ORIGINAL ARTICLE

Expression of Vascular Endothelial Growth Factor (VEGF) in Melanocytic Skin Alterations

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ABSTRACT

Introduction. The study of growth factor expression allows further development of therapeutic modalities in the treatment of malignant diseases of the skin. This study aims to determine the relationship between the level of VEGF expression and morphological parameters (biological behavior of lesions, histological type, the defect on the surface, the density of inflammatory infiltrate, mitotic index, stage of growth and cell type) in melanocytic nevi and melanomas of the skin in different regions.
Methods. The study included skin biopsy material of 73 patients, divided in two groups (group I-melanomas, group II- nevi). The following parameters were determined: histological type, thickness of alteration (Breslow), Clark level, pTNM stage, the width of alteration, the density of lymphocytic infiltration of the tumor, mitotic index, stage of tumor growth, the presence of ulceration, tumor cell type, location and level of expression of VEGF.
Results. Most of benign melanocytic alterations in the skin shows low expression levels of VEGF in 91.18% of cases. In the group of melanomas, a high level of expression was seen in 61.54 % of cases. Nodular and acral lentiginous type of melanoma more often showed a high level of expression of VEGF, while superficial spreading melanoma often showed a low level of VEGF expression.
Conclusion. Benign melanocytic alterations have low, while malignant melanocytic alterations have high level of expression of VEGF.

KEY WORDS

Vascular endothelial growth factor; skin; melanocytic alterations; prognosis.

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Nevomelanocytic nevi are formed by nevomelanocytic clusters in the epidermis (junctional nevus), in the dermis (intradermal nevus), or on both places (compound nevus).[1] They are distinguished from other nevi by the ability of malignant alteration towards skin melanoma.[2] Melanoma is a heterogeneous disease of the skin and mucous membranes which shows a significant increase in worldwide incidence in the past decades (from 2.7 to 6.0 of 100 000 residents per year in men and from 4.6 to 8.5 of 100 000 residents in women).[3] Because of clinical and biological characteristics, the World Health Organization (WHO) has offered the classification of melanoma, where because of the frequency,are described as superficial spreading melanoma, nodular, and acral lentiginous melanoma.[4]

The skin retains the ability of rapid neovascularization, or secondary angiogenesis in response to numerous pathological stimuli, injury, inflammatory dermatoses, and neoplasia.[5]Processes that occur during the angiogenic cascade are regulated by various factors, stimulators and inhibitors, whose balance limits the process.[6] Stimulators of angiogenesis are: growth factor of endothelial cells lining the blood vessels (VEGF), basic and acidic fibroblast growth factors (b-FGF, aFGF, FGF-2, FGF-1), endothelial cell growth factor originating from platelets (PDECGF), angiopoetin-1, and others.[7]

The VEGF family consists of five isoforms: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor. VEGF-A, also known as vascular permeability factor, or simply VEGF, was described as a potent endothelial cell

mitogen which stimulates the proliferation and migration of endothelial cells. VEGF is overexpressed in almost all solid tumors and correlates with vascularity, grade, and prognosis. Several studies have examined the expression of members of the VEGF signaling pathway in melanoma. Secretion of VEGF occurs during progression of early cutaneous melanocytic lesions, with low VEGF expression in benign nevi increasing significantly in dysplastic nevi and more so in malignant melanoma. [8] The transition of melanomas from the radial to the aggressive vertical growth phase is also marked by increased VEGF production. [9,10] Tumor blood flow in melanomas thicker than 0.9 mm was detected using Doppler ultrasound, and endogenous VEGF expression and secretion in melanoma tumor cells were later established. [11]Statistical analysis showed that the expression rate of VEGF in choroidal melanoma was much higher than that in the control group, and was dependent on tumor size, which suggested that VEGF played a role in the progression of choroidal melanoma by stimulating angiogenesis required forpromotion of tumor growth. [12]

The study of growth factor expression allows further development of therapeutic modalities in the treatment of malignant diseases of the skin. Targeted vascular treatment decreases the possibility of creating new blood vessels in tumor, and thus indirectly affects tumor cells and slows tumor growth and development.[13]

The aim of this study was to determine the correlation between the level of VEGF expression and morphologic parameters (biologic behavior of lesion, histological type, surface defects, inflammatory infiltrate density, mitotic index, stage of growth, and cell type) in melanocytic nevi and skin melanoma fromdifferent anatomical regions.

Materials and methods

Patients

The research was done on bioptic skin samples of 73 patients with melanocytic skin alterations, taken at the Clinical Center, Banjaluka between 2004 to 2007. Based on histopathological analysis, patients were divided into two groups: group I - 39 patients with melanoma and group II - 34 patients with nevi.

Morphological analysis

In all specimens, the following was determined: the histological type- determined by the analysis of histological samples according to the WHO histological classification;[4] the thickness of alteration; Clark level- determined histologically and by the layers of tumor location (level I to level V); pTNM stage- on the basis of histological analysis and insight into the history of the disease according to the 7th pTNM classification;[14] tumor infiltration by lymphocytes; mitotic index: the number of mitoses was determined in 10 visual fields at high magnification; the estimation of growth phase: radial or vertical growth phase[4] ;the presence of surface defect: presence or absence of ulceration; cell type of alteration: epitheloid cells, spindle cells, mixed type (epitheloid + spindle cells);localization: alterations have been classified according to the localization into the following subgroups: head and neck, trunk, extremities.

Immunostaining

To detectthe primary antigen VEGF,commercial mouse monoclonal anti-human VEGF antibody (Daco M7273) was usedat a dilution of 1:25. For visualization, we used the LSAB + (Daco K0690) system and chromogen DAB Liquid (K3466).

The presence or absenceand the intensity of vascular endothelial growth factor was assessed by semi-quantitative ranking using a scale from 0 to 3, taking the level of immunostaining of keratinocytes as an internal control. The quantification was as follows: score of 0, no difference in immunostaining for VEGF between melanocytes and ke-

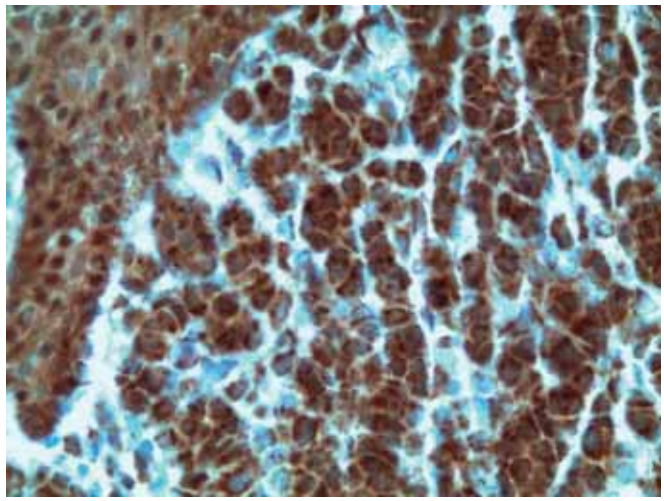

Figure 1. Medium level of VEGF expression in melanocytic nevus, score 2 (anti-VEGF x 400)

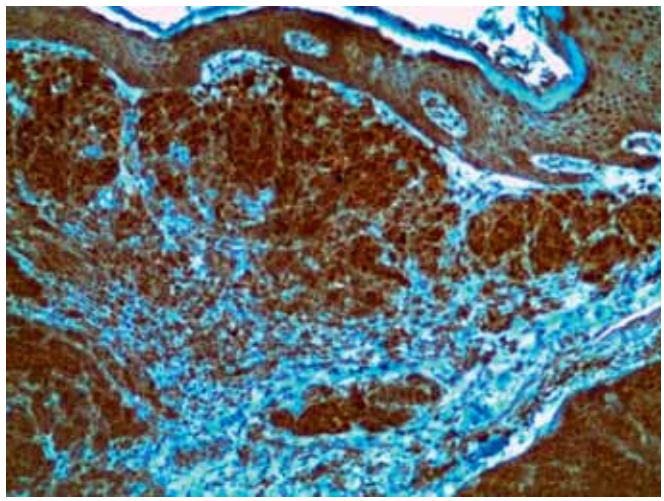

Figure 2. High level of VEGF expression in melanoma, score 3 (anti-VEGF x 200)

ratinocytes; score of 1 - less than 25% tumor cells show an expression of higher intensity compared to the level of staining of keratinocytes; score of 2 - 25 - 75% of tumor cells show an expression of higher intensity compared to the level of staining of keratinocytes (Figure 1); score of 3 - more than 75% tumor cells show an expression of higher intensity compared to the level of staining of keratinocytes (Figure 2).

Statistical analysis

The results were analyzed by methods of descriptive and correlative statistics. Statistical analysis was performed using the SPSS software version 15.0, and the following tests were applied: $\chi 2$ and the related methods of analysis of categorical variables (Fisher's exact test, Kendall tau rank correlation coefficient) and Mann Whitney U- test.

Results

The average age of examinees was 45 years. The gender distribution is 1,92:1 in favor of women.

Most of benign melanocytic alterations in the skin showed low expression level of VEGF (score of 0 and 1) in 91,18% of cases. In the group of melanomas, high expression levels were found in 61.54% of cases (level 2 and 3). A statistically significant difference exists in the expression of VEGF in groups. In group I, expression was often high (score of 2 and 3), and in group II, more often the expression was low (score of 0 and 1) ($\chi 2=21,658$; df= 1; $p<0.001$)[1]

Histological type. A statistically significant difference was not found in level of VEGF expression when different histological types of nevi were compared ($\chi 2=2.062$; $p=0.724$). Nodular and acral lentiginous melanomas more often showed a high level of expression of VEGF, while superficial spreading melanomas often showed a low level of VEGF expression ($\chi 2 = 6.858$, $p = 0.032$) [*].

The presence of surface defects. A statistically significant difference was not present regarding the level of expression of VEGF and the presence of a defect in nevi (Fischer's test, p = 0.101). Unlike nevi, in the melanoma group there was a statistically significant difference in the level of VEGF expression and the presence of ulceration was found ($\chi 2= 4.545$ p = 0.033)[*].

The thickness of alteration. Based on statistical analysis, we can conclude that there was no statistically significant correlation between the expression of VEGF and the thickness of nevi ($\chi 2= 1.009$, p = 0.604). A higher level of expression was present in melanomas that were thicker (higher stage according to Breslow) ($\chi 2=11.211$, p = 0.011, $p<0,05$). Based on the analysis of the Mann-Whitney test, we can conclude that there was no statistically significant difference in the level of expression of VEGF and the width of benign melanocytic lesions (U = 38.000 for the significance of 0.605) and in the melanoma group (U = 142.000 for the significance of 0.273).

Tumor infiltration by lymphocytes. Analysis showed no statistically significant differences between nevi with different densities of lymphocytic infiltration in relation to the expression of VEGF (Chi-square of 1.019, p = 0.601). On the basis of statistical analysis, in the group of melanomas, we can conclude that there was a statistically significant difference in the level of expression of VEGF and the density of lymphocytic infiltration (Chi-square of 8.555, p = 0.014, $p<0,05$). The low level of expression of VEGF is more common in melanomas with dense lymphocytic infiltration, while a high level of expression was found in melanomas with rare lymphocytic infiltrate[*].

Mitotic index. In the studied material, mitotic activity in benign melanocytic alterations was verified in only one case (2.94%), and level of VEGF expression in this case was 1. In the group of melanomas, based on Kendall tau rank correlation coefficient, there was no statistically significant difference in the level of VEGF expression in relation to mitotic activity (t= 0.256, p = 0.060)[*].

Estimation of growth phase. Analysis revealed that there was no statistically significant difference in the level of VEGF expression and the growth phase of nevi (Chi-square of 5, p = 0.07). Melanomas presented with vertical growth phase had showed a higher level of expression of VEGF (Chi-square of 4.840, p = 0.028, $p<0.05$)[*].

Cell type of alteration. All examined nevi had an epitheloid cell type. Analysis of Chi square analysisshowed a statistically significant difference in the level of VEGF expression and cell type of melanoma (Chi-square of 8.871 p = 0.031, $p<0.05$). A high level VEGF expression was more often verified in melanomas with epitheloid cells[*].

Localization. Analysis using Chi square test showed there was no statistically significant difference in expression of VEGF, with respect to the localization of nevus (Chi-square of 2.765, p = 0.251). In the melanoma group based on Chi square test, we could conclude there was a statistically significant difference in the level of expression of VEGF and localization of melanoma (Chi-square of 7.831, p = 0.05, $p<0.05$). Melanomas localized on the extremities showed a higher level of expression of VEGF (score 2 and 3), while melanomas localized on the head, neck and trunk showed a low level of expression of VEGF[*].

Based on analysis of Kendall tau rank correlation coefficient, no significant difference in the level of expression of VEGF and the level of invasion according to Clark was found (t= 0.244, p = 0.063)[*].

[*] Contact the corresponding author for the detailed data.

Statistical analysis using Kendall tau rank correlation coefficient showed a statistically significant difference in the level of VEGF expression and pT stage melanoma (t= 0.259, p = 0.050). Melanomas in higher pT stage of the disease showed higher expression of VEGF (score 2 and 3). **

Discussion

Early diagnosis and differentiation between benign and malignant tumors of the skin is of utmost importance. So far there is an insufficient number of studies that would indicate that routine screening for skin may be important in the prevention of malignant skin tumors and contribute to better treatment of patients suffering from these diseases.[15]

In this study, we have found that melanocytic nevi showed expression of VEGF in the most cases (79.41%). The expression is usually at low grade (grade 1 immunostaining). In the group of melanomas, a low expression of VEGF is present in 38.46% of the cases (score 0 and 1), while a high level of expression was present in 61.54% of the cases (score 2 and 3). Carazo and Peyri[16] reported that the majority of melanoma had showed lower levels of expression (score 0 and 1), which is different from our results. A logical explanation for thisis that the authors examined the selected group of melanomas ("thin melanomas" ie. Breslow less than 1 mm), while we presented results from anunselected group (Breslow thicknesseven greater than 1 mm). The results of Einspahr and associates suggest that the level of VEGF expression may be a significant parameter which indicates the malignant transformation of melanocytic skin alterations. The study demonstrated that the level of VEGF expression in benign melanocytic alterations is low or absent, while in dysplastic nevi it is significantly higher, and the expression is much higher in malignant melanocytic alterations (melanoma). Thus, increased expression of VEGF may be a good indicator of preneoplastic changes in melanocytic alterations.[17] Brychtova and collaborators determined the presence of VEGF expression in benign and malignant melanocytic alterations. More often, the high level of VEGF expression can statistically be verified in melanomas in relation to nevi.[18]

The difference in the level of VEGF expression and morphological parameters (histological type, the defect on the surface, the density of inflammatory infiltrate, mitotic index, stage of growth and cell type) has not been demonstrated in the examined nevi. In the melanoma group, a statistically significant difference exists in the level of VEGF expression and the presence of ulceration and thickness according to Breslow. Boone and associates failed to demonstrate a positive correlation between the expression of VEGF-C and the presence of ulceration, tumor thickness according to Breslow, and the level of invasion according to Clark.[19] In our study, nodular and acral lentiginous types of melanoma were more likely to exhibit high VEGF expression level, while superficial spreading melanoma often showed a low level of expression of VEGF. These results are in line with literature data.[19]

In the examined material, we did not find malignant changes in Clark level I. A number of melanoma cases showed a high expression of VEGF (score 2 and 3) in 24 (61.54%) cases. The cases with higher expression are generally at higher Clark level. Based on analysis of Kendall's tau b test, we found no significant difference in the level of expression of VEGF and the level of invasion according to Clark. Salven and associates also did not find any differences in the manifestation of VEGF (measured by immunohistochemical methods) between small and large primary melanomas.[20] However, this contrary to other reports. Redondo and associates believe that the more Clark's or Breslow's level increases, the percentage of positive immunostaining for VEGF increases, thus linking it with the development of primary tumors, although a prognostic study has not been performed.[16,21]

Melanomas presented with vertical growth phase showed a higher level of VEGF expression. Looking at the value of immunostaining for VEGF according to the Breslow level, we found very important information which we consider fundamental: melanomas in radial growth phase, and those are the ones thathave not undergone change to malignant eclipse, show less VEGF, which is significantly different as measured by precise Fisher's test (p = 0.002) compared to those melanomas who have already penetrated.[22]

In our series, benign melanocytic lesions were located on the trunk in 26 (76.47%) cases, followed by the extremities in 5 (14.71%) casesand head and neck in 3 (8.82%) cases. Melanomas in our material were located on extremities in 15 (38.46%) cases, followed by head and neck in 13 (33.33%) cases and on the trunk in 11 (28.21%) cases. Melanomas located on the extremities showed a higher level of expression of VEGF (score 2 and 3), while melanomas located on the head, neck and trunk show a low level of expression of VEGF.

Melanocytic alterations show the expression of VEGF, regardless of their clinical behavior. Benign melanocytic alterations often indicate low, while malignant melanocytic alterations often show high level of expression of VEGF. Presence and level of expression of VEGF show no difference regarding to histological type, surface defect, density of inflammatory infiltrate, mitotic index, growth phase, cell type, and location of nevi. A high level expression of VEGF is present in the nodular and acral lentiginous types

** Individual morphological parameters and VEGF expression in the nevi and melanomas, with statistical analysis, may be obtained from the corresponding author.

of melanoma, in melanomas with ulceration and rare inflammatory infiltrate in the stroma, high mitotic index, in higher stage disease (Breslow, Clark, pT), and in melanomas located on extremities.

Authorship statement

RG had full access to all data in the study and as corresponding author takes full responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: RG, VG, ZK, IS, TB. Acquisition of data: VG, ZK, IS, TB. Analysis and interpretation of data: RG, VG, ZK. Drafting of the manuscript: RG, IS, TB. Critical revision of the manuscript: VG, ZK. Statistical expertise: IS, TB. Administrative, technical, or material support: RG, VG, IS. Study supervision: ZK, TB.

Financial disclosure

No potential conflicts of interest was reported.

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Vaskularni endotelni faktor rasta (VEGF) u melanocitnim kožnim promjenama

APSTRAKT

Uvod. Istraživanje faktora rasta je značajno za dalji razvoj terapijskih modaliteta u liječenju malignih bolesti kože. Cilj ove studije je da odredi odnos između nivoa ekspresije VEGF-a i morfoloških parametara (biološko ponašanje lezije, histološki tip, defekt površine, gustina inflamatornog infiltrata, mitotski indeks, stadijum rasta i ćelijski tip) u melanocitnim nevusima i melanomima kože različitih regija.

Materijal i metode. Ispitivanja su urađena na biopsijskim materijalima kože 73 pacijenta, koji su podijeljeni u dvije grupe (grupa I- melanomi, grupa II- nevusi). Određivani su sljedeći parametri: histološki tip, debljina promjene (prema Breslow-), Clark-ov nivo, pTNM stadijum, širina promjene, gustina limfocitnog infiltrata u tumoru, mitotski indeks, stadijum tumorskog rasta, prisustvo ulceracije, ćelijski tip tumora, lokalizacija i nivo ekspresije VEGF-a.

Rezultati. Većina benignih melanocitnih promjena kože pokazuje nizak nivo ekspresije VEGF-a u 91.18% slučajeva. U grupi melanoma, visok nivo ekspresije je uočen u 61.54 % slučajeva. Nodularni i akralni lentiginozni tip melanoma češće pokazuju visok nivo ekspresije VEGF-a, dok površinski šireći melanom obično pokazuje nizak nivo ekspresije VEGF-a.

Zaključak. Benigne melanocitne promjene imaju nizak, a maligne visok nivo ekspresije VEGF-a.

KLJUČNE RIJEČI

Vaskularni endotelni faktor rasta; koža; melanocitne promjene; prognoza.