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Prediction of immune molecules activity during burn wound healing among elderly patients: in-silico analyses: experimental research

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Introduction: Burn injuries lead to dysregulation of immune molecules, impacting cellular and humoral immune pathways. This study aims to determine the prediction of immune molecule activity during burn wound healing among elderly patients.

Methods: The current study utilized the Gene Expression Omnibus (GEO) database to extract the proper gene set. Also, the literature review was conducted in the present study to find immune signatures. The study used the "enrich r" website to identify the biological functions of extracted genes. The critical gene modules related to mortality were identified using the weighted gene co-expression network analysis (WGCNA) R package.

Results: The appreciated GSE was extracted. According to the data, the most upregulated signatures were related to natural killer (NK) cells, and the most downregulated signatures were associated with M1 macrophages. Also, the results of WGCNA have shown that the most related gene modules ($P < 107$ and score 0.17) to mortality were investigated, and the modules 100 first genes were extracted. Additionally, the enrich r analysis has demonstrated related pathways, including the immune process, including regulation of histamine secreted from mast cell ($P < 0.05$), T helper 17 cell differentiation ($P < 0.05$), and autophagy ($P < 0.05$) were obtained. Finally, by network analysis, the critical gene "B3GNT5" were obtained (degree > ten and "betweenness and centrality" > 30 were considered).

Conclusion: The study identified significant changes in macrophage and NK cell expression patterns post-burn injury, linking them to potential improvements in clinical outcomes and wound healing. The gene B3GNT5, associated with mortality, was highlighted as a key marker for prognostic evaluation.

Keywords: burns, immune molecules activity, wound healing, wounds and injuries, wounds

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HIGHLIGHTS

- The appreciated GSE was extracted. According to the data, the most upregulated signatures were related to natural killer (NK) cells, and the most downregulated signatures were associated with M1 macrophages.
- The results of weighted gene co-expression network analysis (WGCNA) have shown that the most related gene modules ($P < 107$ and score 0.17) to mortality were investigated, and the modules 100 first genes were extracted.
- The enrich r analysis has demonstrated related pathways, including the immune process, including regulation of histamine secreted from mast cell ($P < 0.05$), T helper 17 cell differentiation ($P < 0.05$), and autophagy ($P < 0.05$) were obtained.
- By network analysis, the critical gene "B3GNT5" were obtained (degree > ten and "betweenness and centrality" > 30 was considered).
- The current study uses a bioinformatics approach and programming methods in biology, two immune cell signatures (NK cell and macrophages), pathways linked to immune molecules, and five genes associated with mortality.

Introduction

Burn injuries represent a widely acknowledged global health concern with substantial societal ramifications[1–13]. These injuries encompass damage to the skin and underlying tissues, typically stemming from exposure to fire, electricity, radiation, or chemical agents[14–30]. Notably, they inflict severe pain and can precipitate adverse physical and psychological repercussions on affected individuals[31–46]. Extensive burn wounds, defined as covering more than 20% of an adult's total body surface area (TBSA), pose a particularly grave threat as they affect both the epidermis and deeper layers of tissue. They rank among the most severe forms of trauma and contribute to ~330 000 fatalities annually worldwide[47]. Noteworthy is the markedly diminished survival rate among patients with extensive burn injuries, with mortality rates soaring to 97.8% in instances where the affected area encompasses over 70% of the TBSA[48]. Furthermore, managing such wounds presents formidable challenges owing to the pronounced and prolonged systemic dysfunction they induce[49–68]. Indeed, burn wounds disrupt the delicate balance of the immune system, precipitating suppression of both humoral and cellular immunity[42]. The magnitude and severity of burns directly correlate with the extent of immune suppression, which may contribute to mortality outcomes[69].

Immune molecules are a diverse group of proteins, peptides, and signalling molecules that play essential roles in the immune response, including immune cell communications, pathogens recognition, and immune system regulation. Different immune cell types produce these molecules, including antibodies, cytokines, chemokines, complement proteins, major histocompatibility complex (MHC) molecules, toll-like receptors (TLRs), pattern recognition receptors (PRRs), immune signatures, and molecules involved in immune cell differentiation[70]. Dysregulation of immune molecules or immune mediators can give rise to defects or malfunctions within the innate or adaptive immune response, thereby causing an array of illnesses or

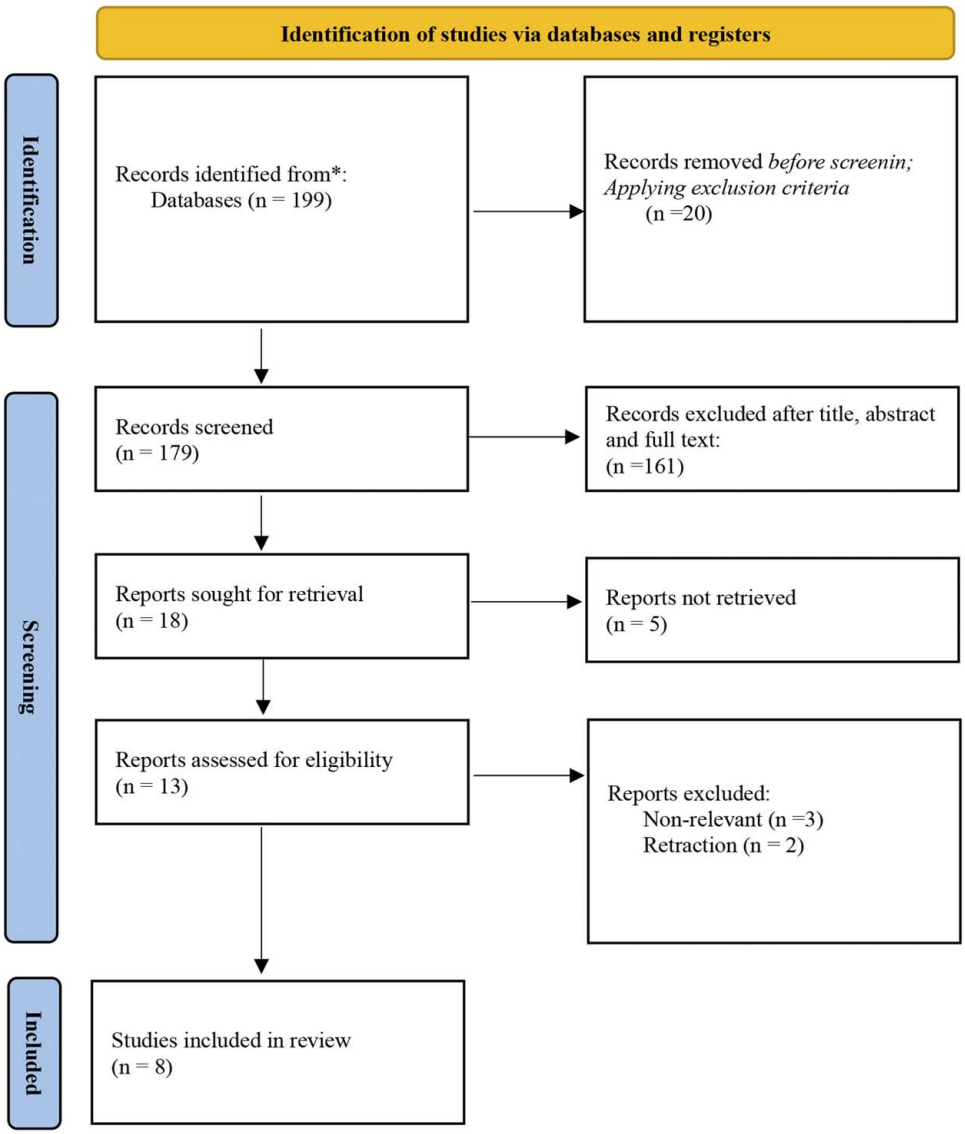

Figure 1. Flowchart of the search strategy.

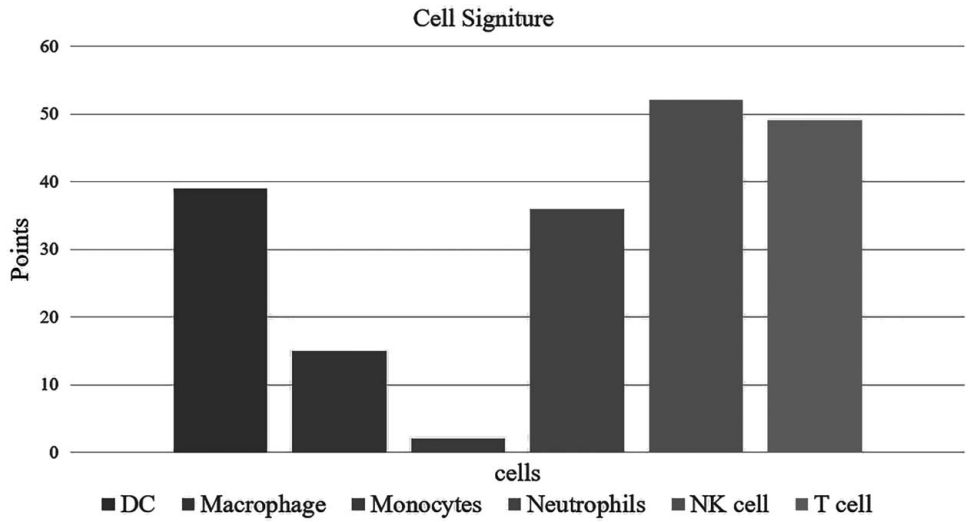

Figure 2. The most upregulated cell signature (The points are related to the number of upregulation times in different periods). DC, dendritic cell; NK, natural killer.

diseases. In this regard, Hypersensitivity reactions, autoimmunity, immunodeficiency, cancer, and burns are among the conditions that can trigger such dysregulation[71]. Also, Burn injuries can cause immune molecule dysregulation, affecting both cellular and humoral pathways. These injuries result in significant changes to the leucocyte transcriptome, activating genes associated with the innate immune system, including pro-inflammatory and anti-inflammatory genes, while suppressing adaptive immune responses[72]. Furthermore, Following the burn injury, there is a marked elevation in pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), which are integral to the initiation of the immune response. These biomolecules facilitate the recruitment of immune cells to the site of injury, playing a critical role in defending against infections. Nevertheless, an exaggerated or prolonged inflammatory response may precipitate complications such as systemic inflammatory response syndrome (SIRS) or sepsis. The role of anti-inflammatory cytokines, particularly interleukin-10 (IL-10), in modulating the immune response and fostering healing underscores the critical equilibrium between pro-inflammatory and anti-inflammatory responses in recuperating burn injuries. This balance is vital for averting infection while ensuring efficient wound healing and recovery[73–76].

In this present investigation, the authors endeavour to utilize bioinformatics methodologies alongside the R programming language to discern immune molecules implicated in the mortality linked with burns and burn wounds. The primary objective is to delineate potential therapeutic targets aimed at ameliorating clinical outcomes of burn wounds, particularly among elderly patient cohorts.

Research questions

(1) Which immune cell is related to the highest gene signature upregulation (during 168 h)?

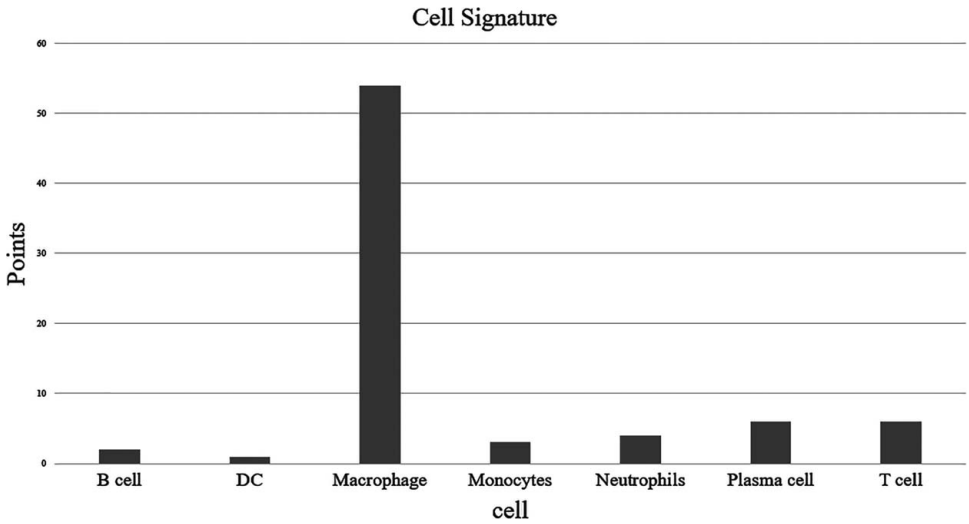

Figure 3. The most downregulated cell signature (The points are related to the number of downregulation times in different periods) DC, dendritic cell.

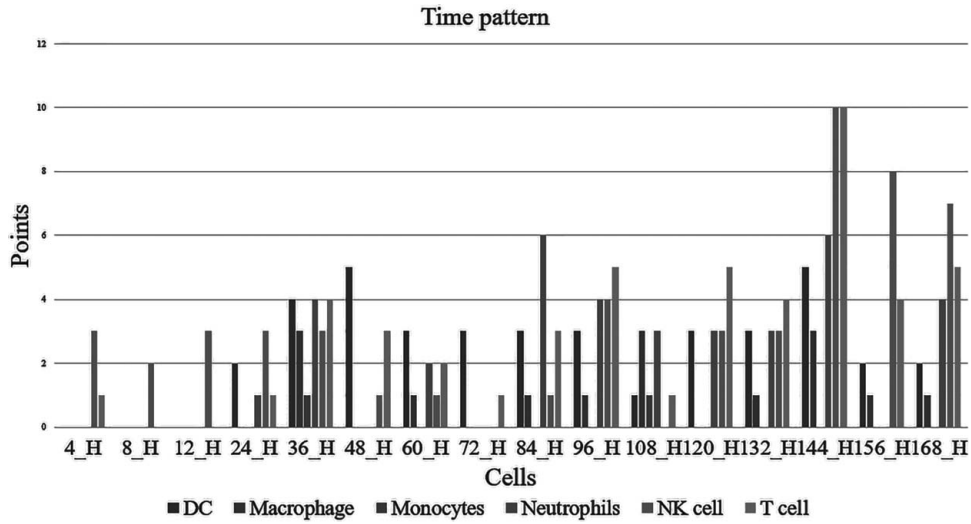

Figure 4. The time pattern of upregulated. DC, dendritic cell; NK, natural killer.

(2) Which immune cell is related to the highest gene signature downregulation (during 168 h)?
(3) What are the most critical immunity genes involved in post-burn mortality (during 168 h)?
(4) Which immune signalling pathway is involved in post-burn mortality (during 168 h)?

Methods

Gene expression omnibus (GEO)

In the current in-silico analyses and ecological study, the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) database was utilized to extract the proper gene set. The worldwide public repository GEO stores and freely distributes high-throughput gene expression and other functional genomic information. Due to the rapid growth of technology, GEO has evolved and can currently take high-throughput data for numerous data applications, such as those investigating genome methylation, chromatin structure, and genome-protein interactions[77]. The term "burn" was scouted as queries in GEO. To choose appropriate "gene series accessions" (GSE), cases with intervention methods, samples under 10, and "without any control group" were disqualified[78].

Literature review

The literature review was conducted in the present study to find immune signatures. The keywords including "immune signature", "T cell immune signature", "B cell immune signature", "Lymphocyte immune signature", "Monocyte immune signature", "Basophil immune signature", "Dendritic cells immune signature", "Macrophage immune signature", "T helper immune signature", "Natural killer cells Neutrophils signature", and "Plasma cell immune signature". The original papers were

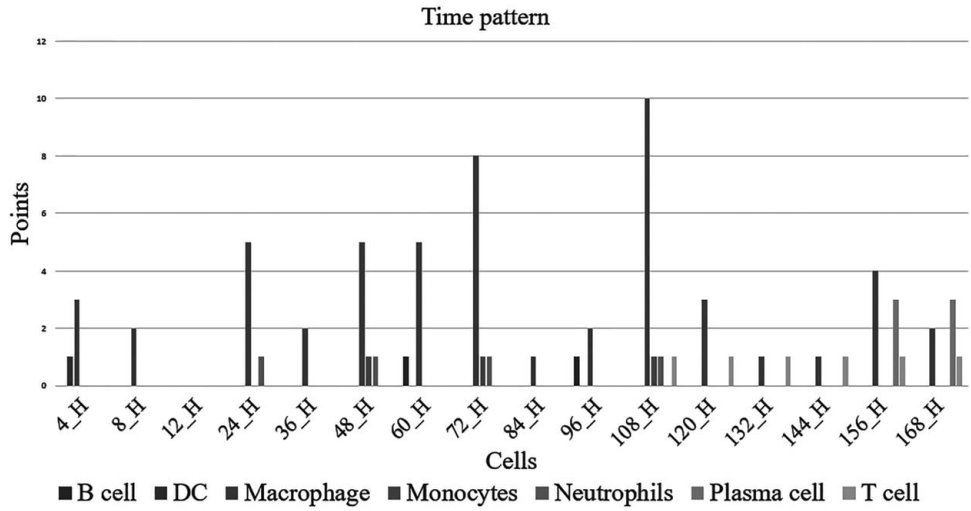

Figure 5. The time pattern of downregulated. DC, dendritic cell.

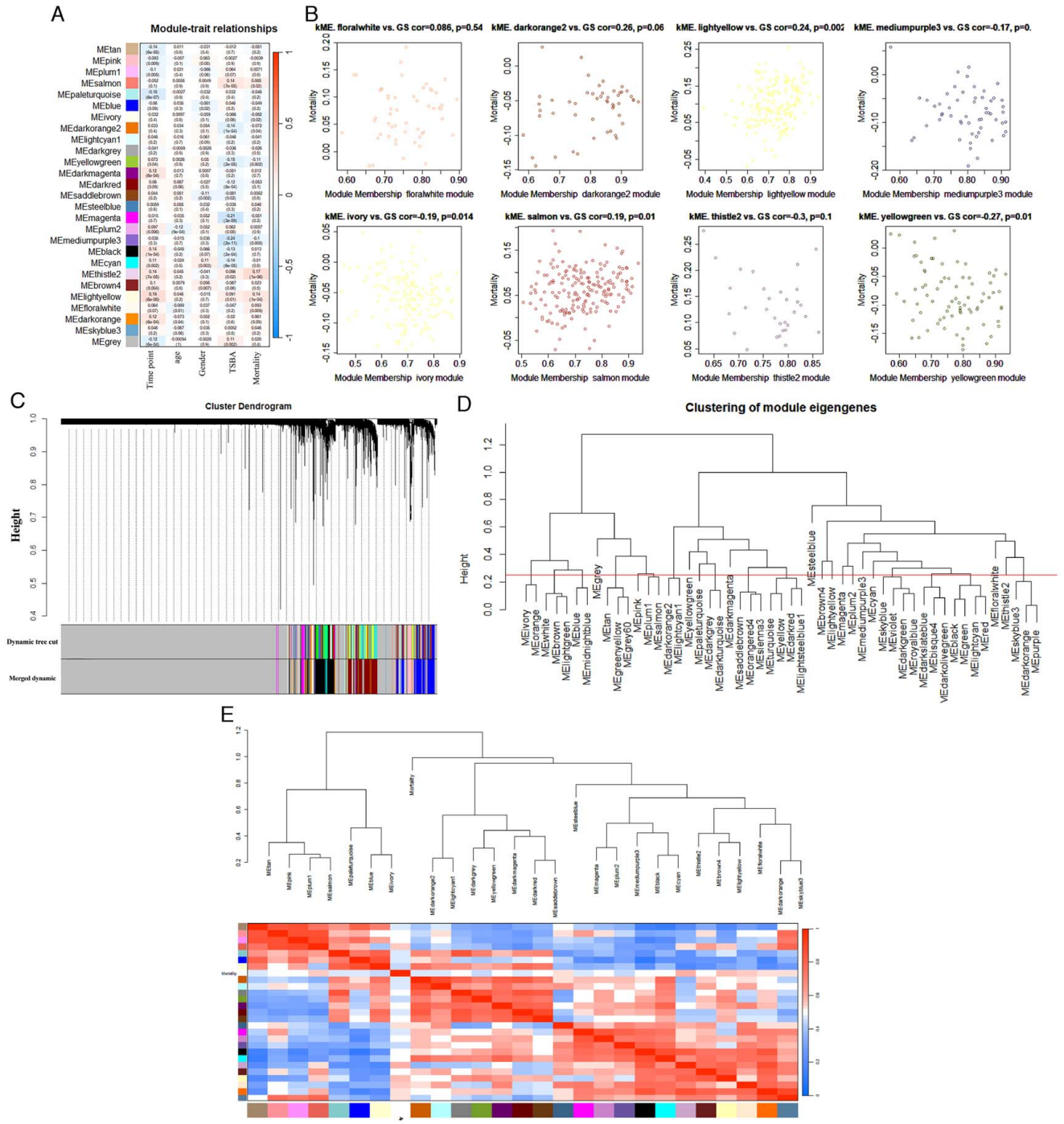

Figure 6. Weighted gene co-expression analysis. (A) The heatmap illustrated by weighted gene co-expression network analysis; note the scores; (B) correlation analysis between module membership and gene significance in mortality; (C) gene cluster dendrogram clustered by weighted gene co-expression network in different colours; (D) co-expression modules; (E) Heatmap of the top 100 genes. Different colours represent different expression trends.

included, and review and bioinformatic investigation were excluded. Ultimately, eight articles were achieved (Fig. 1).

Enrich r

The study used the "enrich r" (https://maayanlab.cloud/Enrichr/) website to identify the biological functions of extracted genes. Enrich r is a gene set online platform that permits the querying of tens of millions of annotated gene sets. Enrich r uniquely synthesizes information on mammalian genes and gene sets by integrating knowledge from prominent studies[79]. As a classification, three categories of genes were provided, including upregulated genes, downregulated genes, and selected genes from the top module, and for valid process selection, $P < 0.05$ were considered[42].

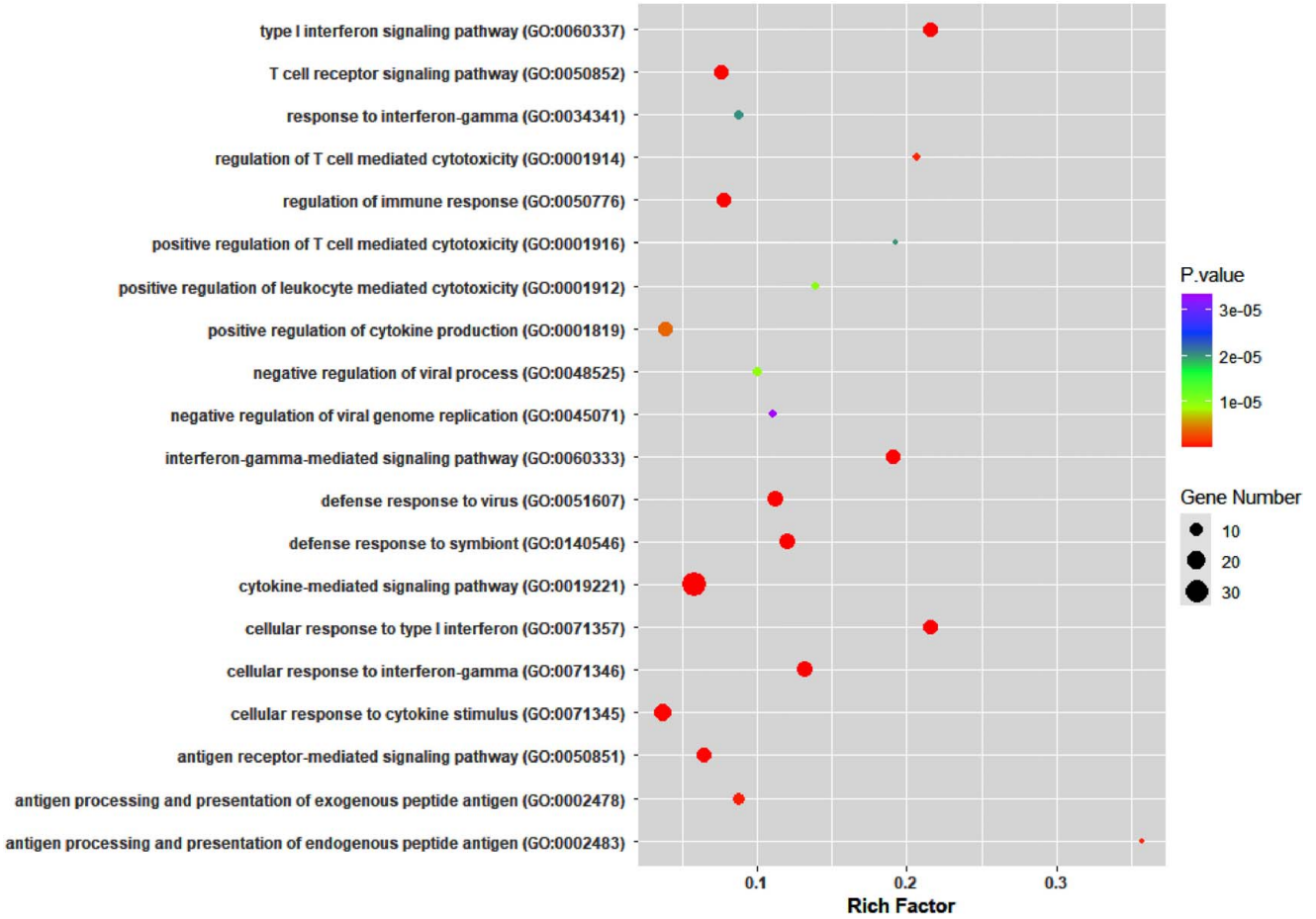

Figure 7. The results of enrich r analysis (upregulation).

Weighted gene co-expression network analysis (WGCNA)

The critical gene modules related to mortality were identified using the WGCNA R package. The WGCNA R software package is a complete collection of R functions for weighted correlation network analysis's many elements. The program comprises procedures for network creation, module identification, gene selection, computations of topological attributes, data simulation, visualization, and software interfacing. In addition to providing the R package, we also offer R software tutorials[80]. Also, WGCNA is a bioinformatics tool that uses statistical and computational approaches to analyze gene expression data and identify co-expressed gene modules. While it involves advanced computational techniques, it is often used with other AI and machine learning tools to analyze large datasets and generate insights into complex biological systems[81].

Network illustration and analysis

The Gephi software (V 0.10.0) was applied for network illustration. Gephi is a freely available software application for visualizing and analyzing extensive networks and graphs. It allows users to import network data from various sources, encompassing spreadsheets, databases, and social media platforms. Furthermore, it permits the creation of an array of diverse visual representations, including node-link diagrams, matrix views, and circular layouts[82].

Study procedure

In the current investigation, after locating an appropriate GSE, To identify molecule modifications among elderly patients, samples of adults over 50 years of age were recruited[83]. In addition, to better comprehend the process of gene expression changes, the following hours after the burn were chosen: 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156 and 168. Moreover, the *P* greater than 0.5 and log fold change (FC) (log FC > 1 and log FC-1) were used to find the appropriate genes. The obtained genes were analyzed as the next step to investigate the expression pattern during the timeline. After extracting "immune gene signatures" by literature review, the pattern of signature expression (up or downregulated) was analyzed by searching among total genes, and signature expression patterns were identified at different hours. Also, using enrich r analysis, the biological function of the discovered genes was investigated. Next, applying WGCNA illustrated the heat map of found total genes, and essential modules were obtained. Further, The module's 100 critical genes with the highest score in terms of mortality were selected[84] and applied for network illustration and "enrich r" analysis to investigate immunity function. Finally, by using

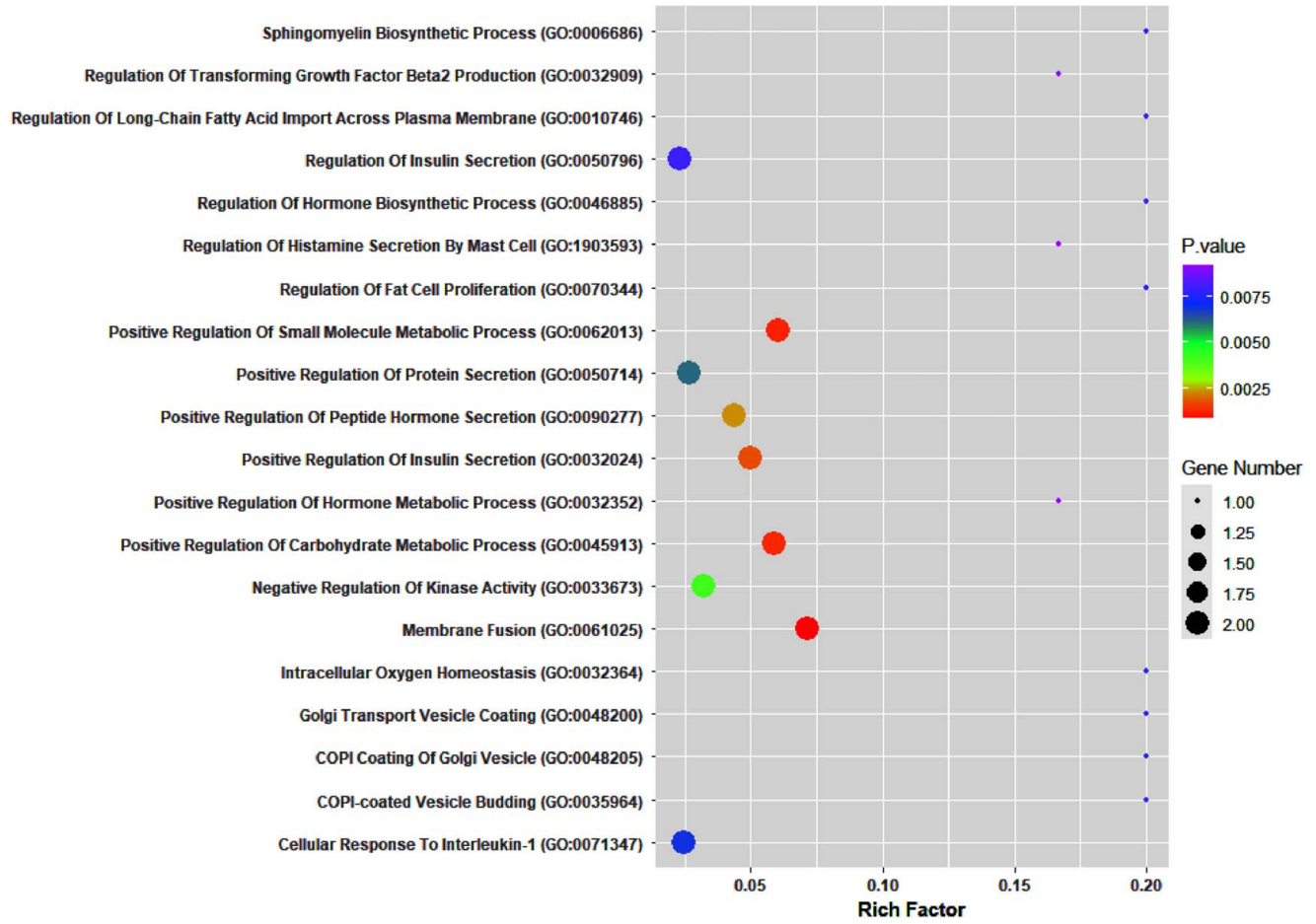

Figure 8. The results of enrich r analysis (downregulation).

Gephi software, the network illustrated, and degree greater than 10 and "betweenness and centrality" greater than 30 were considered[78].

Ethical approval

This article does not contain any studies with human or animal subjects performed by any authors and does not require ethical approval and consent.

Results

Gene set identification

Using proper keywords and criteria, the current study extracted the appropriate GSE, including GSE GSE182616. Also, the upregulated and downregulated genes were investigated in the serial timeline.

Immune molecules extraction and cell-based analysis

According to the data, the most upregulated signatures were related to natural killer (NK) cells, and the most downregulated signatures were associated with M1 macrophages (Figs. 2 and 3). Also, according to timeline analysis, the trend of signature expression during 168 h was investigated and was depicted in diagrams three and four (Figs. 4 and 5).

WGCNA results

The results of WGCNA are shown in Fig. 6. The most mortality-related modules ($P < 107$ and score 0.17) were identified, and the first 100 genes were extracted from the obtained module.

Enrich r results

According to enrich r data, the obtained gene from GEO2R correlated to immune processes with P less than 0.0001, in the upregulated section, including; the T-cell receptor signalling pathway, regulation of T-cell-mediated cytotoxicity, interferon gamma-mediated signalling pathway, and antigen receptor-mediated signalling pathway (Fig. 7), also, in downregulated section, neutrophil-mediated immunity, neutrophil degranulation, neutrophil activation involved in immune response and defense response to the bacterium (Fig. 8). Further, by analyzing the first 100 genes of obtained modules from WGCNA in "enrich r", the immune process, including regulation of histamine secreted from mast cell ($P < 0.05$), T helper 17 cell differentiation ($P < 0.05$) and autophagy ($P < 0.05$) was achieved (Fig. 9).

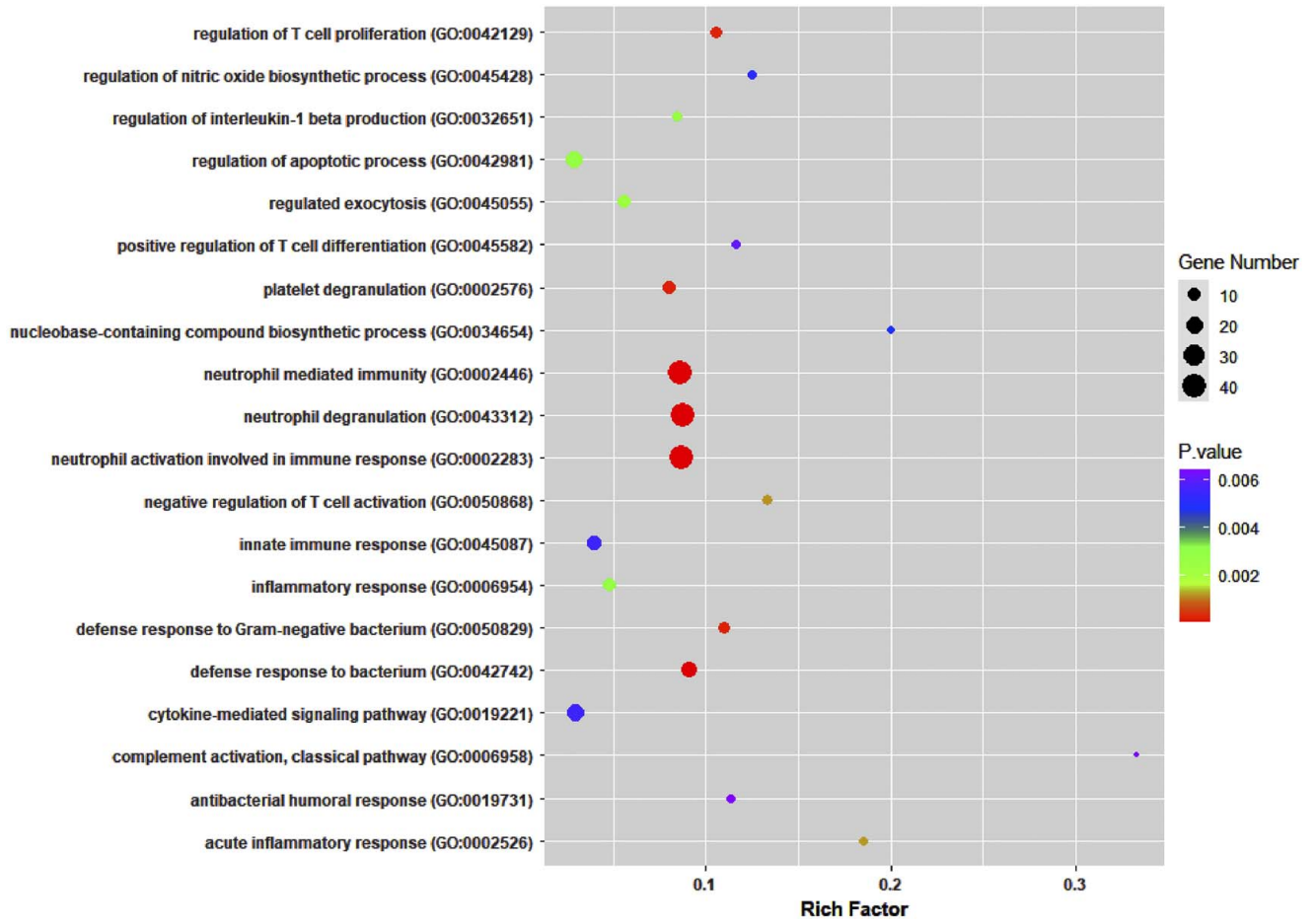

Figure 9. The results of enrich r analysis (critical module).

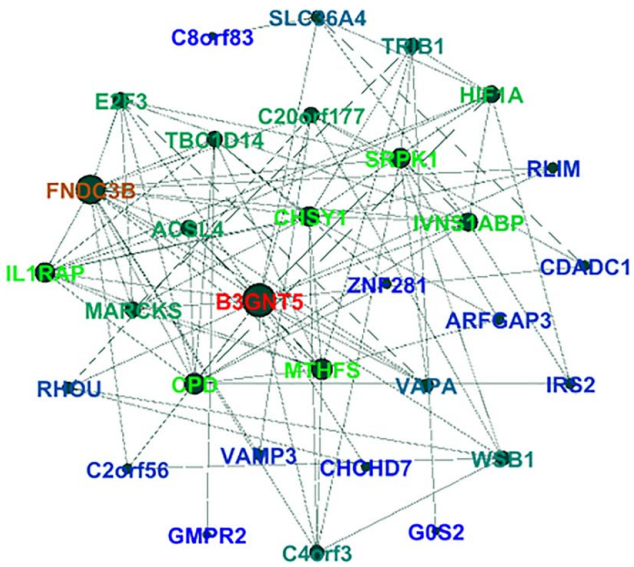

Figure 10. Network illustration and analysis powered by Gephi.

Network illustration and results

The network was illustrated. Also, the B3GNT5 gene was considered the most critical (interacted and centrality) gene in the obtained network (Fig. 10).

Discussion

Burn injuries can have profound physical and emotional impacts, often giving rise to a range of psychological challenges, with anxiety being a prominent issue[85–102]. Nowadays, immune molecules are recognized as a new target for managing various disorders and diseases[103]. In addition, burn wounds, as an inflammatory challenge for the immune system, can affect the immune molecules and ultimately affect the clinical outcomes among burn patients[104]. The current study achieved two immune cell signatures (NK cells and macrophages), the shared pathways between immune molecules and mortality, and a critical gene (B3GNT5) associated with mortality among elderly patients using a bioinformatics approach and programming methods in biology.

The present study obtained expression patterns of two essential cell signatures related to the immune molecules' activity during burn wound healing. At first, the NK cell signature is the most upregulated among immune signatures during 168 h after burn injuries. Teisserenc *et al.* revealed the functional impairment

of NK cells during burn injuries and its association with septic shock after burning[105]. However, Barrett *et al.*[106] demonstrated that non-sever burn injuries didn't impact NK cell activities, and accordingly, burn severity can be an influential factor on NK cell activity or count. Also, inconsistent with our findings, Devine *et al.*[107] showed an extreme count reduction of NK cells after severe burn injuries. In this context, divergence may be attributed to the presence of confounding variables, such as disparate treatments administered or sampling time during the burn period. Also, Patil *et al.*[108] have shown that burn injuries cause an increase in NK cell counts, which is related to infection prevention. Regarding this issue, the induction of NK cell activity was observed after the "cluster of differentiation 3 (CD3) antibody-induced activated" treatment[109]. In addition, it seems that the healing of burn wounds is related to the increased NK cell count. Moreover, based on the present results, according to the investigation of gene signatures behaviour during 168 h, It appears that the activity of NK cells increased with the wound-healing process.

Furthermore, 168 h after burn injuries, the downregulation of the M1 macrophage signature was observed, which may be related to inflammation reduction. Berg *et al.*[110] reported the increased M1 count and gene signature during the early hours after burn injuries, indicating the induction of inflammatory conditions. Cuddihy *et al.*[111] also revealed the role of M1 macrophage during burn wound healing. On the other hand, evidence supports that as inflammation subsides and burn wounds heal, the genetic profile of macrophage inflammatory states, also known as phenotype M1, diminishes, which corroborates the findings of this study[112,113]. Notably, M1 macrophages play a crucial role in promoting corneal angiogenesis by secreting TNF-α, IL-6, and VEGF, which stimulate the proliferation and migration of endothelial cells. It is, therefore, imperative to focus on the modulation of M1 macrophage activation as a promising treatment for burn wound injuries[114]. However, it appears from the obtained results that additional research is required to investigate the immune cell behaviours during burn injuries and their influence on the clinical outcomes of burn wound healing.

In the present study, the most critical gene, B3GNT5, was obtained that strongly related to mortality during 168 h after burn injuries. β1,3-N-acetylglucosaminyltransferase V (B3GNT5) constitutes an essential enzyme that is involved in the biosynthesis of complex carbohydrate chains on glycolipids, specifically the lacto- or neolacto-series, and Its primary function is to participate in the biosynthesis of carbohydrate structures such as Human Natural Killer cell (NHK)-1 and Lewis X[115]. The relation between B3GNT5 and various diseases mortality was shown formerly[116,117]. However, to the best of our knowledge, the relationship between B3GNT5 and burn wounds was not investigated. Nevertheless, the B3GNT5 is associated with NK cell function through (NHK)-1 carbohydrate[118]. On the other hand, the relationship between NK cell dysfunction and burn mortality was indicated previously[72], and the mentioned relationship can be a proposed pathway for the connection between burn wound-related mortality and the gene. Meanwhile, the gene may be a promising candidate for further burn wound healing research investigations.

In the conducted study, the pathways, including "regulation of histamine secreted from mast cell" and "T helper 17 cell differentiation," were achieved that correlated to mortality-related critical modules. Räntfors *et al.*[119] revealed the role of histamine and histamine release on the mortality rate after burn injuries via hyperinflammation and anaphylactic response. Sasaki *et al.*[120] also indicated the Th 17 inflammatory response induction after burn injuries. In addition, Rendon and colleagues have shown that Injuries such as burns can lead to widespread effects on body physiology, some of which can disrupt the intricate processes that regulate Th-17 immunity. These disruptions may increase the risk of infection, multiple organ dysfunction, and even death[121]. In this regard, these pathways can be considered a platform for developing burn wound management methods.

Conclusion

Overall, in the current study, using a bioinformatics approach, two cell signatures, including macrophage and NK cells, were achieved that had the most changes in the expression pattern during 168 h after burn wound incidence. The above changes may be related to clinical outcomes so that the alterations occurring in these cells (count and function) could avert negative prognostic scenarios and expedite the process of wound healing through the modulation of inflammatory responses and the prevention of septic complications. Additionally, the gene, B3GNT5, was extracted, which was found to be correlated with mortality, and although it has demonstrated efficacy in enhancing NK cell activity, it can be regarded as a dependable indicator for prognostic assessment of burn wounds. Finally, the two immune pathways, including "regulation of histamine secreted from mast cell" and "T helper 17 cell differentiation," were obtained, which have a significant relationship with mortality-related genes and can be applied as a concept for medication development.

Ethical approval

This article does not contain any studies with human or animal subjects performed by any authors and does not require ethical approval and consent.

Consent

Not applicable.

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There was no source of funding for this systematic review study.

Author contribution

Study concept and design by all authors; Data acquisition by all authors; Data interpretation by all authors; drafting the manuscript by all authors; Revision of the manuscript by all authors; the final version of the manuscript is approved by all authors.

Conflicts of interest disclosure

The authors have no conflicts of interest to declare.

Research registration unique identifying number (UIN)

We could not register our manuscript in the Research Registry UIN: www.researchregistry.com due to internet access restrictions and international sanctions. we live in Iran. We hardly even meet the basic needs of our daily life. We do not receive any funding for our research and we cannot pay for our research. Please excuse us from registering this manuscript in the Research Registry UIN: www.researchregistry.com.

Guarantor

Ramyar Farzan.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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