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VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-3 (VEGFR-3) EXPRESSION PATTERNS IN PSORIASIS

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ABSTRACT

Introduction: Vascular endothelial growth factor receptors (VEGFRs; including VEGFR1, VEGFR2, and VEGFR3) are generally expressed in the epidermis in humans and are associated with keratinocyte proliferation and migration. However, the role of VEGFR3 in psoriasis is unclear. **Aim:** This study aimed to investigate the expression patterns of VEGFR3, its role in psoriasis, and its association with disease severity. **Materials and methods:** This was a prospective single-centre study. We evaluated40 patients who visited the outpatient clinic at the Department of Dermatology Fayoum Hospital, between 2014 and 2015.The patients provided a detailed medical history, and underwent clinical examination and histopathological investigations. Quantification of VEGFR3mRNA expression was performed using quantitative real-time reverse transcription–polymerase chain reaction (qRT-PCR). The relative quantification of gene expression in each sample was analysed by the $2^{-\Delta\Delta Ct}$method, and expressed as the ratio of target gene to β-actin mRNA expression. Between-group comparisons were performed using one-way analysis of variance and Kruskal–Wallis test, and multiple-group comparisons were performed using the Chi-square test. Associations among variables were determined using bivariate Pearson's correlation analysis. **Results:** qRT-PCR analysis revealed VEGFR3 expressionto besignificantly higher in lesional areas than in non-lesional areas. **Conclusions:** The results of this study indicated that VEGFR3 is a potential disease marker affecting the pathogenesis of psoriasis owing to its effect on skin vascularity in lesional and non-lesionalregions of patients with psoriasis.

KEYWORDS: keratinocytes; psoriasis; vascular endothelial growth factor 3.

1. INTRODUCTION

Vascular endothelial growth factor receptors (VEGFRs; including VEGFR1, VEGFR2, and VEGFR3) are generally expressed in the epidermis in humans, and are associated with keratinocyte proliferation and migration.[1] VEGF expression is upregulatedin lesional psoriatic skin, and the serum levels of the circulating VEGF protein are significantly increased in patients with acute disease.[2] Moreover, serum VEGF levels are directly correlated with disease severity.[3] Yalçin et al.[1] hadevaluated VEGF, VEGFR3, and cyclooxygenase-2 expression in 43 patients with psoriasis, and reported VEGFR3 to be generally expressedin psoriatic and non-lesional skin, including the epidermis and dermis. However, they did not detect any significant difference in VEGFR3expressionin accordance with the psoriasis area severity index (PASI). Thus, the association between VEGFR3 expression and psoriasis severity remains unclear.

This study aimed to investigate VEGFR3expression in patients with psoriasis and its association with disease severity.

2. MATERIALS AND METHODS

2.1. Patients

Ethical considerations

The permissions to perform this study were obtained from the ethics committee of Fayoum University and from the Department of Dermatology, Andrology and STDs, Faculty of Medicine, Fayoum University. Verbal consents were recorded from all the participants (patients and controls) before examination and the method of examination was explained. The participants in both the groups were clearly explained that they had the right to not participate in the study. All the collected medical data were kept confidential. Treatment was prescribed when indicated and the method of use was explained.

Forty patients (20 with psoriasis vulgaris with varying degrees of severity in accordance with the PASI score[9 males and 11 females] and 20 healthy controls [13 males and 7 females]) who visited the outpatient clinic at the Department of Department of Dermatology, Fayoum University Hospital between 2014 and 2015 were enrolled herein. Patients in the psoriasis group were aged 6–67 years (mean age, 32.95 years), while those in the control group were aged 19–45 years (mean age, 32 years). None of the patients had received systemic medication since six months before the study.

2.2. Clinical examination and medical history

For all patients, a detailed medical history, including age, sex, occupation, residence, marital status, onset and duration of illness, previous episodes of the disease or history of other skin or other systemic diseases, and family history was obtained. They were then evaluated for clinical manifestations suggestive of systemic disease. Dermatological examinations, including those of the hair, nails, and mucous membranes, were performed; local examination of psoriatic lesions was performed on the basis of PASI scores;[3] and disease severity was classified as mild, moderate, or severe depending on the extent of skin damage.

2.3. Histopathological analysis

Biopsy specimens from lesional and non-lesional skin regions were obtained from each patient in the disease group, whereas a single skin biopsy specimen was obtained from each participant in the control group. Briefly, the selected area was cleaned with alcohol and a local anaesthetic (xylocaine 2%) was applied. A 3.5-mmpunch biopsy was performed to obtain the specimens that were incubated at −80°C without any preservative, at the dermatology Department, fayoum University (to determine VEGFR3 expression.

Only 14 patients consented to provide two additional biopsy specimens (one from the lesionalarea and one from the non-lesionalarea) for histopathological assessment. These specimens were fixed in 10% formalin, embedded in paraffin, sectioned at 5-µm thickness, and stained with haematoxylin and eosin. The sections were then examined using a light microscope (Accu-Scope # 3025; Olympus, Tokyo, Japan) with a built-in camera (E-330 SLR; Olympus) to determine vascularity, at the dermatology Department, fayoum Hospital.

2.4. Analysis of VEGFR3 expression via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Skin biopsy specimens were weighed and homogenised with extraction buffer, and total RNA was extracted using anRNeasy Mini kit (Qiagen, Frankfurt, Germany) in accordance with the manufacturer's instructions. RNA concentration was then determined by measuring the absorbance at 260 nm using a NanoDrop® ND-1000 instrument (Wilmington, DE, USA). Thereafter, an aliquot containing 0.2 µgoftotal RNA was used for reverse transcriptionthat was performed using theSuperScript First-StrandcDNA synthesis system (Fermentas, Helsinki, Finland) in accordance with the manufacturer's instructions. VEGFR3expression was then determined using aqRT-PCRplatform(QIAplex, Frankfurt, Germany). The sequences of oligonucleotide primers and probes used in the study areas follows: VEGFR3

(NM_008029), forward: 5′-TGGTACCGGCTCAACCTCTC-3′ and reverse: 5′-CACGTTTTTGCAGTCCAGCA-3′; β-actin (X03765), forward: 5′-CACTATTGGCAACGAGCGG-3′ and reverse: 5′-TCCATACCCAAGAAGGAAGGC-3′.

All primers wereprocuredfrom Invitrogen (Carlsbad, CA, USA). qPCR analyseswere performedusing a total reaction volume of 25 µL containing 3 µL of synthesised cDNA solution, 12.5 µL of 2× Probe PCR Master Mix (Qiagen, Germany), 500 nM (each) primer, and 250 nM TaqMan probe. The cDNA was mixed with the primers and SYBR Green PCR master mix, and amplified by polymerase chain reaction (PCR) using ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: initial denaturation at 95°C for 10 min followed by45 cycles at 95°C for 15 s and 60°C for 1 min. The calculated threshold cycle (Ct) value for each transcript was normalised against the corresponding β-actin Ct value. The relative quantification of gene expression in each sample was analysed by the $2^{-\Delta\Delta Ct}$ method and expressed as the ratio of targetgene to β-actin mRNAlevels.

2.5. Statistical analysis

Data were collected and coded to facilitate data manipulation, and then double-entered into Microsoft Access. Data analysis was performed using SPSS software version 18 for Windows 7. Simple descriptive analysis (numbers and percentages) was performed for qualitative data, and arithmetic means and standard deviations were determined as the measures of central tendency and variability, respectively, for quantitative parametric data. Student's *t*-test was used to compare two independent groups, and one-way analysis of variance was used for comparing more than two groups. For non-parametric data, the Kruskal–Wallis test was used to compare more than two groups, whereas the Mann–Whitney test was used to compare two groups. For qualitative data, the Chi-square test was used to compare two or more groups, and bivariate Pearson's correlation tests were used to determine associations across variables. Results with *P* values of ≤0.05 were considered statistically significant.

3. RESULTS

The mean disease duration in the psoriasis group was 4.7 years (range, one month to 30 years). Family history was negative in 17 cases (85%) and positive in three cases (15%). PASI scores ranged from 1.2 to 34.8 (mean, 9.3). Four patients (20%) presented with mild disease (psoriasis affecting less than 3% of the body), 11 patients (55%) presented with

moderate disease (psoriasis affecting 3–10% of the body), and five patients (25%) presented with severe disease (psoriasis affecting more than 10% of the body).

qRT-PCR analysis revealed that VEGFR3 mRNA levels were significantly higher in lesional areas than in non-lesional areas in the psoriasis group (5.8 ± 3.7 [range, 1.1–11.7] Ct units vs. 2.0 ± 0.71 [range, 1.0 ± 3.7] Ct units, respectively; P <0.05). In the control group, VEGFR3 mRNA levels ranged from 1 to 1.5 Ct units (mean, 1.2 Ct units). Thus, VEGFR3mRNA levels were significantly higher in non-lesional and lesional skin biopsy specimens obtained from patients with psoriasis than in specimens obtained from healthy controls (P <0.05 each). No significant difference in age and sex distribution, and VEGFR3 expression between lesional and non-lesional regions,in accordance with family history or disease severity, was observed between the patients and controls (P >0.05). However, positive correlations (P <0.05) were observed between VEGFR3 expression and PASI score in lesional areas (mean: 9.3 ± 8.9; range: 1.2–34.8), and between VEGFR3 expression inlesional and non-lesional areas, indicating that increased PASI scores were associated with *VEGFR3* upregulation in lesional areas in patients with psoriasis. Notably, no correlation was observed between VEGFR3 expression in lesional areas and age or disease duration in patients with psoriasis (P >0.05). Similarly, no significant correlation was observed between VEGFR3 expression in non-lesional areas and age, disease duration, or PASI score in patients with psoriasis.

Finally, VEGFR3expression was significantly (P <0.05) upregulated in lesional and non-lesional areas of patients with severe disease than in those with mild disease.

4. DISCUSSION

Angiogenesis has been reported to be a contributory factor in the pathogenesis of psoriasis vulgaris.[4] The association between angiogenesis and chronic inflammatory diseases such as psoriasisis an important phenomenon implicated in such pathogenesis.[5]

VEGFRs are among the most powerful components regulating vascular growth.[6] Gene therapy approaches involving VEGF to promote therapeutic angiogenesis are under consideration for conditions ranging from ischemic heart disease to non-healing skin ulcers. A surprising observation was that the transgenic delivery of VEGF to the skin results in a profound inflammatory skin condition with many cellular and molecular features of psoriasis, including characteristic vascular changes, epidermal alterations, and inflammatory infiltrates.[7]

Local and systemic increase in VEGF levels have been demonstrated in the skin and plasma of patients with psoriasis, and are known to correlate with improvement following some traditional treatments (methotrexate, steroids, cacinurin inhibitors) for psoriasis.[8] Several VEGF inhibitors such as: Bevacizumab(Avastin), Ranibizumab (lucentis), Axitinib and Sunitinib have beenapproved for the treatment of malignancies and eye disease, and isolated case reports have suggested that psoriasisin some individuals may possibly improve when exposed to these agents.[8] Although these VEGF inhibitors have not yet been approved for the treatment of psoriasis in humans, experimental data support their potential to affect relevant aspects of human cell biology such as endothelial cell differentiation and alleviate skin diseases in animal models. Given the multi-factorial nature of psoriasis, it is unlikely that VEGF inhibitors will be effective in all patients; however, they have the potential to be a valuable addition to the therapeutic arsenal in select cases.[8]

Current VEGF inhibitors in clinical use are associated with several potentially serious side effects including hypertension, left ventricular dysfunction, and gastrointestinal perforation.[8] Such side effects require careful consideration, particularly considering growing concerns regarding the possible association between psoriasis and increased cardiovascular risk.[8]

VEGF, the most critical angiogenic factor, is believedto play important rolesin the pathogenesis of psoriasis and may therefore be a promising therapeutic target for treating psoriasis. Therefore, targeting VEGF/VEGFRs has been proposed to lead to the development of new treatments for psoriasis.[9]

Under normal conditions, VEGFR2 stimulation results in the angiogenesis of blood vascular endothelial cells (BECs), whereas stimulation of VEGFR3 elicits a similar response in lymphatic ECs (LECs). Tie receptors play context-dependent roles in EC survival, and in the stabilisation and remodelling of blood and lymphatic vessels.[10] Two other receptor tyrosine kinase (RTK)families play important roles in angiogenesis, namely the platelet-derived growth factor (PDGF) receptors and erythropoietin-producing human hepatocellular (Eph) receptors.[10]

The VEGF family currently includes VEGF-A, B, C, D, E, and F, and placenta growth factor (PlGF) that form in a distinct pattern by binding to three structurally related receptor tyrosine kinases: VEGF receptor-1, 2, and 3. VEGF-C and VEGF-D also play a crucial role in lymphangiogenesis.[11] VEGF is a specific mitogen for ECs. The VEGF–VEGFR system is a

key component of angiogenesis that also includes many other stimulators, inhibitors, and angiogenic modulators.[12] VEGF-3 and its ligands VEGF-C and VEGF-D are important regulators of lymphangiogenesis, while PlGF has been associated with arteriogenesis.[11]

A major role of VEGF in the pathogenesis of psoriasis was further corroborated by the phenotype of transgenic mice with epidermis-specific overexpression of VEGF. VEGF transgenic mice showed enhanced skin vascularity and vascular permeability, and at about six months of age, these mice spontaneously developed chronic inflammatory skin lesions that histologically closely resembled human psoriasis.[13]

VEGFRs, including VEGFR1, VEGFR2, and VEGFR3, are expressed in the normal human epidermis, and are associated with the proliferation and migration of keratinocytes.[14] Among the genes expressed predominantly in the lymphatic endothelium, VEGFR3 was first identified, and is still considered one of the best lymphatic markers and a key regulator of the lymphatic system.[15] VEGFR3 plays important roles in both lymphangiogenesis and angiogenesis.[15]

In this study, we examined the expression of VEGFR3 and its role in psoriasis. The results showed that VEGFR3 mRNA level issignificantly increasedin lesional skin specimensfrom patients with psoriasis, and positively correlated with the PASI score. Man et al.[13] hadpreviously reported that VEGFRsare upregulatedin non-lesional, perilesional, and lesional psoriatic keratinocytes in all viable epidermal strata in vivo. Moreover, VEGFR1, VEGFR2, and VEGFR3 mRNAs and protein levelswere increasedin psoriatic epidermis. Furthermore, Zhu et al.[3] had evaluated the effects of ultraviolet (UV) irradiation on changes in VEGFR expression in skin keratinocytes from patients with psoriasis and reported that NB-UVB therapy down regulated VEGFR expression, initially in the basal epidermis and gradually in the upper part of the epidermis of patients with psoriasis. Similarly, this study showed that VEGFR3 was significantly upregulated in psoriatic and non-lesional skin specimens of the epidermis and dermis; there was significant concordance between VEGF and VEGFR3 expressionin the psoriatic lesions.

Man et al.[13] used skin samples from 17 patients with chronic plaque psoriasis and 11 normal controls, and found that VEGFRs are overexpressed in lesional psoriatic epidermal keratinocytes. Further, they found that both calcium and VEGF regulate VEGFR expression in psoriatic epidermis. More importantly, calcium was found to be a potential regulator for

VEGFR independent of VEGF. They performed immunofluorescence analysis of VEGFRs in non-lesional, perilesional, and lesional psoriatic skin, which showed that in normal skin, VEGFR1 and VEGFR2 were localised in the basal and suprabasal layers. In upper stratum spinosum and granulosum, few keratinocytes exhibited signals for VEGFR1 and VEGFR2. However, in psoriatic skin, VEGFR1 and VEGFR2 strongly labelled non-lesional, perilesional, keratinocytes in all layers of the epidermis except the stratum corneum, andlesional keratinocytes in all the viable layers, including the parakeratotic stratum corneum. A uniform expression pattern of VEGFR3 was detected in both normal and psoriatic epidermis except for stratum corneum.[13]

In summary, this study demonstratedthat VEGFR3 is an important factor in the pathogenesis of psoriasis, owing to its effect on skin vascularity in both lesional and non-lesional skin areas of patients with psoriasis, and may serve as a potential biomarker for the disease. However, further studies are required to determine the role of VEGF/VEGFR3 in the pathogenesis of psoriasis byusing a larger study population. Moreover, it would benecessary to evaluate the efficacy of anti-VEGF medications, including anti-angiogenic therapy, in the treatment of chronic inflammatory disorders of the skin such as psoriasis.

Declaration of conflict of interest

The authors do not have any conflicts of interest to declare.

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