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Original Article

An Investigation of Vascular Endothelial Growth Factor (VEGFR-1 and VEGFR-2) in Burn Wound Healing

Abdulazeem, L[1], Tariq, A[2], Abdalkareem Jasim, S[3]*

1. DNA Research Center, University of Babylon, Babylon, Iraq
2. Biology Department, College of Science, University of Babylon, Babylon, Iraq
3. Medical Laboratory Techniques Department, Al-Maarif University College, Al-Anbar, Iraq

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Corresponding Author: saade.a.j@uoa.edu.iq

Abstract

Burn damage is a complicated trauma that causes local and general tissue edema as a result of cell breakage and capillary leak syndrome. Angiogenesis plays a key part in the mechanisms that are initiated by tissue damage (e.g., burns) since it works directly and precisely on endothelial cells. The primary mediators of angiogenesis are vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1 and VEGFR-2). This study aimed to figure out what functions VEGF and its receptors play in wound healing after burn, and the systemic release of VEGF in people following severe burn damage. This study included 23 burnt adult serum and 20 healthy controls. The enzyme-linked immunosorbent test was used to assess circulating VEGF serum levels and its receptors (VEGFR-1 and VEGFR-2). VEGF serum levels were considerably higher in this study, compared to VEGF levels in healthy controls. The levels of VEGFR-1 and VEGFR-2 have significantly risen; moreover, VEGF and its receptors have a significant impact on edema-related problems in severely burned individuals. Burn is a frequent disease that damages the skin and induces the production of mediators that cause neovasculature in the majority of patients. VEGF, which causes vasculogenesis and angiogenesis, is one of the most important factors in the skin.

Keywords: Angiogenesis, Burns, VEGF, VEGF receptors (VEGFR-1 and VEGFR-2)

1. Introduction

One of the most frequent and debilitating types of trauma is burn injury. It is a type of skin cell and tissue injury that is damaged or destroyed. When the skin comes into touch with flames, chemical electricity, or radiation, it is usually fatal. External sources of heat, such as fires, scalding liquids, or steam, can produce thermal burns. Thermal burns are most frequently the outcome of a drunk driving accident (1). Cutaneous wounds, also known as ulcers, are a frequent treatment issue that can be caused by a variety of factors, including acute or chronic mechanical trauma, physical or chemical burns, frostbite, and infections. Rheumatism, diabetes, peripheral vascular disease, lipodermatosclerosis, and malignant tumors are some of the diseases and disorders. The cost of delayed cutaneous wounds is one of the most significant health care costs (2, 3).

Vascular endothelial growth factor (VEGF) is produced by a variety of cell types, including monocytes, macrophages, and endothelial cells (4). According to immunohistochemistry, VEGF was predominantly expressed in monocytes during the exudation phase of burns. Chemokines and cytokines have been found to be crucial in the early stages of leukocyte-endothelial infiltration, and VEGF has been proven to improve monocyte function. Endothelial cell attachment allows the transmigration across the

endothelium and activation of the inflammatory response (5). VEGF receptors (VEGFR-1 and VEGFR-2) are mostly expressed by vascular endothelial cells (ECs) and mediate VEGF's biological effects. When VEGF binds to one of these receptors, it stimulates them and causes intracellular signaling (6). Although VEGFR-1 has a high affinity for VEGF, it has considerably lower tyrosine phosphorylation in response to VEGF than VEGFR-2; therefore, VEGFR-2 is considered to mediate the majority of its functional effects (7). VEGF promotes the development of ECs originating from arteries, veins, and lymphatic vessels in physiological conditions. However, because of its propensity to enhance capillary leakage, VEGF is also known as an avascular permeability factor, and this permeability-enhancing action activates significant functions for this molecule in inflammation and other clinical diseases (7, 8).

Keratinocyte proliferation and epidermal barrier homeostasis may potentially be affected by VEGF (9). Burn healing is a multistep biological process that includes hemostasis, inflammation, cell proliferation, and differentiation. Angiogenesis/vasculogenesis, vascular permeability, endothelial cell proliferation and migration, as well as leukocyte adherence, are all stimulated by collagen VEGF (10-12). This 54-dalton protein VEGF also known as the "vascular permeability factor" is made up of two subunits generated by endothelial cells and does not encourage cell proliferation in other cell types. Alternative splicing of mRNA from a single gene with eight exons produces human isoforms with different numbers of amino acids (13) (VEGF-121, 145, 165, 189, 206). The only soluble isoforms are VEGF-121 and VEGF-165, which are also the most prevalent. The most potent activator of endothelial cell growth is VEGF-165 (14). The increase in vascular permeability is caused by VEGF-121 (15). There are five distinct VEGF family members that have been identified, such as VEGF-A (=VEGF), VEGF-B, VEGF-C, VEGF-D, and VEGF-E, which are the several types of VEGF. Only VEGF-A, -B, and -E were shown to affect vascular angiogenesis and permeability. Lymphocytic organogenesis is influenced by VEGF-C and VEGF-D (14, 15).

2. Materials and Methods

2.1. Serum Collection

Venipuncture was used to draw 5 mL of blood from each patient and healthy participant under sterile circumstances. The serum was extracted from the entire blood by centrifugation at 1000 rpm and kept at -20˚C until needed. Lysed cells were centrifuged for 1 h at 4˚C or at 12000g f.

2.2. Enzyme-Linked Immunosorbent Assay

The enzyme-linked immune sorbent assay (ELISA) was used to detect VGEF, VGEFR-1, and VGEFR-2 in serum. The quantitative sandwich ELISA kit (Abcam/UK) was used in this investigation. Subsequently, samples and standards were placed in the wells, and the antibody mix was added. The wells were cleaned after incubation to eliminate any loose material. TMB substrate was added, which was catalyzed by HRP during incubation, resulting in blue. The addition of stop solution then ceased the reaction, completing any color shift from blue to yellow. The signal's strength was proportional to the amount of bound analytic and was measured at 450 nm.

2.3. Subjects

During terrorist attacks at Hilla Teaching Hospital, 25 patients with burns were included in this study. In the morning, blood samples were taken from the patients and control groups. At the beginning of the experiment, consent was received from the participants in the experiment.

2.4. Control Group

A total of 15 healthy individuals took part in the study, and they were tested to see whether they had any medical or mental issues.

2.5. Statistical Analysis

The obtained data were analyzed in SPSS software (version 17.0, Chicago, USA) through descriptive statistics (mean±SD). The chi-square test was used to investigate the relationships between the category variables. A *P*-value of less than or equal to 0.05 was considered statistically significant.

3. Results and Discussion

Inflammation, cell migration and proliferation, extracellular matrix formation and remodeling, as well as neovascularization are all parts of the tissue healing process following burn injury (16). As a result of opening endothelial intercellular connections, VEGF produces noticeable morphological alterations in endothelial cells and increases capillary and venular leakage (14). These processes are of fire caused organ or tissue damage because endothelial cell injury results in permeability alterations, and eventually, edema development. Endothelial cell destruction following fire injury may be caused in a variety of ways. In the current investigation, it was found that following burn injury, blood levels of circulating VEGF were higher than those in healthy controls. Since VEGF has the potential to enhance vascular permeability and may function at extremely low concentrations (17), it may have a role in the development of edema following burn injury.

According to studies, VEGF is an essential mediator of inflammatory processes, and high VEGF concentrations are linked to the degree of organ dysfunction, and an increase in VEGF levels is linked to burn patients (18). VEGF has previously been demonstrated to cause vascular hyperpermeability and monocyte movement (e.g., monocyte-endothelial cellular adhesion) (Figure 1). The results also revealed that the levels of serum VEGFR-1 and VEGFR-2 in burn patients were substantially higher than those in the control group in this study (Figures 2A and 2B).

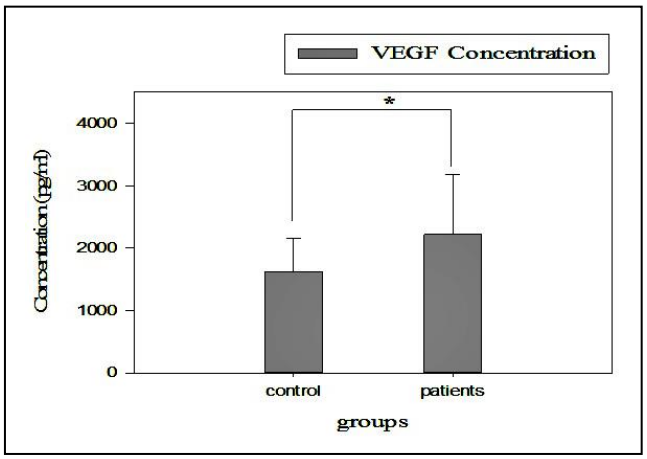

Figure 1. Serum Vascular Endothelial Growth Factor Concentration Showing Extremely Significant Differences between Burn Patients and Controls (P<0.001)

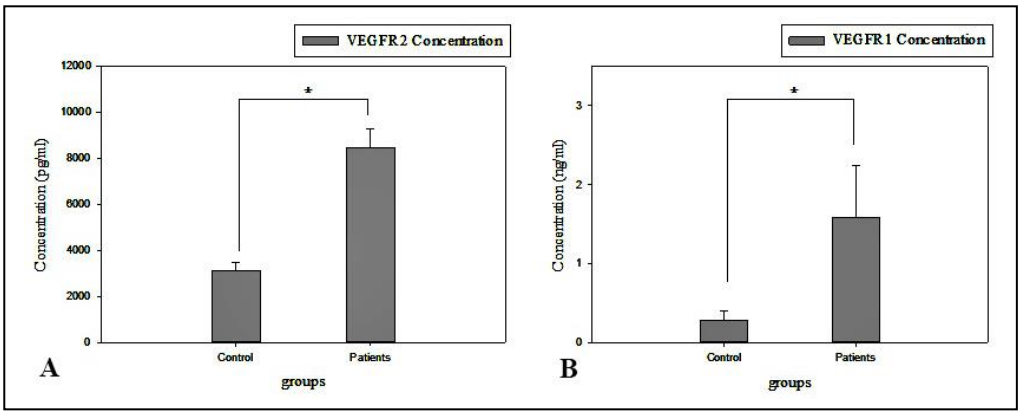

Figure 2. 2A and **2B** Serum Vascular Endothelial Growth Factor Receptor (VEGFR-1 and VEGFR- 2) Concentrations Showing Extremely Significant Differences between Burn Patients and Controls (P<0.001).

VEGF signals are sent by trans-membrane receptors, such as VEGFR-1 and VEGFR-2. These VEGFR-1 molecules are found in monocytes (19). VEGFR-1 is primarily responsible for separating VEGF from VEGFR-2. The cellular signaling processes are controlled by VEGFR-2. The serum of burned patients stimulated the production and release of physiologically active VEGF and its receptors. Our findings revealed that burnt serum increases VEGF levels and activity. Inducing vascular leakage, VEGF is more potent than histamine (20). The relationship between VEGF expression patterns and permeable blood vessels in injured skin shows that VEGF is a strong inducer of skin vascular permeability (21). By regulating the expression of selection and intercellular adhesion molecules on endothelial cells, VEGF promotes leukocyte rolling and adherence to the endothelium during the early phases of healing (22).

To understand how this works, it was considered that VEGF activity during the proliferative phase of healing causes various cell types to collaborate to repair the epidermal and dermal layers of the tissue and drink the proliferative phase of healing, eventually leading to full wound healing. During re-epithelialization, keratinocytes multiply and move to maintain the epidermal barrier. The development of granulation tissue begins with the repair of the dermis, and during this period, inflammation cells, fibroblasts, and new blood vessels abound in this area. These new arteries deliver blood carrying the oxygen and nutrients needed to keep the various cell types engaged in the healing process active (19).

4. Conclusion

Burns are a common ailment that causes skin damage and promotes the generation of mediators that induce neovasculature in the majority of patients. One of the essential factors in the skin is VEGF, which induces vasculogenesis and angiogenesis. The relevance of VEGF in the healing process, particularly as a pro-angiogenic agent, has been thoroughly recognized. The majority of research has shown that VEGF and its receptors have a positive function in burden conditions, leading to the notion of supplementing VEGF and its receptors in nonhealing burns.

Authors' Contribution

Study concept and design: A. T.
Acquisition of data: A. T.
Analysis and interpretation of data: L. A.
Drafting of the manuscript: S. A. J.
Critical revision of the manuscript for important intellectual content: L. A.
Statistical analysis: A. T.
Administrative, technical, and material support: L. A.

Ethics

The study protocol was approved by the Ethics Committee of the DNA Research Center, University of Babylon, Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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