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Evaluation of the role of laminin α1 in wound healing of skin mice by using Immunohistochemical assessment.

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ABSTRACT

Wound healing is a process through which the skin and subcutaneous tissue restore itself after it has been injured wound healing can be illustrated in a progression of physical characteristics (phases) that constitute the post-trauma repairing process. Aim: To know and evaluate the role of laminin α1 during the process of wound healing in the skin according to days of wound injuries**.** Materials & Methods**:** Thirty-two male mice, about 3-6 months, were divided into four groups according to days of wound injury. Each group has eight mice. Group (A) collects specimens on the first day of injury. Group (B) after one week of injury. Group (C) after two weeks. Group (D) control group collects specimens without injury. Tissue Sections were treated with laminin α1. Used Aperio Scope software to assess immunohistochemical reactivity. For statistical analysis used SPSS program**.** Results: immunohistochemical study reveals group (A) animals a very low expression laminin α1 at (0.15±0.008pixel/(micron)^2) compared with group (B) showing high expression laminin α1 after one week at (0.69±0.02pixel/(micron)^2). group (C) show week expression laminin α1 after two weeks (14day) at (0.39±0.02pixel/(micron)2). In group (D) (control group) expression of laminin α1 at (0.36±0.01pixel/(micron)2). Conclusion**:** the laminin α1 protein has an important role during injury healing, through significantly elevated laminin α1 in the first week and beginning to descend at the end of the second week. Can be used this timing as a guideline for injection or topical use of laminin α1 to promote wound healing after injury.

1. Introduction

skin is the largest organ of the human body. It is rather intricate in its arrangement since it is made of three layers in total: the first layer which is known as the epidermal layer, the second one which is known as the dermal layer, and the third layer which is known as the subcutaneous layer. They each have a part to play in maintaining the cutaneous barrier. As a collective unit, it is involved in the skin's overall business which includes its capacity to remake itself following healing of a skin lesion. A wound is defined as damage or disruption of the structure and function of cells and tissues caused by many factors such as chemical, physical, thermal, microbial, or immunological damage. Such disruption of epithelial integrity can also influence the general structure and form of the underlying tissue (Mulkalwar et al., 2015; Earley., 2008; Velnaret al.,2009). Wound healing represents a multicomponent biological process in which cells, cytokines, growth factors, and elements of the extracellular matrix act sequentially and in parallel (Olczyk et al., 2014; Nyman et al., 2019). Laminins are the most important proteins present in ECM and have essential roles in wound healing (Iorio et al., 2015). It is the most diverse and the most plentiful protein of the basement membrane and has paramount functions in the organization of tissue location and firmness, giving cells a base

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framework and playing a role in two critical functions of the healing process re-epithelialization and angiogenesis (Ishihara et al., 2018). This study aims to investigate the laminin α1 role in wound healing after injury.

2. Methodology

2.1 Animal care

The study was performed on (32) male albino mice collected from the Biotechnology Research Center, Al-Nahrain University, weighing between 25-28±50 g, aged 3-6 months, the animals were put in a plastic cage, effortless to clean with food (standard pellet diet) and free arrival to water (fresh tap water). Ten animals in each cage were kept with illumination variance (10 dark/14 light) and room temperature (20±2 C º) in a clean and well-ventilated room.

2.2 Experimental design

Thirty-two adult healthy male mice (albino mice), were divided into four groups according to days of wound injury. Each group has 8 male mice:

Group A: Collected the specimen from the area of skin injury on the first day of injury.

Group B: Collected specimens from the area of skin injury after one week (7 days) of injury.

Group C: Collect specimens from the area of skin injury after two weeks (14 days) of injury.

Group D: The animals are the control group collect specimens of skin without injury.

Induce adequate anesthesia through inhaled anesthesia. Shaved back of anesthetized mouse with hair trimmer and made linear incision in back length about 2-3 cm of mice, in 3 different times injury was tread, after 1 day, first week, second week and control group (without injury). The skin was collected and specimens were fixed chemically by immersion into 10% neutral buffered formalin. Then further histological procedure was done to obtain a paraffin block of specimens for immunohistochemical assessment.

2.3 The preparation of the paraffin section

The skin specimens were histologically willing for paraffin section as follows: start with the process of fixation, dehydration, clearing, impregnation, embedding in tissue block, sectioning on glass slides, de-waxing, hydration, staining, and mounting (Suvarna et al., 2019).

2.4 Immunohistochemical staining

The laminin α1 antibody (ab210954) supply by ABCAM. This antibody is applied for revealing the laminin α1 as antigen which is present in the skin. It is utilized with an immunohistochemistry detection kit called rabbit and mouse-specific HRP/DAB detection IHC kit. The prepared histological tissue slide stained with IHC for laminin α1 was examined for histological evaluation and estimation using a light microscope.

2.5 Statistical Analysis of Data:

All statistical analysis systems use the Statistical Package for Social Sciences (SPSS). Software program. The data have come across as mean and standard error. Variances between the mean percentages of the laminin α1protein expression of skin were calculated for statistical significance using ANOVA the variance in the mean positively ± standard Error between groups according to the day of injury (Babbie et al., 2018).

2.6 Immunohistochemical examination

Aperio image scope verasion12 Used to enumeration algorithms program to measure colors amount in sections of tissues which depend on parameters of brown color quantification in three ambits of intensity (strong positive, positive and weak positive) and negative (blue color) for unstained tissue and measured the total positive of the slide for three colors.

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3. Results and discussion

3.1 *Immunohistochemical expression of laminin α1*

Laminin α1 protein is a family of large multi-domain glycoproteins, these proteins are significant structural proteins of BM and ECM. The laminin α1 is a bioactive component of basal lamina that participate actively in cell adhesion, migration as well as differentiation. Laminin α1 is a protein present in the layers of skin (dermis, epidermis) intercellular and basement membrane of skin. The expression immunohistochemical staining of laminin α1 was performed using monoclonal primary antibody against laminin α1. the group (A) animals they reveal a deficient expression of laminin α1 in area wound injure at the first day of injury at (0.15±0.008pixel/(micron)^2) [table (1-1), fig. (1-1) A&B] compared with group B which recorded high expression of laminin α1 in area wound injure after one week (7 days) of injured at (0.69±0.02pixel/(micron)^2 at p-value (0.001) [table (1-1), fig. (1-2) A&B]. group (C) show week expression of laminin α1 in area wound injure after two weeks (14 days) of injured at (0.39±0.02pixel/(micron)^2) at p-value (0.001) [table (1-1), fig. (1-3) A&B]. In group (D) (control group) skin without injured expression of laminin α1 at (0.36±0.01pixel/(micron)^2 [table (1-1), fig. (1-4) A&B].

Table 1: Reveals the Mean ±SE positivity of laminin α1 expression in skin injury for animal groups according to day of injury.

GROUPS	Mean ±SE pixel/(microns)²
First day	**0.15±0.008**
One week	**0.69±0.02**
Two weeks	**0.39±0.02**
Control	**0.36±0.01**
**(p≤0.001)	

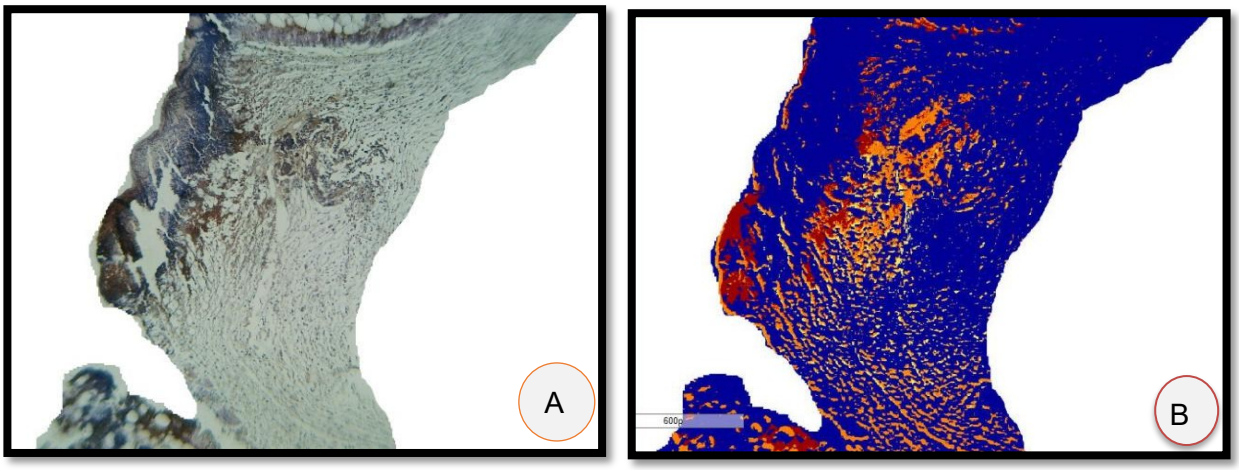

Figure -1: A- cross-section skin in group A (first day) shows a very low expression of laminin α1 at the incision area of skin 100X. B- snap shoot for section (A) as interpreted by aprio image J software program; blue=-negative, yellow= weak positive, orange= positive, brown= strong positive

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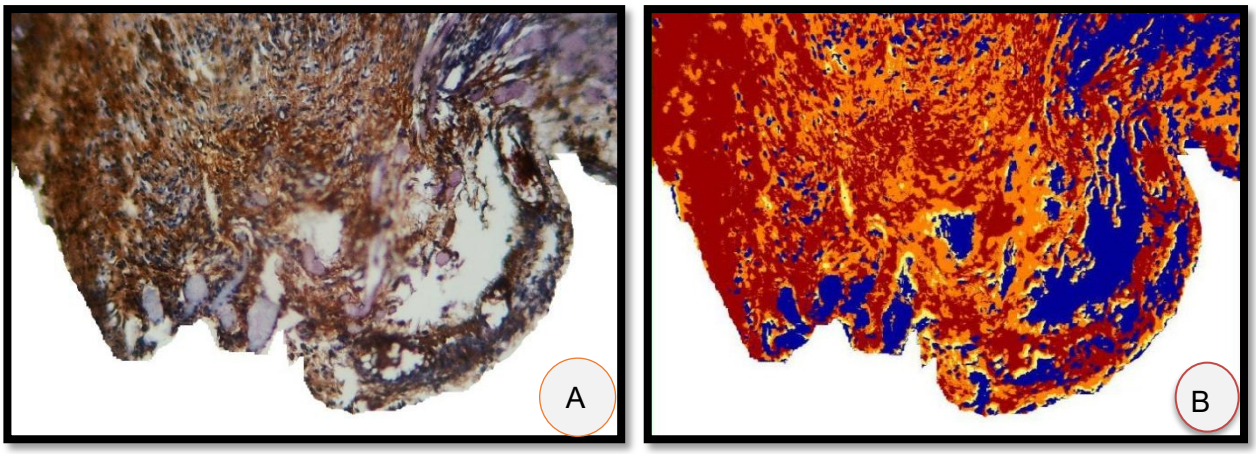

Figure 2: A a-cross-section of skin in group B (one week) shows a marked expression of laminin α1 at the incision area of skin 100X. B- snap shoot for section (A) as interpreted by aprio image J software program, blue=negative, yellow=weak. positive, orange= positive, brown= strong positive

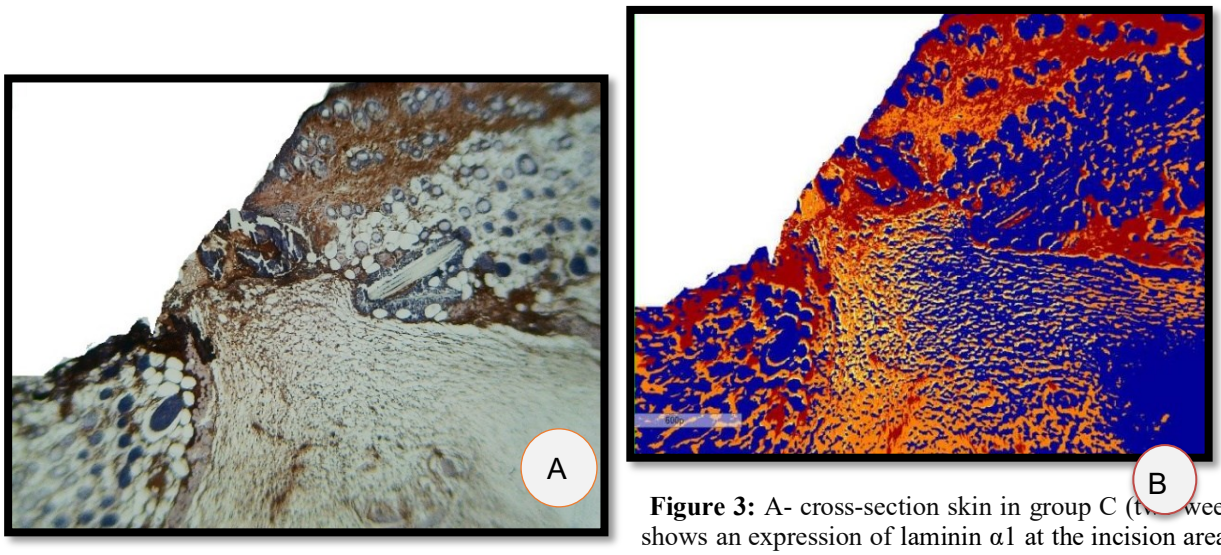

Figure 3: A- cross-section skin in group C (two weeks) shows an expression of laminin α1 at the incision area of skin 100X. B- snap shoot for section (A) as interpreted by aprio image J software program; blue=-negative, yellow= weak positive, orange= positive, brown= strong positive

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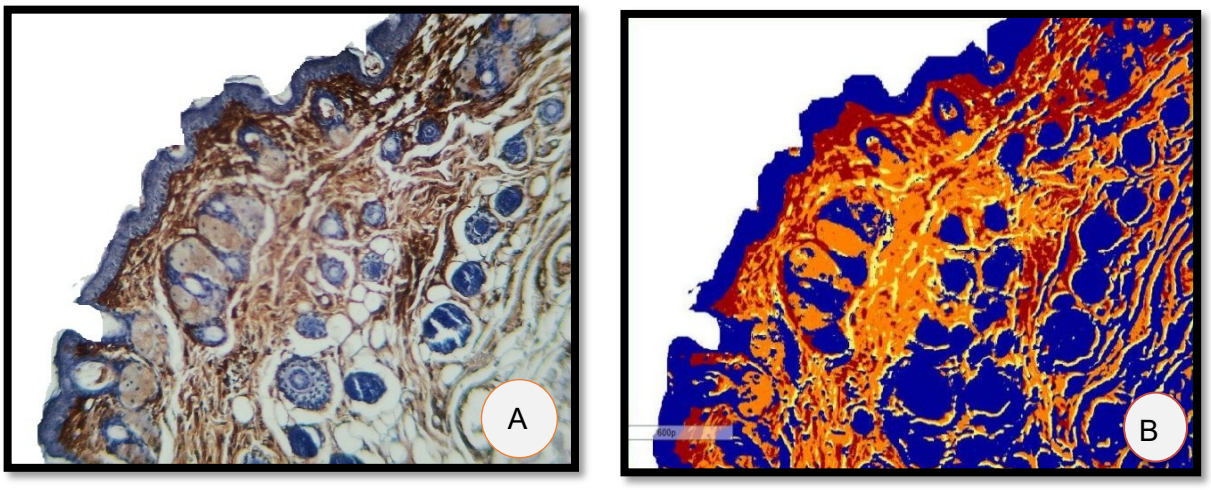

Figure 4: A- cross-section skin in the control group (D) shows an expression of laminin α1 at the incision area of skin 100X B- snap shoot for section (A) as interpreted by aprio image J software program, blue=-negative, yellow= weak positive, orange= positive, brown= strong positive.

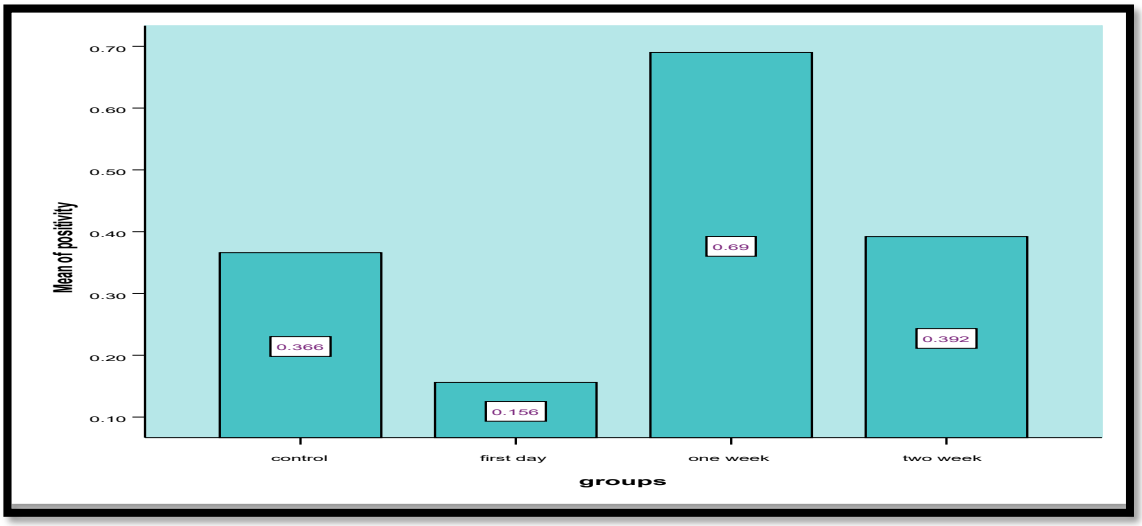

Figure 5: Histogram shows the difference in the reactivity of laminin α1 at the injury of the skin of experimental animal groups.

3.2 discussion effect of laminin α1 on wound healing

In this study, we found that the expression of laminin α1 on the first day of the wound was weak compared to its measurement on the seventh day (first week) of the wound. This is because wound healing goes through several stages and specific periods for each stage, and this is consistent with researchers in other studies that revealed that wound healing has three phases which are inflammation, proliferation, and remodelling (George Broughton et al., 2006). The first phase is called the inflammatory phase which includes hemostasis and inflammation, the injury in the skin quickly triggers a cascade of clotting that provides a temporary fibrin blood clot barrier to the wound. (Heng., 2011; Reinke& Sorg., 2012). Furthermore, the inflammation phase is revealed by the migration of leukocytes to the injured site. The response is very quick and is in phase with the main indicators pointing to inflammation – erythema and edema in the area of the lesion. Under normal circumstances, cell response is initiated in the first 24 hours and can be sustained for two days. Also, there is a fast development of

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the immune cells present on the tissue level as it does with mastocytes. Inflammation is intact and localized tissue response is released by lesion which is tissue demolition. Inflammatory cells work in the process of repairing the wounded area and are involved in the release of lysosomal enzymes and reactive oxygen species and the cleanup of different cellular debris (Medrado et al., 2003; Mason et al., 2002). This study revealed the highest expression of laminin α1 after one week (seven days) than other groups due to in this phase reepithelization occurs this agrees with the authors, who found the second phase is the proliferative phase is characterized by vascular network restoration and granulation tissue formation, this phase begins about (3 to 10 days) after injury. Various growth factors and cytokines play a role in this phase such as the transforming interleukin (IL) family, angiogenesis factors, and growth factor-beta family [Reinke & Sorg., 2012; Wang., 2018) so revealing the abilities of laminins to influence cell behavior, including angiogenesis and their participation in determining cell motility (Taniguchi et al., 2016). Moreover, in this stage, the common proliferating cells are endothelial cells and fibroblasts. As a result of cell proliferation, there occurs a necessity for a sufficient supply of blood to the affected tissues. Thus, an angiogenic response starts at the same time (Demidova-Rice et al., 2012; George Broughton et al., 2006). In addition to that Angiogenesis is an arranged process, including proliferation of the endothelial cell, rearrangement, and disruption as well as, provisional basal lamina, and migration, and are related to tubular architecture states (Rosen., 2002). Li J., et al authors revealed the epithelial coating cells are equivalent to all of the aforementioned proceeding, by the response to a cytokine, which moves and proliferates along the edges of the wound with the purpose of its healing, which is known as re-epithelialization. The wound re-epithelialization by keratinocytes, the collection of the over-proliferation stage with the cell migration near the lesion(Li et al., 2007). So, immunohistochemical assessment for the wound after two weeks found the expression of laminin α1 in (group-C) near to the expression of the control group and minimum than the expression of the week group, in this period the phase remodeling tissue and scar tissue formation, the final or third stage of wound healing is re-modeling, which begins in two to three weeks after the first appearance of the lesion and may take one year or more. Its most basic purpose is to obtain the maximal tensile strength through the degradation, reordering, and reparative synthesis of the ECM. The final phase of the lesion's healing is characterized by the ability of the granulation tissue to progressively remodel as well as the structure of normal tissue and be withdrawn and form the scar less into vascular and minimal cellularity (Cotran et al., 2005). Moreover, this stage includes proportion in the relation between synthesis and degradation, including the proteins and collagen laid down in the wound site, and is progressively more systematic. Finally, they will area structure very similar to that observed in normal unscarred tissue which gives type 3 collagen in place of type 1 collagen. However, the wounds never reach the same level of tissue density, as the diagram to the right illustrates. (Young & McNaught., 2001).

4. Conclusions

The laminin α1 protein has an important role during injury healing, through significantly elevated laminin α1in the first week and beginning to descend at the end of the second week. Can be used this timing as a guideline for injection or topical use of laminin α1 to promote wound healing after injury.

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