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VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-3 EXPRESSION PATTERNS IN PSORIASIS:

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Abstract

Psoriasis is a no communicable disease and appears as an inflammatory skin disease. The disease is characterized by the appearance of skin lesions of specific and severe red in the size of the cash coin, and the Pests often appear on the elbow, knee, scalp, hand and foot. Recently, vascular endothelial growth factor receptors (VEGFRs, including VEGFR-1, VEGFR-2 and VEGFR-3) have been found to be expressed in normal human skin and are associated with the Psoriasis. The aim of this research is to investigate the expression patterns and relationship of vascular endothelial growth factor receptor-3 (VEGF-R3) in psoriasis compared to its expression patterns in control and its relation to disease severity. This study was carried out on 40 subjects, selected from the attendants of outpatient clinic, in the Department of Dermatology, Andrology and STDs, Faculty of Medicine, Fayoum University Hospital, between the periods of 2014 to 2015. The results of the study indicate that VEGFR3 could be an important factor in the pathogenesis of psoriasis due to its effect on skin vascularity on both lesional and non lesional skin of psoriatic patients as well. Also it could be used as a marker for the disease. The study also recommended that there be a need to be done on a large number of patients to detect the role of VEGF\VEGFR-3 in pathogenesis of psoriasis.

Kay word: psoriasis VEGFR3, expression, patterns

1. Introduction

Psoriasis is a chronic inflammatory disease that affects the small joints and skin. It has an autoimmune mechanism, automatic antigen is not identified. Therefore it can affect two to four percent of the population of Caucasian origin (Ghoreschi et al., 2003). Most patients present psoriasis lesions on the knees, elbows and scalp (Griffiths and Barker, 2007).

Signs of skin anatomy include psoriasis: infiltration of multiple immune cells, corneal cell proliferation, activated mast cells, and prominent blood vessels in the dermis. Psoriasis is also commonly associated with a prominent permeable barrier, and excessive production of VEGF (Biedermann et al., 2000).

One of the pathogenic mechanisms of psoriasis is VEGF vascular generation. Psoriasis patients have high serum levels of VEGF and ESAF. The severity of psoriasis is associated with VEGF serum levels (Nofal et al., 2009). Other facts supporting the role of VEGF in psoriasis are the strong relationship between multiple forms of individual nucleotides from the VEGF gene, causing psoriasis (Young et al, 2006) and the expression of VEGFR-1/2/3 in the skin in psoriasis patients. Furthermore, VEGFRs were strongly classified in non-parenteral, pathogenic, and parenchymal cells in all skin layers in vivo.

The balance between pro and antimatal factors regulates the formation of new blood vessels. Transient physiological blood vessels occur, for instance in pregnancy or wound healing. In pathological processes such as tumor growth or blood vessel inflammation chronic progression facilitates disease (Heidenreich et al., 2008).

2. Research Problem

Conducting such a research regarding this topic is expected to have high positive reflections; therefore, the problem of this study comes from the significance of patient safety which represent a worldwide issue and an important area of research, and that can be summarized as in the following:

1) This study will be fruitful source of information on the disease psoriasis, which will help in advancing patient safety as long as patient safety represents an imperative area in health care.

2) This study would represent a good reference for the future studies as long as it would provide the subsequent researchers and interested scholars in the field of patient safety with valued literature, recommendations and suggestions that are important for their proposed studies in the light of contemporary health-care safety thoughts.

3. Research Questions

The main question of this study is: **"How to investigate the expression patterns and relationship of vascular endothelial growth factor receptor-3 (VEGF-R3) in psoriasis?"**

This main question is subdivided into the following sub-questions:

1. How it is compared the vascular endothelial growth factor receptor-3 (VEGF-R3) to other expression patterns in control of disease?
2. What is the relation of vascular endothelial growth factor receptor-3 (VEGF-R3) on disease severity?
3. How is psoriasis disease severity measured and classified as mild, moderate or severe?
4. How it is detected the vascular endothelial growth factor receptor - 3 level in tissues using RNA extraction and RT-PCR of VEGFR-3mRNA?

4. Research Objectives

The main objective of this study is: **"To investigate the expression patterns and relationship of vascular endothelial growth factor receptor-3 (VEGF-R3) in psoriasis."**

This main objective is subdivided into the following sub-objectives:

1. To compared the vascular endothelial growth factor receptor-3 (VEGF-R3) to other expression patterns in control of disease
2. Identify a relation of vascular endothelial growth factor receptor-3 (VEGF-R3) on disease severity.
3. To learn how is psoriasis disease severity measured and classified as mild, moderate or severe.
4. To learn how it is detected the vascular endothelial growth factor receptor - 3 level in tissues using RNA extraction and RT-PCR of VEGFR-3mRNA.

5. Research Significance

The importance of research is to provide a basis for specific discussion, the expression patterns and relationship of vascular endothelial growth factor receptor-3 (VEGF-R3) in psoriasis, its prevalence, causes, history, quality of life, relevance to health, diagnosis and management, research needs and implications for health care services, as well as actions that can be taken at the country level to promote the care of psoriasis patients.

6. Background & Literature Review

Psoriasis is a common chronic inflammatory disease of the skin. Its pathogenesis has not been completely elucidated **(Perera et al., 2012).**The angiogenic stimulus is thought to be derived from the hyperplastic epidermis, and in 1994, vascular endothelial growth factor (VEGF) was identified as a major epidermis-derived vessel-specific growth factor that was strongly up-regulated in psoriatic skin lesions, in this issue, Young et al provide the first genetic evidence-based on the analysis of single nucleotide polymorphisms of the VEGF gene in psoriatic and in healthy individuals-that an "angiogenetic constitution" might determine psoriasis susceptibility **(Young et al., 2004)**.

Vascular endothelial growth factor receptors (VEGFRs, including VEGFR-1, VEGFR-2 and VEGFR-3) were found to be expressed in normal human epidermis and are associated with proliferation and migration of keratinocytes **(Man et al., 2008)**.

VEGF involvement in psoriasis

Many factors with prophylactic effect are expressed in high psoriatic skin levels: VEGF, TNF, TGF-α, HIF, ESAF, interleukin (IL-8, IL-17 and angiopoietins (Creamer et al., 2002). The joint action of these agents stimulates ECs of the dermis to form new blood vessels. Interactions between agents supporting vascular disease are very complex; for example, TNF contains pro-vascular prophylaxis by stimulating other pro-vascular agents in ECs, such as basal cell growth factor (bFGF), IL-8, and VEGF (Armstrong, 2011).

The over-expression of VEGF in skin biopsies for psoriasis patients is an argument for the important role VEGF plays in maintaining the normal functioning of the epidermal barrier and the relation between VEGF skin and corneal hypertrophy.

Thus, it may be sustainable that VEGF is involved in the proliferation of keratinocytes [6,. As well as, it has been shown by Detmar et al. (1994) that VEGF stimulates in vitro splint activity of keratinocytes and is produced in both neurons and keratinocytes in VEGFR-1 and VEGFR-2 expression. VEGFR-1 and -2 are detectable in skin lesions for psoriasis patients. Thus, because VEGF increases the expression of VEGFR in keratinocytes and regulates keratinocytes of VEGF expression, we can support the idea that VEGF has an autosomal effect on keratinocyte proliferation (Sabat, 2007).

Skin barrier disorder alone is not enough to produce psoriasis. Other disorders of the immune system contribute to the creation of a complete psoriasis phenotype (Bachelez, 2005). There is also a perpetuation of the inflammation of psoriasis: VEGF increases the expression of cell adhesion molecules from the capillaries in the formation and increases vascular permeability, thus favoring the transfer of white blood cells to psoriasis; this process leads to increased oxygen consumption, And activation of hypoxic vasodilators such as HIF-1, and sustaining this vascular / vascular cycle of psoriasis (Armstrong et al., 2011).

7. Research Methods

This study was carried out on 40 subjects, selected from the attendants of outpatient clinic, in the Department of Dermatology, Andrology and STDs, Faculty of Medicine, Fayoum University Hospital, between the period of 2014 to 2015. They were divided into:

Psoriasis group: included 20 patients with psoriasis vulgaris of various degrees of severity according to PASI score. Patients were of both sexes (males and females) of different age groups and those who had taken systemic medications in the last 6 monthes before the study were excluded.

Control group: included 20 healthy volunteers of both sexes (males and females) and of different age groups were included as a control group. They were selected from apparently healthy volunteers presenting to Dermatology Outpatient Clinic, Fayoum University hospital (Fayoum University).

Patients were subjected to the following:

1-Detailed history taking.

2-Careful clinical examination:

- Careful general examination for clinical manifestations suggestive of systemic disease.

- Dermatological examination including:

1- Examination of the hair, nails and mucous membranes.

2- In case of psoriatic group, local examination of psoriatic lesion that evaluated by PASI score.

3- Classifying the disease severity:

Psoriasis is usually graded as mild (affecting less than 3% of the body), moderate (affecting 3–10% of the body) or severe (more than 10% of the body skin) **(Louden et al., 2004)**.

Several scales exist for measuring the severity of psoriasis. In general, the degree of severity is based on the following factors: the proportion of body surface area affected; disease activity (degree of plaque redness, thickness, and scaling); response to previous therapies; and the impact of the disease on the person.

The Psoriasis Area Severity Index (PASI) is the most widely used measurement tool for psoriasis. PASI combines the assessment of the severity of lesions and the area affected into a single score in the range 0 (no disease) to 72 (maximal disease). Nevertheless, the PASI can be too unwieldy to use outside of trials, which has led to attempts to simplify the index for clinical use **(Louden et al., 2004).**

3- Histopathological investigations:

Skin biopsies were taken from each patient (one from lesional skin and one from nonlesional skin), and one skin biopsy from controls. The selected area was cleaned with alcohol and a local anaesthetic (Xylocaine 2%) was injected subcutaneously. A 3.5 mm. punch biopsy was used to obtain the specimen. The biopsies were kept directly at -80 without any preservatives at the Biochemistry department, Faculty of Medicine, Cairo University, for detection of vascular endothelial growth factor receptor - 3 level in tissues using RNA extraction and RT-PCR of VEGFR-3mRNA. Only 14 patients agreed for taking additional two biopsies (one from lesional skin and one from nonlesional skin), for histopathological assessment.

a) Total RNA extraction:

Skin biopsy was weighted and homogenized with the extraction buffer using the RN easy Mini kit (Qiagen, Germany) as described by the manufacture's instruction.

b) RNA quantitation:

The concentration of RNA was determined by an absorbance at 260 nm and using Nanodrop ND-1000 instrument (Wilmington, USA).

c) cDNA Synthesis

An aliquot containing 0.2 µg of total RNA was used for the reverse transcription reaction, which was conducted using the superscript first- strand cDNA synthesis system (Fermentas, Finland) according to the manufacturer's instructions. The sequences of oligonucleotide primer and probe are as follow:

Primers used in the Methods:

VEGFR-3 (NM_008029): F 5- TGGTACCGGCTCAACCTCTC-3'R5' CACGTTTTTGCAGTCCAGCA-3. β-actin (X03765): F 5- CACTATTGGCAACGAGCGG-3R5 TCCATACCCAAGAAGGAAGGC-3.

The quantification of VEGFR-3mRNA was performed using a Real time PCR (Qiaplex, Germany).

All primers were from Invitrogen. Q-PCR reactions were performed in a total volume of 25 µl containing 3 µl of synthesized cDNA solution, 12.5 µl of 2x Probe PCR Master Mix (Qiagen, Germany), 500 nM of each primer and 250 nM of the TaqMan probe. The cDNA was mixed with primers and SYBR Green PCR master mix (Applied Biosystems), and amplified by PCR using an ABI Prism 7700 instrument (Applied Biosystems). PCR conditions were 95°C 10 min – (95°C 15 s– 60°C 1 min) × 45. The calculated threshold cycle (C_T) value for each transcript was normalized against the corresponding β- actin C_T value. The relative

quantification of gene expression in each sample was analyzed by the 2ΔΔCt method and expressed as the ratio of related gene to β- actin mRNA.

Histopathological Assessment:

Light microscope (Accu-Scope #3025 Five Headed "A 3025-5"-OLYMPUS) (Fig. 7), with a built-in camera (Olympus, digital camera E-330 SLR, Japan) (Fig. 8), were used to examine and photograph the sectioned skin biopsies), at the dermatopathology unit of the Dermatology, STD's and Andrology Department, Al-Minya University Hospital.

The fourteen skin biopsies were subjected to: Histologic processing**:** skin biopsies were fixed in formalin 10%, embedded in paraffin, sectioned into 5 µm thick sections, and then stained with Haematoxylin and Eosin (H & E) to be examined under the light microscope for histopathologic findings (vascularity).

8. Results

As regards to VEGFR3–PCR in lesional area was (5.8±3.7) ranged between (1.1 and 11.7), and in non-lesional area was (2±0.71) ranged between (1 and 3.7).

And there were statistically significance difference with p-value <0.05 between VEGFR3-PCR level in lesional and non-lesional areas with high mean among cases of psoriasis.

Interrestingly, our study showed that there is statistically significance difference with p-value <0.05 between different degree of disease severity as regards to VEGFR3-PCR level in lesional and non-lesional areas with high mean among cases of psoriasis with severe degree of disease.

There were statistically significance positive correlation with p-value <0.05 between VEGFR3-PCR level in lesional areas and each of PASI score and VEGFR3-PCR level in non-lesional areas, which indicates increase in PASI score will followed by increase in VEGFR3-PCR level in lesional areas cases of psoriasis.

In this study there were no statistically significance correlation with p-value >0.05 as regards to age, and disease duration.

9. Conclusion

In conclusion of our study, VEGFR3 could be an important factor in the pathogenesis of psoriasis due to its effect on skin vascularity on both lesional and non lesional skin of psoriatic patients as well. Also it could be used as a marker for the disease.

10.Recommendation

Further studies are needed to be done on a large number of patients to detect the role of VEGF\VEGFR-3 in pathogenesis of psoriasis.

Further studies are needed to detect the efficacy of anti-VEGF medications in the treatment of psoriasis.

Further studies are needed to be done to detect the relation between VEGFs\VEGFRs and psoriasis and whether that relation might be caused by one or more of the VEGFs\VEGFRs.

Also further studies are needed to be done to detect the relation between vascular endothelial growth factor receptors (1, 2 and 3) and the pathogenesis of psoriasis, and which one is considered to be the main domain in psoriasis.

Also further studies are needed to be done to detect if stimulation of functional lymphangiogenesis via VEGFR-3, in addition to antiangiogenic therapy, might therefore serve as a novel strategy to treat chronic inflammatory disorders of the skin such as psoriasis.

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