Egyptian Journal of Medical Microbiology Volume 34 / No.3 / July 2025 109-114 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Vascular Endothelial Growth Factor and Neutrophil Gelatinase-Associated Lipocalin as Critical Biomarkers in Patients with Burn Injury

Tamara K.M. Alkhalidi, Ahmed A.J. Aljanaby*
Department of Biology, Faculty of Science, University of Kufa, Iraq

Key words:
VEGF, NGAL, Burn-patients, First-degree, Second-degree

Corresponding Author:
Ahmed Abduljabbar Jaloob Aljanaby
Department of Biology, Faculty of Science, University of Kufa, Iraq
Tel.: 009647816118353
ahmedaj.aljanabi@uokufa.edu.iq

ABSTRACT

Background: *Burn is one of the most common and serious types of injuries, affecting millions of people worldwide.* ***Objective:*** *This study aims to investigate the immunological role of Vascular endothelial growth factor and neutrophil gelatinase-associated lipocalin in patients with first and second-degree burn injuries.* ***Methodology:*** *From 1st June 2024 until the end of January 2025, 88 participants were enrolled in this study as follows: 48 burn patients aged ≥ 18 years old (26 patients with first-degree burn and 22 patients with second-degree burn), and 40 healthy volunteers as controls. VEGF and NGAL have been measured in all participants' blood using the ELISA technique.* ***Results:*** *The results proved that there was a significant increase (P value<0.0001) in serum levels of VEGF in total burns patients (293.4 ± 11.81pg/ml) compared with control (161.0 ± 14.42 pg/ml. The serum levels of VEGF of second-degree burn patients (334.3 ± 20.81 pg/ml) were significantly higher (P0.0004) than serum levels of first-degree burn patients (258.7 ± 8.436 pg/l). Also, the serum levels of NGAL were significantly higher (P < 0.0001) in total patients (4176 ± 182.2 pg/ml) than in healthy individuals (150.1 ± 9.0 pg/ml). Patients who had second-degree burns (5200 ± 84.65 pg/ml) had significantly higher (P < 0.0001) serum levels of NGAL than those who had first-degree burns (3310 ± 211.4 pg/ml).* ***Conclusion:*** *Our findings show that VEGF and NGAL had an important immunological role in second- and first-degree burn patients; these markers might be helpful in burn treatment.*

INTRODUCTION

Burns are a common type of injury that occurs when the skin and underlying tissues are damaged by heat, chemicals, electricity, or radiation[1]. Burn injuries can range in severity from minor to severe, with the most severe cases often requiring hospitalization and long-term treatment [2]. One of the most serious complications of severe burn injuries is the risk of infection, burn victims with infections have a fatality rate twice as high as those without infections, as the dysregulated immune system increases the risk of infection [3]. In fact, sepsis, or systemic infection, is the leading cause of mortality following burn injury, with 42%-65% of burn victims dying from infections. The development of effective burn dressings and treatments is crucial for reducing the risk of infection and improving outcomes for burn patients [4]. Traditionally, burn care has involved the use of antibiotics and topical antimicrobials, but the increasing resistance of microbes to these treatments has led to a need for alternative approaches [5].

Burn injuries are typically classified into three main categories: first-degree, second-degree, and third-degree burns. First-degree burns involve only the outermost layer of skin, the epidermis, and are characterized by redness, pain, and mild swelling [6]. These burns typically heal within a few days without any long-term complications. Second-degree burns, also known as partial-thickness burns, involve both the epidermis and the underlying dermis layer of skin. These burns are more serious, resulting in blistering, intense pain, and a longer healing time of 18-21 days [7].

Vascular endothelial growth factor is a critical signaling protein involved in the regulation of angiogenesis, the process of new blood vessel formation from pre-existing vasculature [8]. This crucial process has significant implications for the treatment of burn injuries, as impaired angiogenesis can lead to delayed wound healing and susceptibility to infection. Burn injuries often result in extensive tissue damage, which can disrupt the normal vascular architecture and impair the body's ability to mount an effective angiogenic response [9]. This can exacerbate the hypoxic conditions within the wound bed, further compromising tissue repair and rendering the area more vulnerable to bacterial colonization.

Neutrophil gelatinase-associated lipocalin, also known as lipocalin-2, is a protein that has gained increasing attention in the field of burn infection research[10]. Acute kidney injury (AKI) is a severe

complication that can occur after major burn injuries, often leading to significant morbidity and mortality. Researchers have explored the potential of NGAL as a biomarker for early detection of AKI in burn patients, as it has been shown to be a sensitive and specific indicator of kidney injury [11]. This study aims to assess the immunological function of VEGF and NGAL in individuals suffering from first and second degree burn injuries.

METHODOLOGY

Patients selection

This study enrolled 88 participants in the age range of 18 to 60 years. It was carried out in the Department of Burns at AL-Najaf Hospital in Al-Najaf City, Iraq, from June 2024 to January 2025. The study included forty-eight burn patients; 26 with first-degree burns (First week injury) and 22 with second-degree burns (Second week injury) diagnosed by the physicians, and 40 healthy volunteers as controls. VEGF and NGAL have been measured in all participants' blood using the ELISA technique.

Ethics consideration

The Institutional Ethics Committees of the College of Medicine at the University of Kufa and the Scientific Committee for Research of the Health Department of Najaf both gave their approval.

Measurement of serum levels of VEGF and NGAL

This was conducted following the directions provided by the manufacturer's instructions (Bioasay Technlogy Laboratry, Shanghaii, China). A sample of five ml of blood was collected from each individual, and a serum sample of two ml was obtained centrifuged at 8000 rpm for 10 minutes [12]. ELISA method has been used to measure VEGF and NGAL concentration in serum of the cases as[13] determination of the optical density (OD value) was done by ELISA reader at 450nm wavelength, then the results were calculated by plotting the standard curve.

Statistical analysis

The statistical analysis and all results were conducted using GraphPad Prism version 6. Statistically significant P values less than 0.05 were considered for the statistical analysis [14].

RESULTS

Age and sex of the participants

There was no significant difference in age groups ($P > 0.05$) between the control (39.65 ± 2.434 years), male 43.50 ± 3.301 years), and female (40.68 ± 2.560 years) (Table 1).

Table 1: Age groups and sex of the participants in this study

Total N=88	Age/Year Mean ± SE	P-value
Control N=40	39.65 ± 2.434	P1 0.1792
Male N=20	43.50 ± 3.301	P2 0.3884
Female N=28	40.68 ± 2.560	P3 0.2483

P1 compared between control and male, P2 compared between control and female, P3 compared male and female.

Vascular endothelial growth factor

Our results showed that the VEGF levels in blood of all burn patients were 293.4 ± 11.81 pg/mL, which was significantly higher ($P < 0.0001$) than the levels in the control group (161.0 ± 14.42 pg/mL) (Figure 1). The serum levels of first-degree cases (258.7 ± 8.436 pg/mL) and second-degree cases (334.3 ± 20.81 pg/mL) were significantly higher ($P < 0.0001$) than the control. The serum levels of VEGF in second-degree burn patients were significantly higher ($P < 0.0004$) than those in first-degree burn patients (Figure 2).

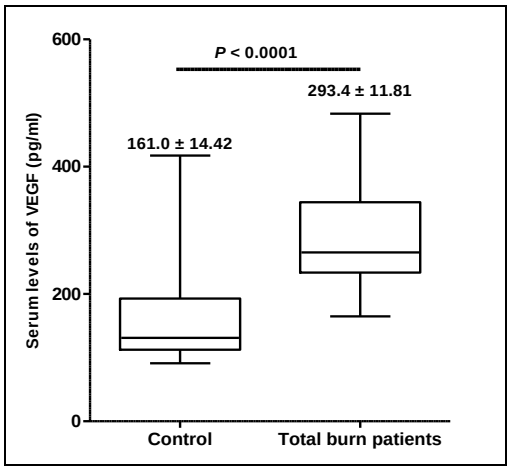

Fig. 1: Serum levels of VGEF in total burn patients and control

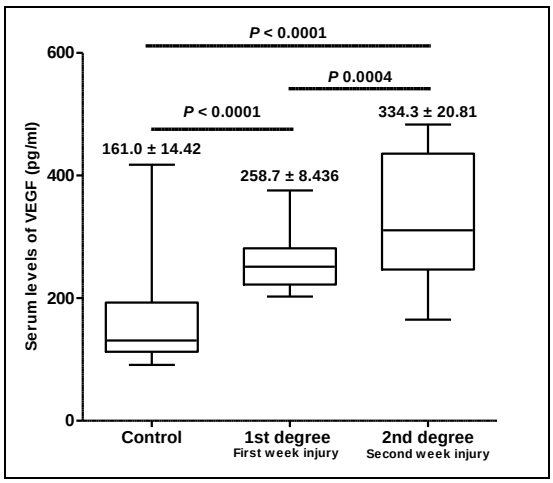

Fig. 2: Serum levels of VGEF in total burn patients and control according to burn degree

Neutrophil gelatinase-associated lipocalin

The current study revealed a significant increase (P < 0.0001) in serum NGAL concentrations among all burn patients (4176 ± 182.2 pg/ml) compared to the control group (150.1 ± 9 pg/ml) (Figure 3). Serum NGAL levels were notably higher in both first-degree burn patients (3310 ± 211.4 pg/ml) and second-degree burn patients (5200 ± 84.65 pg/ml) than in the control group (P < 0.0001). Additionally, second-degree burn patients showed significantly elevated serum NGAL levels compared to first-degree burn patients (P < 0.0001) (Figure 4).

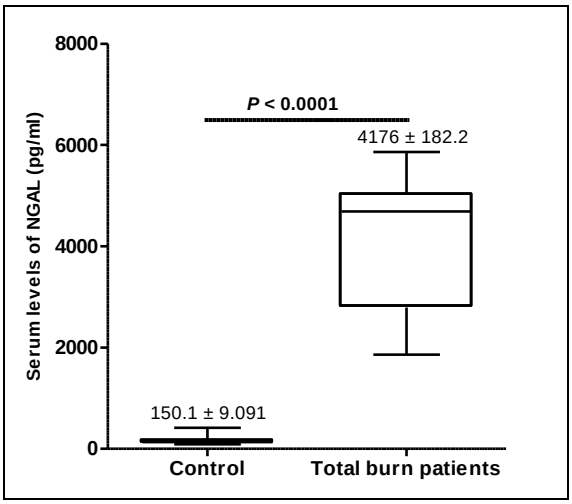

Fig. 3: Serum levels of NGAL in total burn patients and control

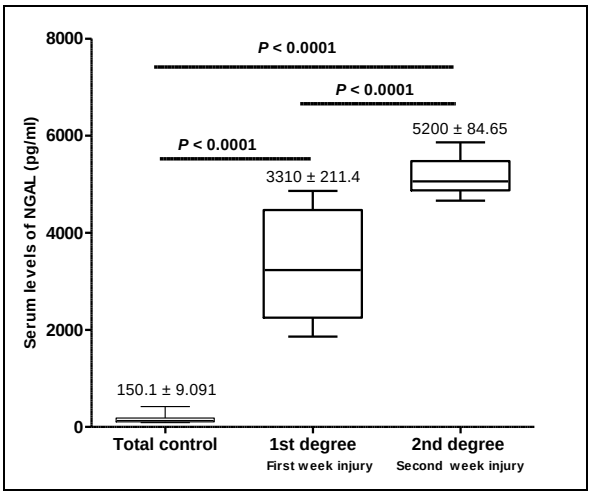

Fig.4: Serum levels of NGAL in total burn patients and control according to burn degree

Our results revealed that the levels of VGEF and NGAL in the blood of all patients were positively correlated with each other, with no statistically significant difference P = 0.7081 (Figure 5). A similar positive correlation was observed between the two markers during the first week of injury P = 0.2022 (Figure 6) and the second week of injury P = 0.1302 as shown in (Figure 7).

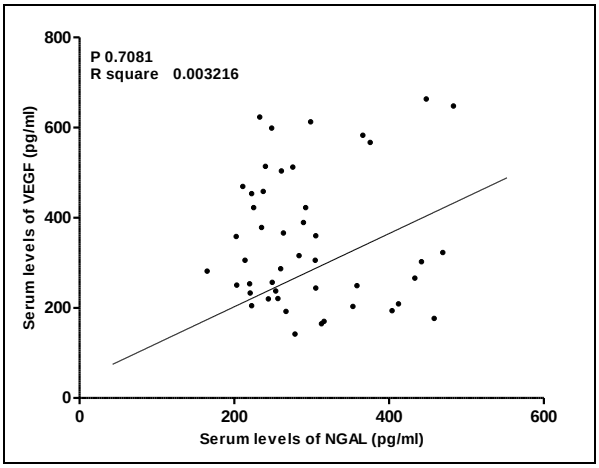

Fig.5: Correlation between serum levels of VGEF and NGAL in total burns patients

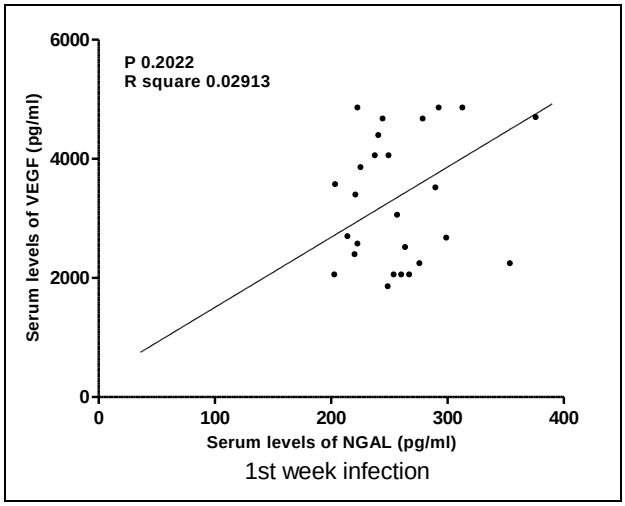

Fig.6: Correlation between serum levels of VGEF and NGAL in burn patients during 1st week injury

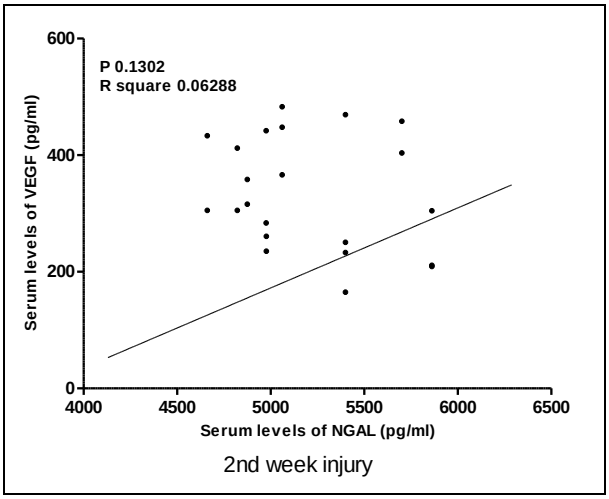

Fig.7: Correlation between serum levels of VGEF and NGAL in burns-patients during 2nd week injury

DISCUSSION

Burns are one of the worst kinds of injuries that can happen to the body, and they have a big effect on the immune system [15]. The immune response in burn victims is complex and changing, involving both innate and adaptive immunity. Understanding these changes in the immune system is important for better treatment and better patient outcomes [16]. Burn injuries elicit complex immunological and inflammatory responses, ranking among the most severe types of trauma. The immune system must manage the systemic repercussions of burns to prevent complications such as infections, sepsis, and multi-organ dysfunction syndrome (MODS) [17]. The key to better clinical care and better patient outcomes after burns is a better understanding of the immune response.

Severe burn injuries often result in compromised immune responses, leading to increased susceptibility to infections [18]. The disruption of the skin barrier, available bacterial nutrients, destruction of vascular supply, and systemic disturbances contribute to the high risk of infection in burn patients. Despite advances in treatment strategies, such as improved resuscitation, wound coverage, and infection control, burn injuries continue to pose significant challenges due to the profound and complex metabolic changes triggered by the initial insult [19]. The hyper metabolic state associated with severe burns is characterized by increased stress, inflammation, glycolysis, proteolysis, and lipolysis. These responses are more severe and prolonged in burn patients compared to other trauma or critically ill populations. The magnitude of the metabolic derangements is directly correlated with the extent of the burn injury, with burns covering more than 30% of the total body surface area being particularly problematic [20]. The disruption of normal physiological processes and the diversion of resources to address the burn injury contribute to impaired immune function, making burn patients highly susceptible to local and systemic infections [21].

Topical antimicrobial agents have played a crucial role in the management of burn wound infections, which were previously a major cause of morbidity and mortality. However, the increasing prevalence of antimicrobial resistance has necessitated the exploration of alternative strategies to prevent and combat burn-related infections [22].

Vascular endothelial growth factors are particularly important in burn injuries, as it increases blood flow to the injured tissues, thus enabling the transport of vital nutrients and immune cells and promoting the healing process [23]. Burn patients have notably higher blood levels of VEGF and its receptors (VEGFR-1 and VEGFR-2) than do healthy people, according to studies. This spike implies that VEGF helps explain the higher vascular permeability and oedema seen in severe burns[24].

Additionally, NGAL levels have been shown to correlate with the severity of burn injuries and the risk of infections, making it a valuable tool for clinical assessment and management of burn patients [25]. The hyper responsive state of pulmonary neutrophils following burn injury has also been an area of interest. Researchers have found that the mammalian target of rapamycin (mTOR) pathway regulates this heightened neutrophil function, leading to increased susceptibility to opportunistic infections like *Pseudomonas aeruginosa* [26].

CONCLUSION

Burn injuries significantly alter the immune response, creating a delicate balance between immunosuppression and inflammation. To facilitate the development of tailored medicines that enhance outcomes for burn patients, it is vital to have a solid understanding of the immunological systems that are at play. At this time, researchers are continuing their investigation into innovative therapies to regulate the immune response and improve recovery in burn patients. VEGF and NGAL have emerged as promising biomarkers for the early detection and management of burn-related complications.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article. This manuscript has not been previously published and is not under consideration for another journal.

Funding: The authors did not earn any funds from any organizations.

REFERENCES

1. Shahriari-Khalaji M, Sattar M, Cao R, Zhu M. Angiogenesis, hemocompatibility and bactericidal effect of bioactive natural polymer-based bilayer adhesive skin substitute for infected burned wound healing. Bioact Mater. 2023;29:177-195.
2. Jeschke MG, van Baar ME, Choudhry MA, Chung KK, Gibran NS, Logsetty S. Burn injury. Nat Rev Dis Prim. 2020;6(1):11.
3. Aljanaby AAJ, Aljanaby IAJ. Prevalence of aerobic pathogenic bacteria isolated from patients with burn infection and their antimicrobial susceptibility patterns in Al-Najaf City, Iraq-a three-year cross-sectional study. F1000Research. 2018;7:1157.
4. Neznansky A, Opatowsky Y. Expression, purification and crystallization of the phosphate-binding PstS protein from Pseudomonas aeruginosa. Acta Crystallogr Sect F Struct Biol Commun. 2014;70(7):906-910.
5. Cronau H, Kankanala RR, Mauger T. Diagnosis and management of red eye in primary care. Am Fam Physician. 2010;81(2):137-144.
6. Cook KA, Martinez-Lozano E, Sheridan R, Rodriguez EK, Nazarian A, Grinstaff MW. Hydrogels for the management of second-degree burns: Currently available options and future promise. Burn trauma. 2022;10:tkac047.
7. Proksch E, Jensen JM, Crichton-Smith A, Fowler A, Clitherow J. Rationale Behandlung von Patienten mit Verbrennungen 1. Grades. Der Hautarzt. 2007;58(7):604-610.
8. Hartono SP, Bedell VM, Alam SK, et al. Vascular endothelial growth factor as an immediate-early activator of ultraviolet-induced skin injury. In: Mayo Clinic Proceedings. Vol 97. Elsevier; 2022:154-164.
9. Wasiak J, Cleland H, Campbell F, Spinks A. Dressings for superficial and partial thickness burns. Cochrane Database Syst Rev. 2013;(3).
10. Sen S, Godwin ZR, Palmieri T, Greenhalgh D, Steele AN, Tran NK. Whole blood neutrophil gelatinase–associated lipocalin predicts acute kidney injury in burn patients. J Surg Res. 2015;196(2):382-387.
11. Cho SY, Hur M. New Issues With Neutrophil Gelatinase-associated Lipocalin in Acute Kidney Injury. Ann Lab Med. 2023;43(6):529-530.
12. Mohammad AK, Aljanaby ŸAJ. A Key Role of Interleukin 34 in Patients with Chronic Kidney Disease and Lupus Nephritis. Egypt J Med Microbiol. 2025;34(1):289-293.
13. Al-janabi DRA, Aljanaby AAJ. Bacteriological investigation of pyelonephritis in AL-Najaf Governorate, Iraq: a cross-Sectional study. In: BIO Web of Conferences. Vol 84. EDP Sciences; 2024:3014.
14. Al-janabi DRA, Aljanaby AAJ. Comparison of Escherichia coli and Proteus mirabilis in patients infected with chronic pyelonephritis in Al-Najaf Governorate, Iraq. In: BIO Web of Conferences. Vol 139. EDP Sciences; 2024:6006.
15. Girardot T, Rimmelé T, Venet F, Monneret G. Apoptosis-induced lymphopenia in sepsis and other severe injuries. Apoptosis. 2017;22:295-305.
16. Paterson HM, Murphy TJ, Purcell EJ, et al. Injury primes the innate immune system for enhanced Toll-like receptor reactivity. J Immunol. 2003;171(3):1473-1483.
17. Ni D, Lin X, Deng C, et al. Energy metabolism: from physiological changes to targets in sepsis-induced cardiomyopathy. Hell J Cardiol. Published online 2024.
18. Hazeldine J, Naumann DN, Toman E, et al. Prehospital immune responses and development of multiple organ dysfunction syndrome following traumatic injury: A prospective cohort study. PLoS Med. 2017;14(7):e1002338.
19. Bouma G, Burns SO, Thrasher AJ. Wiskott–Aldrich syndrome: immunodeficiency resulting from defective cell migration and impaired immunostimulatory activation. Immunobiology. 2009;214(9-10):778-790.
20. Wan J, He J, Chen L, Qiu L, Wang F, Chen XL. Retrospective study from a single center on the efficacy of pulsed lavage following excision of burns≥ 30% of the total body surface area in 63 patients. Med Sci Monit Int Med J Exp Clin Res. 2022;28:e937697-1.
21. Wang YB. Emphasis on the exploration and research of the protective strategy of organs after burns in the early stage. Zhonghua Shao Shang za zhi= Zhonghua Shaoshang Zazhi= Chinese J Burn. 2019;35(3):161-162.
22. Cancio LC. Topical antimicrobial agents for burn wound care: history and current status. Surg Infect (Larchmt). 2021;22(1):3-11.
23. Abdulazeem L, Tariq A. An investigation of vascular endothelial growth factor (VEGFR-1 and VEGFR-2) in burn wound healing. Arch Razi Inst. 2022;77(2):747.
24. Zheng X, Liang GUO, Siyi LAI, et al. Emodin suppresses alkali burn-induced corneal inflammation and neovascularization by the vascular endothelial growth factor receptor 2 signaling pathway. J Tradit Chinese Med. 2024;44(2):268.

25. Rakkolainen I, Vuola J. Plasma NGAL predicts early acute kidney injury no earlier than s-creatinine or cystatin C in severely burned patients. Burns. 2016;42(2):322-328.

26. Dunn JLM, Kartchner LB, Gast K, et al. Mammalian target of rapamycin regulates a hyperresponsive state in pulmonary neutrophils late after burn injury. J Leukoc Biol. 2018;103(5):909-918.