones [1]. However, the pathogenesis of melasma is not yet fully understood.

Previous reports have demonstrated that lesional melasma skin shows more prominent solar elastosis compared with normal skin, suggesting that the dermal change may influence the development of melasma [2]. Very recently, we suggested that dermal inflammation induced by accumulation of UV irradiation may be associated with activation of fibroblasts, which result in the up-regulation of stem cell factor in melasma dermal skin leading to increased melanogenesis [3]. These studies have given us new insights on the pathogenesis of melasma, in particular, that the dermal environment may have an important role in the development of melasma.

Recent studies have suggested that a connection between vessels and cutaneous pigmentation could exist. Human melanocytes may respond to angiogenic factors because normal human melanocytes express functional vascular endothelial growth factor (VEGF) receptors [4]. Also, it has been reported that the topical plasmin inhibitor, tranexamic acid, is effective in the treatment of UV-induced hyperpigmentation [5]. Localized microinjection of tranexamic acid improved melasma *in vivo* [6]. Tranexamic acid prevents the binding of plasminogen originating from endothelial cells, to keratinocytes, which is thought to be a possible mechanism for melasma treatment [5]. These *in vitro* and *in vivo* findings suggest that interactions between the altered cutaneous vasculature and melanocytes may have an influence on the development of hyperpigmentation in the overlying epidermis.

A major clinical characteristic feature of melasma is hyperpigmented patches, but we have observed that melasma patients have additional distinguishing features like pronounced telangiectatic erythema confined to the melasma lesional skin. Therefore, in the present study, we have investigated the vascular characteristics of melasma lesions. Our data confirmed that melasma lesions show significant increase in vascularity. We then investigated the expression of VEGF, a major angiogenic factor of the skin, in altered vasculature of melasma lesions.

## 2. Materials and methods

### 2.1. Patients

We examined 50 Korean women with newly diagnosed melasma, which was determined by physical

examination and confirmed by histological examination. Informed written consent was obtained from each patient prior to skin sampling. This study was approved by the ethical committee of Ajou University Hospital.

### 2.2. Colorimetric measurement

The lesional melasma and perilesional normal skin (usually within 1 cm away from the lesional border) of 30 melasma patients were evaluated. The subjects had a wash-out period for at least 2 weeks from bleaching products, UV light therapy and steroid containing triple agents and a wash-out period for at least 3 months from lasers, dermabrasion, and chemical peeling. The intensity of erythema was measured by skin reflectance with a tristimulus color analyzer (Chromameter CR300, Minolta, Japan) and expressed in the  $L^*a^*b^*$  system. This system allows a color to be quantified according to three axes: white–black or lightness ( $L^*$ ), red–green or chrome ( $a^*$ ) and yellow–blue or hue ( $b^*$ ). The  $a^*$  parameter was used as the measurement of redness.

### 2.3. Immunohistochemistry and image analysis

Skin biopsy specimens were obtained from 50 melasma patients. Two-millimeter punch biopsies from lesional melasma and perilesional normal facial skin (usually within 1 cm away from the lesional border) were obtained from each patient under local anesthesia. Fifty pairs of tissue were prepared for routine and immunohistopathological examination by 10% formalin fixation. Melanin pigment was visualized with the Fontana-Masson stain, performed by the usual methods without an eosin background stain. The amount of pigmentation was measured by the ratio of pigmented area to measured epidermal area (PA/EA) in lesional melasma and perilesional normal skin. The measurement was done under constant magnification at 200 $\times$ .

Paraffin-embedded sections (4  $\mu\text{m}$ ) of both lesional and perilesional normal skin were processed for immunohistochemistry as described earlier [7]. A monoclonal antibody to factor VIIIa-related antigen (Immunon<sup>TM</sup>, Pittsburgh, PA, U.S.A.) was diluted 1:100 and incubated for 20 min at 48 °C. At 100 $\times$  magnification, the number of vessels  $\text{mm}^{-2}$ , the average vessel size and the relative area occupied by blood vessels were determined in the dermis of each slide section. The measurement was performed in an area within 100  $\mu\text{m}$  distance from the epidermal–dermal junction [8]. A monoclonal antibody to VEGF (Santa Cruz Biotechnology, Inc., Santa Cruz,

CA, U.S.A.) was diluted 1:50 and incubated for 20 min at 48 °C. The stained area per epidermal area (SA/EA) was measured in lesional and perilesional skin. Each measurement was evaluated under constant magnification. For each frame, the tracing was repeated three times and the mean was used for evaluation. The image was analyzed using Image Pro Plus Version 4.5 (Media Cybernetics Co., MD, U.S.A.).

## 2.4. Statistics

Comparison of erythema intensity and vascularity between melasma lesional and perilesional normal skin was tested with two-sided unpaired Student's *t*-test. The relationship between pigmentation and vascularity was tested with Pearson's correlation coefficients. A possibility value of less than 0.01 was considered as statistically significant. SPSS 11.0 statistics program was used for analysis.

## 3. Results

### 3.1. Erythema intensity in melasma patients

On physical examination, melasma patients exhibited pronounced telangiectatic erythema within lesions of melasma (Fig. 1). The values of  $a^*$  was higher in the melasma lesion (mean value of Rt. and Lt. cheek area,  $12.92 \pm 1.96$  and  $12.78 \pm 2.19$ ) than



**Fig. 1** Pronounced telangiectatic erythema noticed on melasma lesion.

that of perilesional normal skin (mean value of Rt. and Lt. cheek area,  $10.24 \pm 1.35$  and  $10.53 \pm 1.73$ ,  $p < 0.01$ ).

### 3.2. Immunohistological evaluation of vascularity in melasma patients

To investigate whether vascularity is increased in melasma lesions, we performed immunohistochemistry for factor VIIIa-related antigen. We found increased numbers of enlarged blood vessels in the melasma lesion (Fig. 2B), as compared with perilesional normal skin (Fig. 2A). Computer-assisted image analyses of factor VIIIa-related antigen-stained sections revealed a significant 16.28% increase in vessel size (Fig. 2C) and 33.89% increase in vessel density (Fig. 2D) in melasma skin compared to perilesional normal skin. This resulted in an overall increase of 68.75% in the cutaneous area covered by blood vessels in melasma skin compared to perilesional normal skin (Fig. 2E).

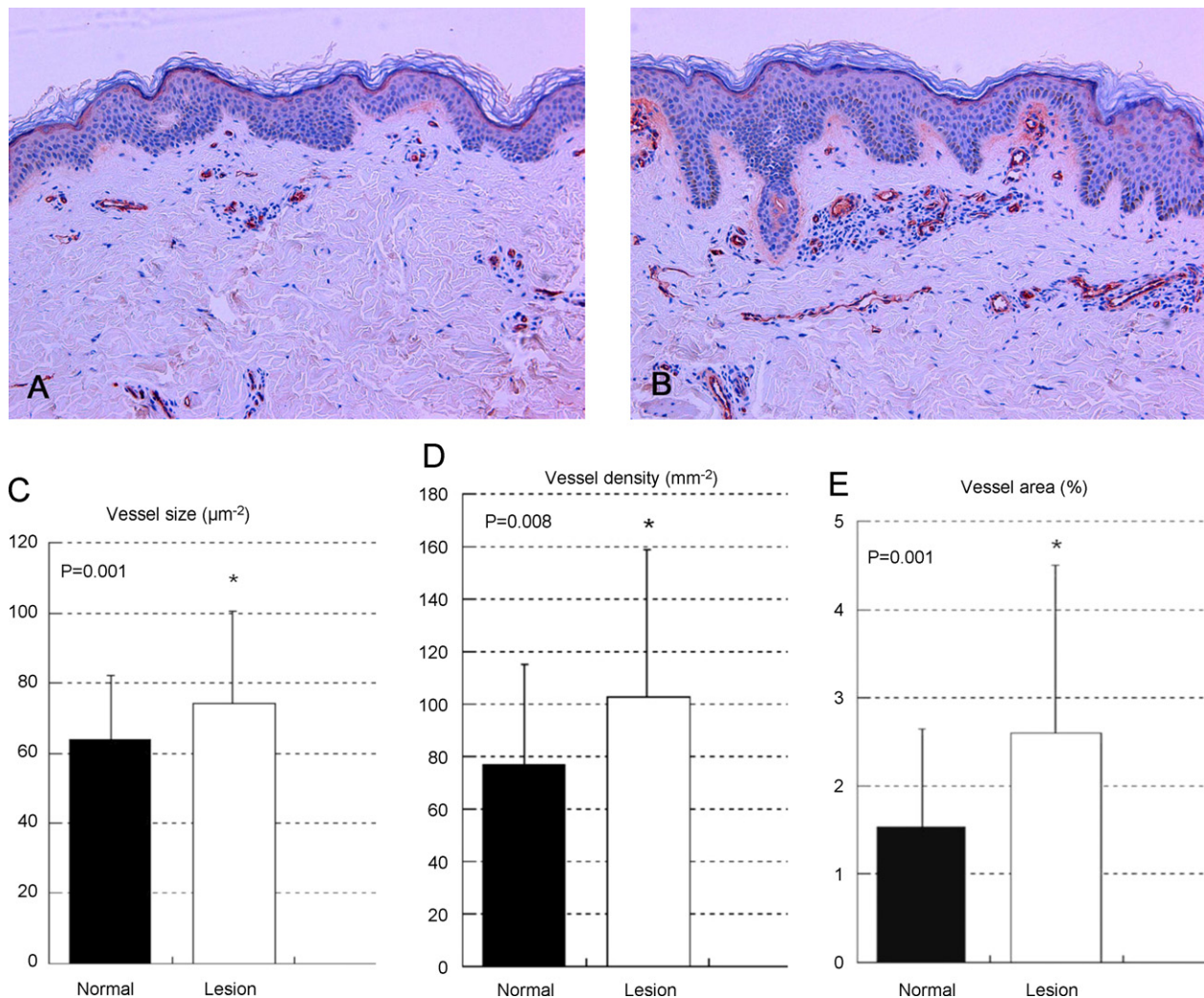
As shown in Table 1, there was a positive relationship between number of vessels and pigmentation in melasma lesional skin. However, no appreciable relationship was found between pigmentation and vessel size or area.

### 3.3. VEGF expression was increased in melasma

To examine whether the increased angiogenesis in melasma lesions is associated with increased expression of VEGF, we examined the expression of VEGF protein using immunohistochemistry. In melasma lesions, distinctly positive immunoreactivity against VEGF was noticed in keratinocytes whereas perilesional normal skin only showed weak immunoreactivity in keratinocytes (Fig. 3). The SA/EA of perilesional normal skin samples was  $0.137 \pm 0.132$ , and that of lesional melasma skin samples was  $0.270 \pm 0.237$ . This difference was statistically significant ( $P = 0.034$ ). In addition, dermal blood vessels, and fibroblasts were also faintly stained. However, the staining pattern of perilesional and lesional skin was similar in papillary and reticular dermis.

## 4. Discussion

We have demonstrated that a significant increase of both the number and size of dermal blood vessels in the lesional skin is one of the major findings in melasma. Interestingly, the increase in the number of vessels was more prominent than the increase in vessel size in lesional melasma skin. This finding



**Fig. 2** Immunohistochemistry for factor VIIIa-related antigen revealed enlarged and elongated blood vessels in the upper dermis (B), as compared with perilesional normal skin (A). Computer assisted morphometric analysis of factor VIIIa-related antigen stained sections revealed a significant increase in vessel size (C), vessel density (D) and the relative area covered by blood vessels (E), as compared with perilesional normal skin.

suggests that the erythema noticed in melasma patients could be due to angiogenesis as well as telangiectasia.

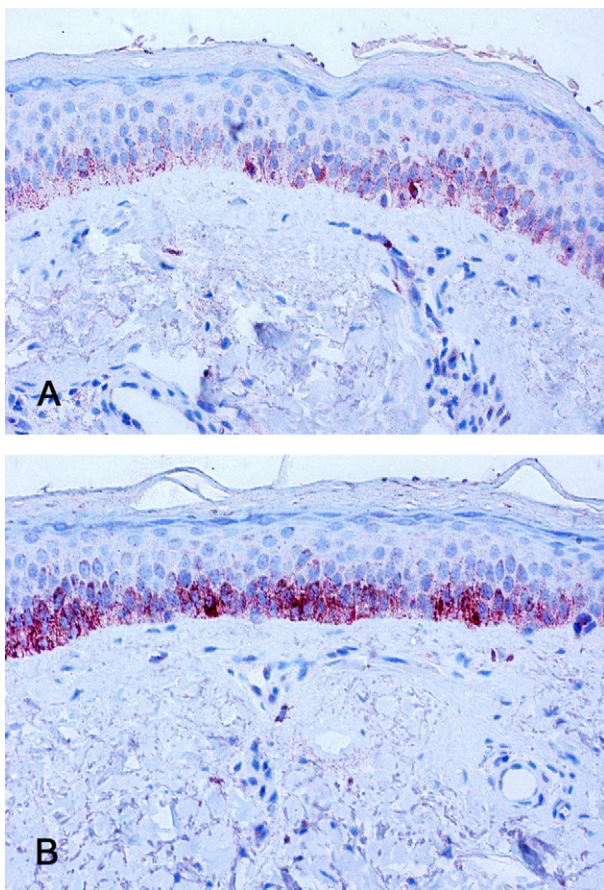
Angiogenesis, the formation of new blood vessels from preexisting vessels, is restricted to the perifollicular vasculature during the growth phase of hair follicles in normal skin [9]. However, the skin can initiate a rapid angiogenic response during wound healing and inflammation. For example,

UVB irradiation of the skin induces an angiogenic switch, associated with the up-regulation of proangiogenic factors such as VEGF, basic fibroblast growth factor, and interleukin-8 [10–12]. In our study, we found that VEGF expression was more upregulated in melasma lesions compared to perilesional normal skin. These results suggest that VEGF is a major angiogenic factor in vascular alteration noticed in melasma.

**Table 1** The relationship between epidermal pigmentation and vascularity

Level of pigmentation	Number of vessels ( $\text{mm}^{-2}$ )	Vessel size ( $\mu\text{m}^2$ )	Vessel area (%)
Perilesional normal skin	0.031	0.179	0.103
Lesional melasma skin	0.467*	0.169	0.163

Each value represents Pearson's correlation coefficient ( $r$ ). There was a significant positive correlation between pigmentation and number of vessels. \* $p < 0.01$ .



**Fig. 3** Immunostaining with an antibody to VEGF. The expression of VEGF was significantly increased at lesional epidermis (B) compared with perilesional normal skin (A). Original magnification 400 $\times$ .

The significance of these increased vascularity in melasma is unclear. It is well known that UV irradiation induces angiogenesis so the vascular alteration in melasma might be just a result of chronic UV accumulation accompanying epidermal hyperpigmentation. However, both lesional and perilesional facial skin had been exposed to chronic UV irradiation, but only lesional melasma skin showed pronounced vascular changes. Furthermore, the number of vessels had a positive relationship with pigmentation in melasma lesional skin. So, it is possible to speculate that the increase in vascularity was not only an epiphenomenon of UV damage but that it may play an important role in the pathogenesis of melasma by an increase in factors released from these proliferated vessels. However, although our patients were newly diagnosed, it should be noted that steroid containing triple reagent can induce telangiectasia because people usually apply triple reagent for the treatment of melasma.

VEGF is known to stimulate the release of arachidonic acid and the phosphorylation and activation of cytosolic phospholipase A<sub>2</sub> [13]. It is possible

that the resulting metabolites from the arachidonic acid pathway may affect melanogenesis [14]. This hypothesis is further supported by a report that normal human melanocytes express functional vascular endothelial growth factor receptors [4]. Therefore VEGF could have a direct influence on melanocyte behavior through its receptor. Also, blood vessels or endothelial cells modified by UV radiation may release cytokines and soluble factors like plasminogen, which might be a possible cause of hyperpigmentation in melasma [15]. The biological role of cutaneous blood vessels in the pathogenesis of melasma remains an interesting topic for future studies.

In summary, we have shown that vascularity is increased in melasma lesions. This present study may offer future perspectives for pathogenesis and therapeutic consideration of melasma.

## Acknowledgment

This work was supported by the Korea Science and Engineering Foundation through Chronic Inflammatory Disease Research Center Ajou University Grant R13-2003-019.

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