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Research Paper

Chitinase 3-Like Protein 1, Th 2 Cytokines (IL-4 and IL-13) in Incised and Lacerated Cutaneous Wounds: Experimental Study

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ABSTRACT

Background: Wounds are associated with increased levels of inflammatory biomarkers. In forensic investigations, biomolecular analysis can help determine the age of a wound. This study aimed to estimate the levels of inflammatory biomarkers: chitinase 3-like 1 (CHI3L1), interleukin-4 (IL-4), and interleukin-13 (IL-13); and to correlate them with immunohistopathological effects.

Methods: Cutaneous incisional (IW) and lacerated (LW) wounds were induced in thirty-six male rats, with eighteen rats allocated to each group. Tissue samples were collected at 4 and 8 days post-injury for histopathological, immunohistochemical, and biomolecular examinations.

Results: IL-4, IL-13, and CHI3L1 demonstrated significant time-dependent upregulation, with expression levels rising four- to six-fold at days 4 and 8 post-injury ($P<0.05$). CHI3L1 showed a biphasic expression pattern, with peak expression on day 4. IW generally exhibited higher gene expression levels compared to LW.

Conclusion: The biphasic time-dependent expression patterns of CHI3L1 in cutaneous injuries could be useful for wound age estimation, supporting their future application in medical and forensic contexts through further human clinical validation.

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Introduction

Although wound age estimation is difficult in forensic pathology, it can help reconstruct murder scenes and identify potential culprits. Forensic wound examination is an essential medicolegal task. It identifies the type of force used, the elapsed time between the infliction of the wound and death. It plays a critical role in both wound age estimation and injury type identification. Each wound has distinct macro and microscopic features, e.g., a lacerated wound results from blunt force trauma with irregular edges and localized pooling of blood in adjacent subcutaneous tissues. In contrast, an incised wound is caused by sharp objects with clean edges (1). However, factors such as skin tension and cleavage lines can alter the morphological appearance of wounds (2).

Biological responses to injury; wound vitality involves a well-coordinated interaction between cells and biomolecules through a sequence of overlapping phases—namely, inflammatory, proliferative, and maturation—that drive tissue reconstitution (3). Assessing these alterations using morphological, histopathological, immunohistochemical, and biomolecular techniques can assist forensic examiners in determining wound age and identifying the likely causative agent (4, 5).

Chitinase 3-like 1, a member of the glycosyl hydrolase 18 gene family, increases from day one to day three after wounding and plays a crucial role in wound healing by regulating cell death and inflammation through an interleukin-13 receptor α2-dependent mechanism (6). The injury-related increase in CHI3L1 expression is linked to the recruitment of immune cells that maintain Th1/Th2 balance and to the activation of macrophages, particularly M2 macrophages, which are crucial for extracellular matrix repair and remodeling (7).

T helper cells exert immune regulatory and tissue-reparative effects that counteract the antimicrobial effects of T helper 1 cells. T helper 2 cytokines, such as interleukin-4, interleukin-10, and interleukin-13, suppress inflammation and promote tissue healing (8, 9). Interleukin-4, a cytokine that activates connective tissue cells and stimulates extracellular matrix accumulation, is expressed after day one of injury, gradually increases up to day 4, and completely disappears by day 21 (10). IL-13 is a major profibrotic cytokine that regulates inflammation and tissue remodeling in a time-sensitive, cell-specific, and site-dependent manner. In the early stages of wound repair, IL-13 promotes collagen degradation via matrix metalloproteases while suppressing proinflammatory pathways, but in later phases, it enhances collagen deposition and prevents its breakdown (9). The ability of T helper cells and interleukin-13 to regulate tissue inflammation and fibrosis can be enhanced by chitinase-like proteins (11). Evaluating chitinase 3-like 1 mRNA/protein and inflammatory cytokine mRNA expression using molecular and histological techniques is useful for assessing wound vitality and could be applied in forensic wound analysis. Additionally, mRNA analysis offers earlier insights into wound vitality than histo-morphological assessments (5, 12, 13).

Although IL-4, IL-13, and CHI3L1 have been associated with inflammation and tissue remodeling, their forensic applicability remains limited by the lack of standardized expression curves in controlled laceration wound models. Wound morphology varies significantly between sharp-force and blunt-force injuries; a model that compares both under identical experimental conditions is needed. A rat model allows precise control of wound biomechanics, consistent wound depth, and accurate sampling. This study, therefore, aimed to fill this gap by assessing the temporal expression of CHI3L1, IL-4, and IL-13 at the edges of incisional and lacerated cutaneous wounds under conditions that could replicate medicolegal scenarios.

Materials and Methods

Ethical considerations

Approval from the Institutional Animal Care and Use Committee at Cairo University, Cairo, Egypt (reference number CU/III/F/11/24), was obtained before animal handling. The National Institutes of Health Guide for the Care and Use of Laboratory Animals was followed.

Animals

Thirty-six male albino rats weighing 150-200 gm (average 12 weeks old) were obtained from the animal house of the Faculty of Medicine, Cairo University, Egypt. The resource equation method was used to calculate the total number of animals in each group; incisional wound (IW) and lacerated wound (LW); using an acceptable range of the inter-subject error (DF) 10–20 and number of groups (k) 12. The calculated number of rats/group was 12, increased to 18 to accommodate attrition rates and ensure statistical reliability (14).

Wound model and sampling

Two groups were anesthetized by intraperitoneal injection of Ketamine-Xylazine (K: 80 mg/kg + X: 8mg/kg) in the same syringe. In the IW group, a full-thickness cutaneous cut injury approximately 2 cm was created in all rats using a surgical blade (no. 15). In LW, an object, approximately 350 g in weight with a1.6 cm striking surface, was dropped through a cardboard tube from a height of 47 cm, striking the skin after about 0.4 seconds. The impact delivered an estimated kinetic energy of 1.6 Joules and an impact pressure of 17 kPa, producing a cutaneous laceration approximately 1.5 cm in length, without involvement of the underlying muscle. The type of the wound was assessed by macroscopic examination (Figure 1).

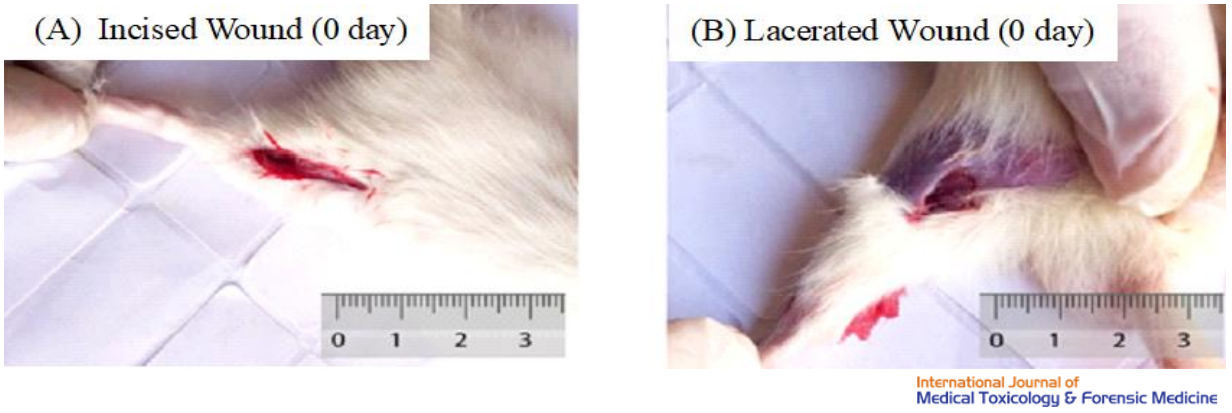

Figure 1. Mean RBG measures in BBI & SSI groups.

The wounds were left undressed, and the rats were housed in their cages with free access to a balanced diet and water ad libitum. Rats in each group were sacrificed by cervical dislocation at 4-and 8-day post-wounding (six rats at each time interval). The 0-day samples served as baseline controls, the 4-day samples represented the progressive inflammatory phase, and the 8-day samples reflected changes associated with the early proliferative stage of wound healing (4).

Samples were collected immediately after scarification. Half of the samples were preserved in liquid nitrogen for RNA expression analysis, while the other half was preserved 10% neutral buffered formalin for histopathological and immunohistochemical examination.

Histopathological examination

After formalin-preserved cutaneous samples were dehydrated and cleared, the tissues were embedded in paraffin and sectioned at 5 µm. The serial sections were stained with hematoxylin and eosin and examined under a light microscope (15).

Immunohistochemical examination

Sections were then dewaxed, immersed in a solution of 0.05 M citrate buffer, pH 6.8 for antigen retrieval, and treated with 0.3 % H_2O_2 and protein block. They were then incubated with the following primary antibodies; polyclonal anti-IL13 antibody (Invitrogen, Cat # PA5-102574, dilution 1/200), anti-IL4 (Invitrogen, Cat # PA5-115416, 1:200 dilution) and rabbit anti-CHI3L1 polyclonal antibody (Invitrogen, Cat # PA5-95897, USA; 1:200 dilution). After rinsing with phosphate-buffered saline, they were incubated for 30 min at room temperature with a goat anti-rabbit secondary antibody (Cat# K4003, EnVision+™ System Horseradish Peroxidase Labelled Polymer; Dako). Slide visualization was performed using a DAB kit, followed by counterstaining with Mayer's hematoxylin (16).

Expression analysis of Chitinase 3-Like Protein 1, Interleukin-4, and -13 gene

RNA extraction

Total RNA was extracted from skin samples by the standard TRIzol® Reagent extraction method (Invitrogen, Germany) according to the manufacturer's instructions. Total RNA was treated with 1 U of RQ1 RNase-free DNase (Invitrogen, Germany) to digest DNA residues, and resuspended in DEPC-treated water. Purity of total RNA was assessed by the 260/280 nm ratio (1.8-2.1). Additionally, integrity was confirmed by ethidium bromide-stain analysis of 28S and 18S bands using formaldehyde-containing agarose gel electrophoresis (17).

Reverse transcription (RT) reaction

According to Hamza et al. (2015) (18), the RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany) was used to obtain cDNA from skin samples using 5 µg of total RNA, following the manufacturer's instructions.

Table 1. The sequences of specific primer of the targeted and reference genes used were designed.

Gene	Primer sequence	NCBI Reference
IL-4	F: CAA CAA GGA ACA CCA CGG AG R: TTT CAG TGT TGT GAG CGT GG	X16058.2
IL-13	F: CCC TGA CCA ACA TCT CCA GT R: AGG TCC ACA GCT GAG ATG TC	NM_053828.1
CHI3L1	F: ACC CCA GAC TGA AGA CAC TG R: TCC GCC TTC AGT TCC TTG AT	NM_001309820.1
GAPDH	F: GAG ACA GCC GCA TCT TCT TG R: ACC GAC CTT CAC CAT CTT GT	XM_063285517.1

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Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

StepOne™ Real-Time PCR System from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA USA) was used using 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template (10, 19). The sequences of the specific primers for the targeted and reference genes used were designed (Table 1).

The relative quantification of the target to the reference was determined by using the $2^{-\Delta\Delta CT}$ method (20, 21).

Statistical analysis

All quantitative RT-PCR data obtained from the gene expression analysis for the tested genes were analyzed using SPSS version 20. The ANOVA with the Scheffé Post Hoc test was used to determine significant differences between groups. The values are expressed as mean ± SD. A p-value < 0.05 was considered significant.

Results

Histopathological findings

The cutaneous wounds underwent significant histopathological inflammatory changes. By day 4 post-injury, both the incised and lacerated groups exhibited features of acute inflammation; the incised wounds showed marked necrosis, neutrophilic infiltration, edema, and fibrin deposition with minimal

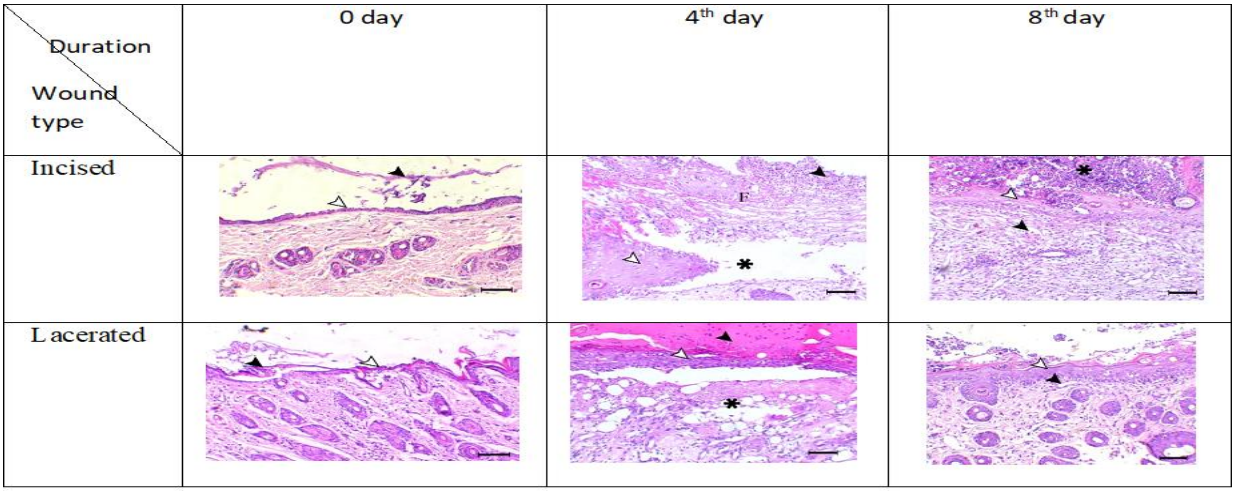

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Figure 2. Photomicrographs illustrating wound healing progression (scale bar = 50 µm) at days 0, 4, and 8 post-injuries in incised and lacerated wounds. At day 0, the epidermal layer in the incised wound showed erosive changes within (white arrowhead) with sloughing of the epidermal epithelium (black arrowhead). The epidermal layer in the lacerated wound showed marked erosive changes (white arrowhead) with desquamated epidermal epithelium. At day 4, the incised wounds exhibited extensive necrotic changes, edema (asterisk), abundant fibrin deposition, and prominent infiltration of neutrophilic inflammatory cells (black arrowhead), with an epithelial tongue extending deeply into the necrotic tissue (white arrowhead). By day 8, these wounds showed persistent neutrophilic infiltration within the overlying scab (asterisk), which covered a rim of regenerating epidermal epithelium (white arrowhead), and the underlying dermis displayed immature granulation tissue rich in newly formed blood vessels and mild collagen deposition (black arrowhead). In the lacerated wounds, day 4 revealed the formation of a protective scab consisting of a proteinaceous eosinophilic layer (black arrowhead) overlying a partially re-epithelialized epidermis (white arrowhead), along with multifocal edema and fibrin accumulation in the dermis (asterisk). By day 8, the scab showed reduced inflammatory cell infiltration, with a fully regenerated epidermis of normal thickness (white arrowhead), and mature granulation tissue characterized by few blood vessels and marked collagen deposition (black arrowhead).

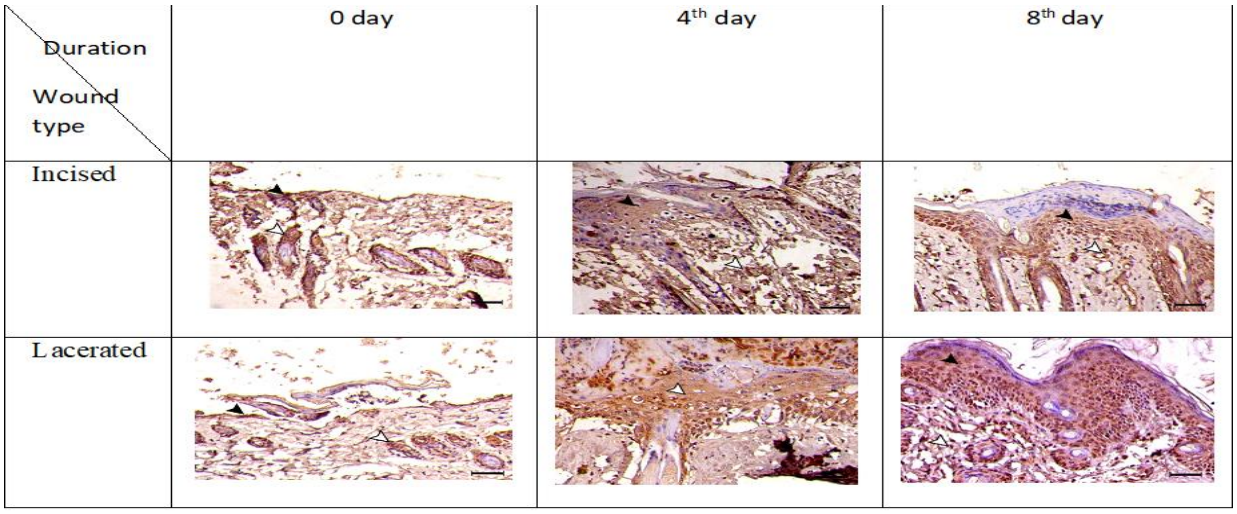

Figure 3. Photomicrographs illustrating IL-4 immunoexpression (scale bar = 50 μm) at days 0, 4, and 8 post-injuries in incised and lacerated cutaneous wounds. In the incised wounds, IL-4 expression was observed at day 0 within the damaged epidermal epithelium (black arrowhead) and in the dermal hair follicle epithelium (white arrowhead). By day 4, marked expression was detected within the proliferating epithelium (black arrowhead) and in the dermal connective tissue (white arrowhead). At day 8, IL-4 immunostaining had persisted in the proliferating epidermis (black arrowhead), with mild expression in the dermal granulation tissue (white arrowhead). In the lacerated wounds, IL-4 expression was detected at day 0 within the epidermis (black arrowhead) and in the dermal hair follicle epithelium (white arrowhead). By day 4, the expression became more prominent in the proliferating epithelium (white arrowhead). At day 8, the marked IL-4 expression was detected in the proliferating epidermis (black arrowhead), accompanied by moderate expression within the dermal granulation tissue (white arrowhead).

Table 2. The expression levels of IL-13, IL-4, and CHI3L1 genes in Incisional wound (IW) and lacerated wound (LW) at different healing durations.

	IL-4 expression		IL-13 expression		CHI3L1expression	
Time points	**Incisional wound**	**lacerated wound**	**Incisional wound**	**lacerated wound**	**Incisional wound**	**lacerated wound**
0h	1.00±0.076^{c}	1.00±0.085^{c}	1.00±0.1^{c}	1.00±0.096^{c}	1.00±0.078^{c}	1.00±0.123^{c}
4 days	6.38±0.181^{b}	6.14±0.257^{b}	4.57±0.313^{b}	4.37±0.245^{b}	5.68±0.215^{a}	5.19±0.251^{a}
8 days	8.21±0.249^{a}	7.93±0.286^{a}	6.23±0.255^{a}	5.83±0.216^{a}	3.82±0.280^{b}	3.55±0.280^{b}

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$^{a,b,c:}$ Mean values within same wound type at different time points with unlike superscript letters were significantly different (P<0.05).

epidermal re-epithelization, while the lacerated wounds developed a protective scab with moderate epidermal re-epithelization and multifocal dermal edema accompanied by moderate fibrin deposition. By day 8, both groups showed healing, with the incised wounds demonstrating ongoing neutrophilic infiltration beneath the scab and obvious epidermal re-epithelization, along with immature granulation tissue marked by increased vascularization and mild collagen deposition. In contrast, the lacerated wounds demonstrated complete epidermal re-epithelization under a well-formed scab, reduced inflammatory cellular infiltration, and a mature granulation tissue with minimal vascularization and prominent collagen deposition, indicating more advanced progression into the proliferative phase of tissue repair (Figure 2).

Immunohistochemical findings

IL-4 expression was observed in both incised and lacerated wounds at all examined time points. In incised wounds, IL-4 was initially detected in the damaged epidermis and dermal hair follicles at day 0, with marked expression in the proliferating epithelium and dermal connective tissue by day 4. By day 8, strong epidermal expressions persisted, along with mild staining in the dermal granulation tissue. In lacerated wounds, IL-4 expression was also present at day 0 in the epidermis and hair follicles, became more prominent in the proliferating epithelium by day 4, and showed marked epidermal and moderate dermal granulation tissue expression by day 8 (Figure 3).

IL-13 expression was detected in both incised and lacerated wounds across all time points. In incised

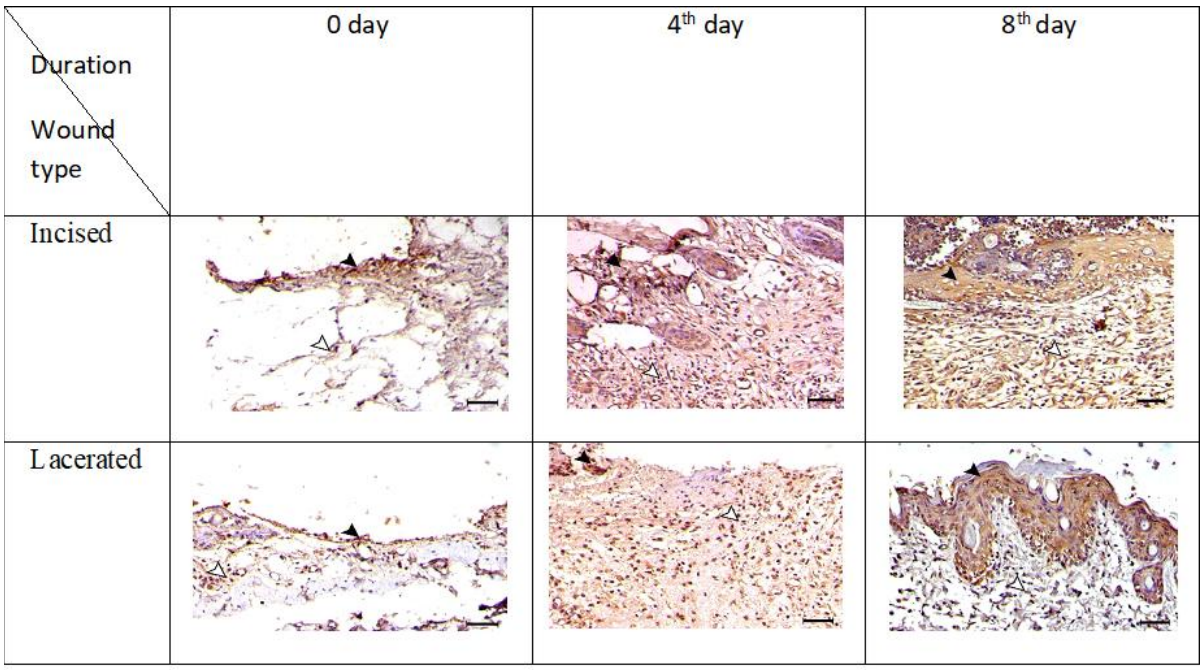

Figure 4. Photomicrographs illustrating IL-13 immunoexpression (scale bar = 50 µm) at days 0, 4, and 8 post-injuries in incised and lacerated cutaneous wounds. In the incised wounds, IL-13 expression was observed at day 0 within the damaged epithelium (black arrowhead) and within the dermal connective tissue fibers (white arrowhead). By day 4, marked expression was detected within the proliferating epithelium (black arrowhead) and in the dermal granulation tissue (white arrowhead). On day 8, IL-13 immunostaining had persisted in the proliferating epidermis (black arrowhead), with expression also observed in the dermal granulation tissue (white arrowhead). In the lacerated wounds, IL-13 expression was detected at day 0 within the damaged epidermis (black arrowhead) and in the dermal fibrous connective tissue (white arrowhead). By day 4, the expression became more marked within the granulation tissue (white arrowhead). At day 8, the IL-13 expression was markedly detected in the proliferating epidermis (black arrowhead), accompanied by moderate expression within the dermal granulation tissue (white arrowhead).

wounds, expression was initially observed in the damaged epithelium and, to a lesser extent, in the dermal connective tissue at day 0, becoming marked in both the proliferating epidermis and the dermal granulation tissue by days 4 and 8, respectively. In lacerated wounds, IL-13 was similarly present in the damaged epidermis and dermal connective tissue at day 0, with increasing expression in the granulation tissue by day 4, and marked epidermal and moderate dermal expression by day 8 (Figure 4).

In incised wounds, CHI3L1 expression was mild at day 0 in the damaged epithelium and dermal follicles, increasing by day 4 with marked epithelial and moderate dermal expression. By day 8, marked expression persisted in the epidermis, with mild staining in the dermal granulation tissue. In lacerated wounds, CHI3L1 was initially observed in the epidermis and dermal connective tissue at day 0, became more prominent in the granulation tissue by day 4, and showed marked epidermal expression with slight dermal staining by day 8 (Figure 5).

Chitinase 3-Like Protein 1, Interleukin-4, and -13 gene expressions

At 0-day, cutaneous IW and LW showed the baseline expression levels of IL-4 (1.00±0.08/1.00±0.09), IL-13 (1.00±0.08/1.00±0.09), and CHI3L1 (1.00±0.08/1.00±0.12) that were nearly identical.

After 4 days post-injury, cutaneous IW and LW showed significantly higher expression levels of the IL-4 (6.38±0.18/ 6.14±0.26), IL-13 (4.57±0.31/ 4.37±0.25), and CHI3L1 (5.68±0.22/ 5.19±0.25) genes ($P<0.01$) compared to control, with approximately four to six-fold increase. Moreover, at 8 days post-injury, the expression levels of IL-4 (8.21±0.25 in IW/7.93±0.29 in LW) and IL-13 (6.23±0.26/ 5.83±0.22, respectively) were significantly increased ($P<0.001$) compared with both day 4 post-injury and the control. In contrast, the CHI3L1 expression in cutaneous IW and LW (3.82±0.30 and 3.55±0.28, respectively) was also significantly higher than in the control ($P<0.001$). Still, it remained significantly lower than that observed at day 4, indicating old injury. Expression levels of IL-4, IL-13, and CHI3L1 genes in

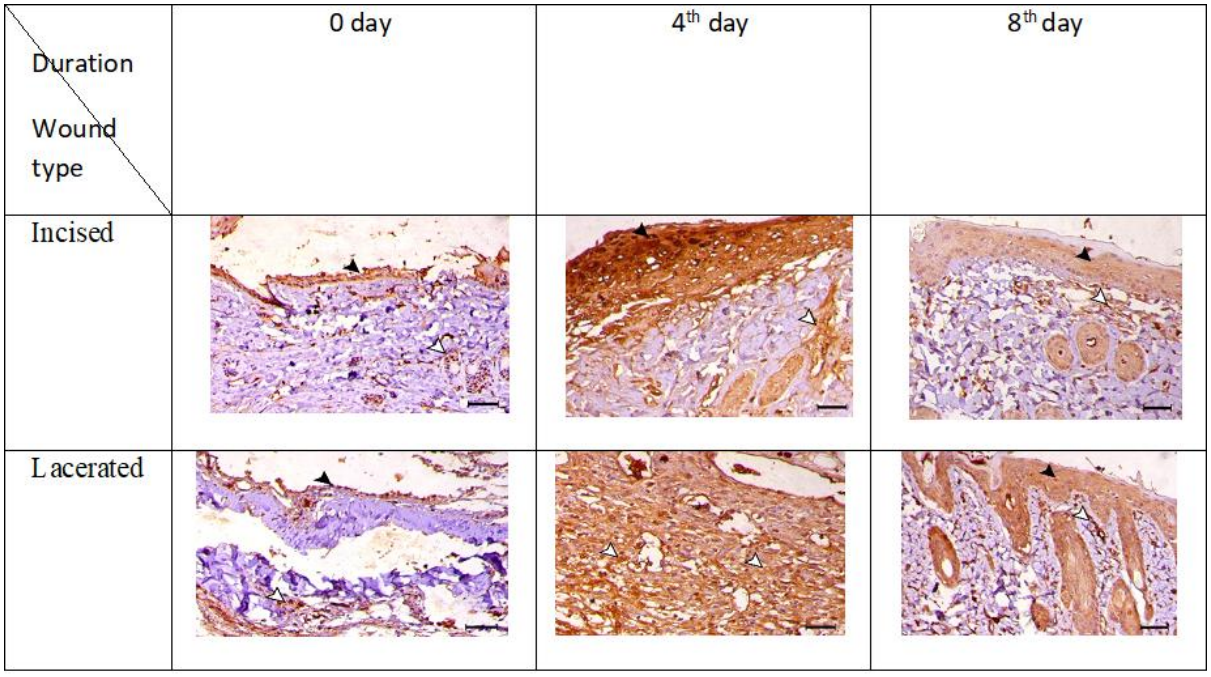

Figure 5. Photomicrographs illustrating CHI3L1 immunoexpression (scale bar = 50 µm) at days 0, 4, and 8 post-injuries in incised and lacerated cutaneous wounds. In the incised wounds, CHI3L1expression was mildly observed at day 0 within the damaged epithelium (black arrowhead) with slight expression within the dermal follicular epithelium (white arrowhead). By day 4, marked expression was detected within the damaged epithelium (black arrowhead) and with moderate expression in the dermal connective tissue (white arrowhead). On day 8, IL-13 immunostaining had persisted in the proliferating epidermis (black arrowhead), with mild expression in the dermal granulation tissue (white arrowhead). In the lacerated wounds, CHI3L1 expression was detected at day 0 within the damaged epidermis (black arrowhead) and in the dermal connective tissue (white arrowhead). By day 4, the expression became more marked within the granulation tissue (white arrowhead). At day 8, the CHI3L1 expression was markedly detected in the proliferating epidermis (black arrowhead), accompanied by slight expression within the dermal granulation tissue (white arrowhead).

IW were generally higher than in LW (Table 2).

Discussion

The wound-healing process is influenced by various mechanical factors, including impact force and wound extent, which affect gene expression, cellular proliferation, growth factor production, and the production of inflammatory mediators (22). These time-dependent molecular responses and their morphological impacts are used in the medical field to estimate wound age (4, 5).

In this experimental animal model, during the early stages of cutaneous wound healing (up to 4 days), the expression levels of IL-4, IL-13, and CHI3L1 increased significantly after injury, reflecting a consistent inflammatory response in both incisional and lacerated wounds. Moreover, the significantly higher expression of CHI3L1 and IL-13 in incisional wounds suggests a distinct healing response, particularly on days 4 and 8 post-wounding, respectively.

IL-4 and IL-13 are key regulators of the immune response, particularly in promoting tissue repair and wound healing by activating M2 macrophages, which are associated with anti-inflammatory and tissue-repair functions. Salmon- Ehr et al. (2000) (10) identified IL-4 in the lower dermis of cutaneous wounds as early as day 1 post-wounding, peaked around day 4, and then gradually decreased until disappearing by day 21. IL-4 has a vital role in early tissue healing by activating lymphocytes and allowing active macrophage polarization. During the proliferative phase of healing, IL-4 enhances cellular proliferation and differentiation, promoting tissue regeneration (23). However, sustained IL-4 expression during delayed healing may be due to underlying pathological conditions or the body's attempt to compensate for impaired healing (24).

Furthermore, IL-13 suppresses the initial inflammatory response. It facilitates the transition from the inflammatory to the proliferative phase by promoting extracellular matrix re-epithelization via transforming growth factor-beta, which enhances collagen production and fibroblast activity (25). However, its extensive expression can lead to fibrosis, hypertrophic scarring, and keloid formation. So, a balanced expression of inflammatory cytokines is required for proper wound healing (9). The

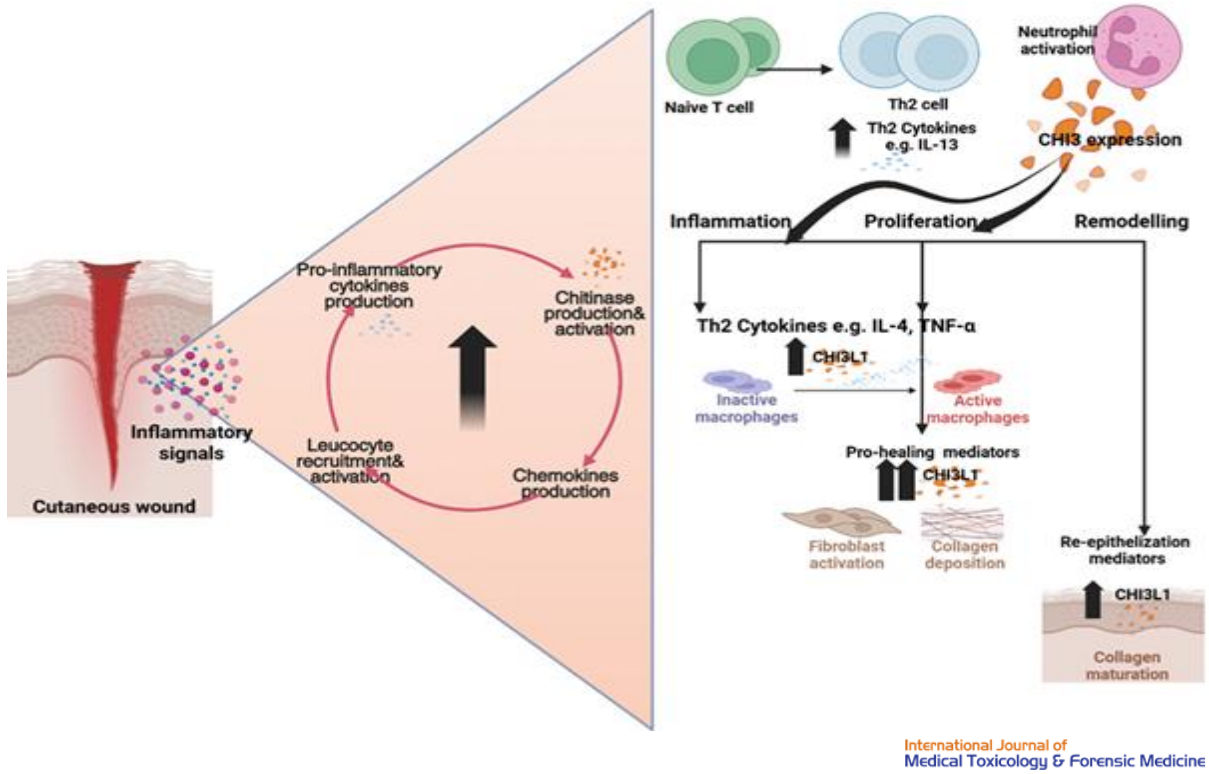

Figure 6. The schematic illustration of the process of acute cutaneous wound healing through its three phases: inflammation, proliferation, and remodeling. During the inflammatory and proliferative phases, immune cell infiltration—including neutrophils, Th2 cells, and macrophages—was observed, coinciding with peak activity of chitinase-3-like 1 (CHI3L1). As fibroblast activation increases and granulation tissue forms, followed by collagen deposition, tissue maturation, and extracellular matrix development, CHI3L1 expression declines, supporting the progression toward proper wound healing.

significantly higher IL-13 expression in incisional wounds compared to lacerated wounds could reflect the immune system's ability to modulate cytokine responses in response to wound extent and specific tissue repair requirements, such as fibroblast activation, collagen synthesis, and tissue remodeling.

CHI3L1, also known as YKL-40, is a glycoprotein involved in tissue remodeling, inflammation, and extracellular matrix formation. Its role in wound healing differs according to the nature of the injury and post-wounding interval (6, 13). Li et al. (2021) (22) identified 15 key genes that regulate cellular proliferation, the inflammatory response, and cytokine activity, particularly IL-17 and the Hematopoietic cell lineage signaling pathway. These genes can predict wound severity and the extent of the lacerated tissue damage. Their findings indicated that healing processes in certain tissues, such as skeletal muscle, experienced similar molecular and cellular alterations that were affected by the extent of tissue damage. In mild injuries, the most upregulated genes were related to the organismal interface and the inflammatory response to external stimuli. Conversely, in severe injuries, the predominant genes were associated with defense mechanisms, the organismal interface, and external biotic stimuli (6, 13, 22, 26).

Moreover, Webb et al. (2001) (27) suggested that the presence of IL-4 and IL-13 in the early wound phase was indirectly involved in tissue remodeling via the IL-4Rα subunit. This pathway was associated with the enhancement of Ym proteins, which are homologous to chitinases and have the capability of recognizing specific carbohydrate molecules on cell surfaces to target cells for destruction or activation(28)

During the early phase of wound healing, Goren et al. (2014) (29) identified high levels of Ym1 (chitinase 3) in murine wound tissue, recognizing it as a marker of the IL-4–activated macrophage phenotype. Similarly, Murase et al. (2017) identified high CHI3L1 expression in an experimental cutaneous wound model until day 3 post-injury. They disappeared by day 5, suggesting that CHI3L1 could serve as a histological marker for estimating wound age in this phase (26). In addition, Murase et al. (2022) observed a clear increase in CHI3L1 expression, starting from day 2 post-injury and reaching a significant peak by day 4 to 6 in human cadaver cutaneous injuries (13). The CHI3L1 expression period could vary by species and wound

duration. The peak activity of CHI3L1 in wound healing occurs during the inflammatory phase, where neutrophilic infiltration occurs within the first 2 days, followed by macrophage infiltration within 3-5 days (30). These cells are responsible for CHI3 expression (13, 26, 29). By day 5, proinflammatory cytokines attract fibroblasts, enhancing their migration to the wound area. This supports granulation tissue synthesis and extracellular matrix formation. CHI3L1 levels subsequently decline, as its sustained overexpression may impair tissue repair and contribute to chronic, non-healing wounds (31-33). This process could explain the decrease in CHI3 expression observed by day 8 post-injury, where its levels decline as the tissue remodeling phase progresses (Figure 6). The higher levels of the assessed markers, IL-4, IL-13, and CHI3L1, in incisional wounds could be related to an enhanced demand for tissue remodeling and matrix formation during cutaneous regeneration after severe damage. These markers are known to be involved in inflammatory and tissue remodeling processes; their expression levels may vary with timing, wound environment, and individual immune responses (21, 25, 33).

This study has some limitations, including the complexity of the wound-healing process and interspecies differences. Despite these limitations, the pattern of IL-4, IL-13, and CHI3L1 expression could serve as an indicator of wound age across different wound types. However, further studies are needed to standardize these biomarkers relative to other inflammatory markers and to conduct preclinical validation across different stages of wound healing in human subjects.

Conclusion

This study identified time-related changes in IL-4, IL-13, and CHI3L1 expression in incisional and lacerated cutaneous wounds, highlighting their potential usefulness as indicators of wound age, particularly on days 4 and 8, respectively. To the best of the authors' knowledge, no studies have been published on the use of CHI3L1 for wound age estimation in lacerated wounds. The identified biphasic pattern of CHI3L1 expression, characterized by a five-fold peak at day 4 followed by a decline by day 8, could be considered as a contribution to medical research, suggesting potential utility for improving wound age determination.

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Conflicts of Interest

The authors report there are no competing interests to declare.

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