THE STUDY OF ANTEMORTEM AND POSTMORTEM WOUNDS: CYTOKINE LEVELS AS MARKERS FOR DETERMINATION OF WOUND AGE AND VITALITY IN ALBINO RATS

By

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ABSTRACT

The determination of wound age and vitality has an important role in forensic practice. **Objectives:** Interleukin-I β (IL1- β), interleukin- 6 (IL-6) and fibronectin (Fn) were estimated (in extracts of skin samples), and histopathological examination of the skin samples in adult male rats for differentiation between antemortem (AM) and postmortem (PM) wounds and determination of wound age. **Methods:** forty two adult male albino rats were divided equally into 7groups. AM (group I) and PM (group II) controls: the samples were taken from the dorsum of intact skin rats. The wound samples were taken 0.5 hour (group III), 3 hours (group IV), 6 hours (group V) and 24 hours (group VI) after infliction of the incised wounds. Group VII: samples taken from PM inflected wounds. The skin biopsies were taken under anesthesia in all AM tested groups and all specimens were biopsied for biochemical and histopathological examination. **Results:** There was significant time dependent increase in IL-1β, IL6 and Fn levels at 0.5, 3, 6 and 24 hours after wound infliction in comparison with the AM control. Specimens from PM wounds showed insignificant changes in IL-1β, IL6 and Fn levels in comparison with PM control. The histopathological examination revealed signs of vitality in AM wound groups that were absent in PM inflected wound group. **Conclusion**: We concluded that the combined assay of IL-1β, IL-6 and Fn may be a useful tool in age estimation of AM inflicted skin wounds. We also concluded that vital reactions that appeared in AM skin wound sections are essential for release of these parameters.

Key wards: Wound age, Vitality, Interleukin-1β, Interleukin-6, Fibronectin, Histopathology.

INTRODUCTION

When a wound is inflicted on a living organism, a series of events is triggered, called vital reaction. Determination of wound vitality and the interpretation of the chronological sequence of events is an important part of the work in forensic medicine. It is important to be able to report with the greatest possible precision when injuries occurred and whether during the vital, or during the postmortem period (**Ohshima, 2000; Kondo, 2007).**

Although classical approach is still an area of concern in forensic medicine, there is a continuous demand for further research and gathering of information to be applied in daily practical work **(Grellner and Burkhard, 2007).** Vitality as one of the central issues in daily forensic practice deals with the question, whether injuries were caused during lifetime of an individual **(Madea and** Grellner**, 2003).** Wound age describes the time interval between the infliction of a wound and the time of death. It can be considered as the survival time of the individual following a physical injury **(Oehmichen, 2004**).

Skin wound healing starts immediately after injury and consists of three phases: inflammation, proliferation and maturation. During the inflammatory phase, platelet aggregation at the injury site is followed by the infiltration of leukocytes such as neutrophils, macrophages, and T-lymphocytes into the wound site. Many cytokines, glycoproteins, growth factors and proteases are closely involved in the wound healing process to complete normal tissue repair after damage **(Singer and Clark, 1999)**. Therefore, monitoring of the markers of wound healing can be adopted to determine wound vitality and wound age in forensic medicine (**Bai, et al. 2008).**

Cytokines are glycoproteins produced by many inflammatory cells such as neutrophils, lymphocytes, macrophages, etc. They have multi-biological functions related

to immune, hematopoietic, endocrine, nervous and inflammatory systems. At present, it has been well-known that cytokines have close relation with wound healing process (**Nishio et al. 2008).**

Interleukin-1β (IL-1β) is the most important proinflammatory cytokine that regulates many aspects of the immune and inflammatory responses which are produced by various kinds of cells such as neutrophils, monocytes, macrophages and fibroblasts. IL-1α and IL-1β expression was strongly enhanced during wound healing (**Ishida, et al., 2006 and Sue, et al., 2013).**

Interleukin-6 (IL-6) is produced in the wound by epidermal keratinocytes, dermal fibroblasts, and macrophages. It affects multiple processes that are related to wound healing. After wounding, IL-6 increases adhesion of neutrophils to dermal fibroblasts and modulate growth factors and its expression is intimately involved in reepithelialization, granulation tissue formation, and inflammation **(Zubair, *et al.*, 2012)**. Fibronectin (Fn) is a glycoprotein component of basement membranes and interstitial connective tissues. It is synthesized by various cell types including fibroblasts, endothelial cells, hepatocytes and macrophages where it plays an important role in cell adhesion and cell migration during wound healing, and promotes phagocytosis by macrophages and fibroblasts**(Grinnell et al. 1981) .**

The main problem of postmortem human studies autopsy samples is associated with the inexactly known survival time. Animal experiments have the advantage of controlled conditions which makes possible to evaluate different parameters under standardized circumstances. Moreover, sequential biopsies can be taken **Grellner and Burkhard 2007).** In the present study, interleukin-1β, interleukin-6 and fibronectin were estimated in extracts of AM skin wounds at 0.5, 3, 6 and 24 hours after infliction of wounds. Moreover, we extended our measurements to include the levels of these mediators in rat skin wounds 6 hours PM (aiming to test for their practical usefulness in the estimation of wound vitality and the duration after its infliction). Moreover, histopathological examination of specimens to show the vital reaction following skin wound.

MATERIAL & METHODS

The current study was conducted at Forensic Medicine& Clinical Toxicology and Medical Biochemistry Departments, Faculty of Medicine, Zagazig University. Forty two adult male albino rats weighing 200- 250 g. were used. They were obtained from the animal house of the Faculty of Veterinary Medicine, Zagazig University. The study had been conducted in the Animal House of the Faculty of Medicine, Zagazig University. All ethically approved conditions used for animal housing and handling was considered. The animals were acclimatized to experimental conditions prior to the start of work for a period of one week. They were kept in cages, in groups with free access to food and water. The study protocol was ethically approved by the Institutional Review Board (IRB) of the faculty of Medicine, Zagazig University.

The animals were divided as follows:

Group I (AM Control): six rats; specimens were excised from the dorsum of intact skin.

Group II (PM Control): Six rats were sacrificed and specimens were excised from the dorsum of intact skin 6 hours PM.

Antemortem inflicted wound groups (n= 24):

Rats were divided equally into four groups and were assigned for collection of wound samples at the indicated time interval as following:

Group III: biopsies were collected 30 minutes after wound infliction.

Group IV: biopsies were collected 3 hours after wound infliction.

Group V: biopsies were collected 6 hours after wound infliction.

Group VI: biopsies were collected 24 hours after wound infliction.

Postmortem inflicted wound group (Group VII): Six rats were sacrificed and PM wounds were inflicted within 10 minutes after death. The skin samples were then collected 6 hours after the incision.

Wounds infliction and specimens' collection:

Rats were anesthetized using diethyl ether inhalation. After shaving the skin on the dorsal region, Nearly 1 cm full-thickness incision was made using sterile scalpels. Complete homeostasis with sterile gauze was done and any clots present were gently removed. The incised wounds were then covered with sterile gauze and the animals were isolated in separate clean cages. The AM wound and control skin biopsies were taken under diethyl ether anesthesia in all the tested groups. Wound specimens were biopsied from all animal groups parallel to the wound margins. Each specimen was divided into two halves Figure (1). Half of each wound specimen was stored at −80°C until ELISA analysis. The other half was kept in 10% formalin for histopathological study.

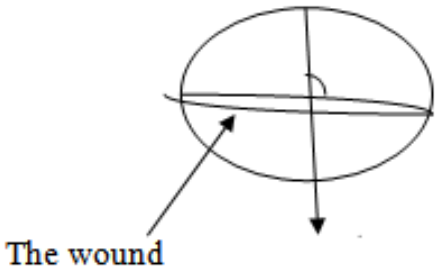

Figure (1): Showing the method of taking biopsy

The frozen skin specimens were homogenized under cooling condition (on ice). **(Nickoloff *et al.*, 1995)**. Measurement of IL-1β **(Dinarello, 1992)**, IL-6 **(Gaines-Das and Poole, 1993)** and Fn **(Prostmann and Tiessing, 1992)** by enzyme-linked immunosorbent assays (ELISA) using commercial kits (Boehringer GmbH, Mannheim, Germany).

Histopathological examination:

Samples of different groups were fixed in 10% buffered formalin solution and processed to obtain paraffin sections of 5µm thickness that were stained with hematoxylin and eosin (**Bancroft and Gamble, 2002).**

Statistical analysis:

Data were represented as means ± SD. The differences were compared for statistical significance by Analysis of variance (ANOVA) and post hoc Tukey's tests. While the comparison of postmortem inflected wound groups and two control groups was done using paired t tests. Difference was considered significant at $p < 0.05$. The statistical analysis was performed using a software (Statistical Package for the Social Sciences Incorporation, Chicago, Illinois, United State of America).

RESULTS

(A) Biochemical Results

The level of IL-1β IL-6 and Fn in samples collected from AM control showed insignificant differences in comparison with that of PM control levels (P>0.05) (Table1)

Table 1: Comparison of IL-1β, IL-6, Fn levels in AM and PM control groups

Groups / N= 6 / Parameters	Group (I) AM Control	(Group II) PM Control	P
IL-1 β	119± 16.6	114.5±6.57	0.508
IL6	105.2± 15.3	101. 4±11.8	0.623
Fn	63.3 ± 11.2	61± 10.5	0.756

Values are expressed as mean± standard deviation (SD) of n = 6 animals, IL-1β I; interleukin-1β, IL- 6; interleukin -6, Fn; fibronectin.

Antemortem inflicted wound groups

Analysis of data of the present study revealed a statistically significant difference between the studied parameter levels at different time intervals and control samples (P <0.0001) for IL-1β, IL-6 and Fn. The level of IL-1β increased non significantly at 0.5 hours (130±23.7) (P>0.05) and increased significantly at 3, 6 and 24 hours after infliction of wound (208± 19.4, 225.1± 26 and 209.4± 12.1 respectively) when compared with that of control group (119± 16.6) (P <0.0001). This increase was time dependent with highest level was observed at 6 hours after infliction of wound (Table 2) (Figure . 2).

As regarding IL-6 its level non significantly increased following incision at 0.5 hour (122.3± 20.1) then increased significantly at 3 hours (203.1±32.5) in comparison to control samples (105.2± 15.3), (P <0.000), then maximally increased to (232.1± 29.8) at 6 hours (P <0.0001). The increase in IL-6 level was maintained high at 24 hour interval (217.3±23.3) (Table 2). (Figure. 2).

Regarding fibronectin, there was a significant and progressive increase in Fn level following incision after 0.5, 3, 6 and 24 hours 112.6±19.1, 169.7± 26.5, 211.2± 16.9 and 225.4± 14.6 respectively) in comparison to control samples (63.3 ± 11.2) (P <0.0001) (table 2) (Figure . 2).

Table 2: Comparison of the mean values of IL-1β, IL-6 and Fn levels in wounded skin at different time intervals of control and AM incisions, by ANOVA and post hoc Tukey's tests

Parameters / Groups / N= 6	IL-1β (pg/mg protein) Mean ± SD	IL-6 (pg/mg protein) Mean ± SD	Fn (pg/mg protein) Mean ± SD
Group I (AM control)	119± 16.6	105.2± 15.3	63.3 ± 11.2
Group III (0.5 hours)	130± 23.7	122.3± 20.1^{cde}	112.6±19.1^{acde}
Group IV (3hours)	208± 19.4^{abd}	203.1±32.5^{abd}	169.7± 26.5^{abde}
Group V (6hours)	225.1± 26^{abce}	232.1± 29.8^{abc}	211.2± 16.9^{abc}
Group VI (24hours)	209.4± 12.1^{abd}	217.3±23.3^{abe}	225.4± 14.6^{abc}
F	43.59	33.83	88.5
P Value	<0.000	<0.000	<0.0001**

Values are expressed as mean± standard deviation (SD) of n = 6 animals, IL-1β I; interleukin-1β, IL- 6; interleukin -6, Fn; fibronectin, a: significant when compared with control group, b: significant when compared with group II, c:significant when compared with group III, d: significant when compared with group IV, e: significant when compared with group V

**: P <0.0001 highly significant

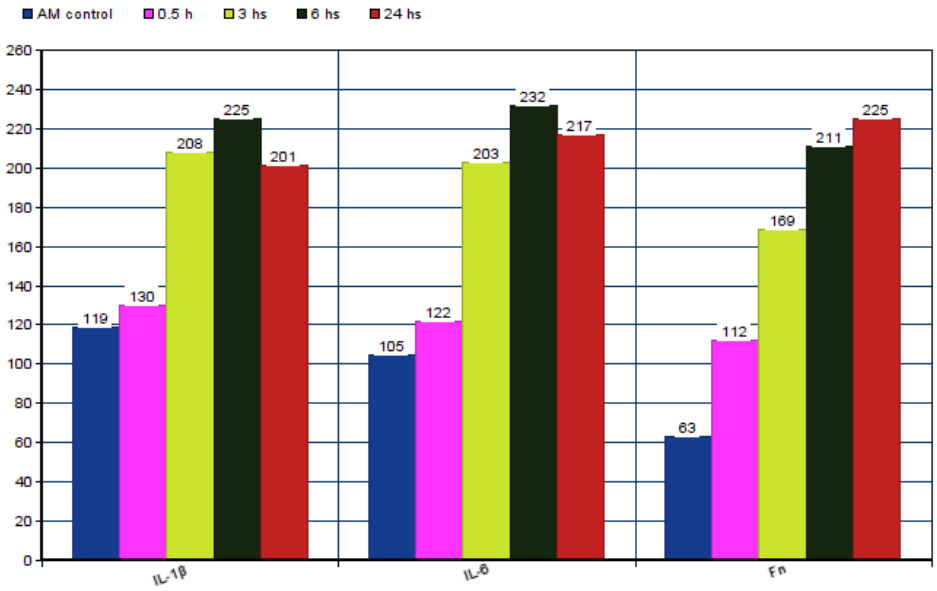

Figure . (2): showing the mean values of IL-1β I; interleukin-1β, IL- 6; interleukin -6, Fn; fibronectin **levels in skin of antemortem control and antemortem wound samples**

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Postmortem inflicted wound group:

the level of IL-1β IL-6 and Fn from skin samples collected from PM inflicted wound 6 hours after the incision showed insignificant increase in comparison with that of corresponding PM control levels (P>0.05) (Table 3).

Table 2: Comparison of IL-1 β, IL-6, Fn levels in postmortem inflected wound

Parameter	Group (N= 6)		T	P
	PM control	PM inflicted wound		
IL-1β	114.5±6.57	122.8± 12.7	0.3169	0.7585
Il6	101. 4±11.8	110.33± 13.1	1.6894	0.1254
Fn	60.33±11.7	65.20± 8.9	0.2363	0.8185

Values are expressed as mean± standard deviation (SD) of n = 6 animals, IL-1β I; interlukine-1β, IL- 6; interlukine -6, Fn; fibronectin, P > 0.05 non significant.

B) Histopathological Results:

Examination of AM control samples (intact skin) showed that the epidermis was thin and only consisted of epidermal cells with underlying dermis that appear with hair follicles. (Figure 3). Wound specimens at 0.5 h post infliction were similar to control samples (Figure 4). Wound specimens at 3 hours post infliction showed mild aggregates of inflammatory cells in the dermis (Figure 5). More progressive aggregation of inflammatory cells in Sections of skin wounds 6hours (Figure 6) and 24 hours post infliction of the incision (Figure 7).

Postmortem samples:

Examination of intact skin at 6 hours postmortem showed little changes in the form of loss of cell integrity, hazy appearance of epithelial cells (Figure 8). Specimens of skin wound at 6 hours postmortem showed hazy appearance of epithelial cells and increased eosinophilia (Figure 9)

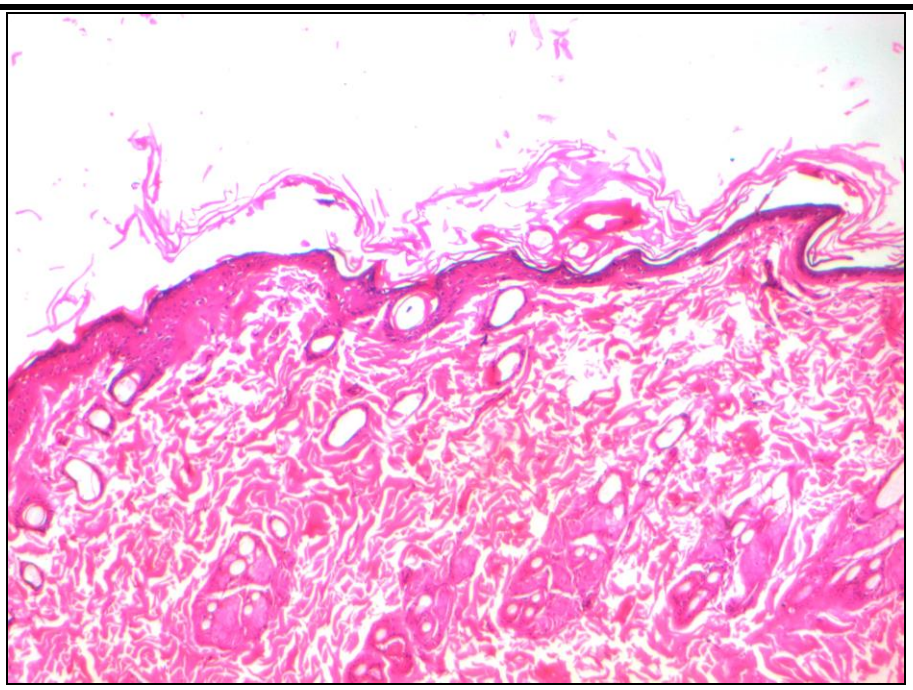

Figure (3): A photomicrograph of intact rat skin sections in AM control group showing thin epidermis that consists of epidermal cells with underlying dermis that appear with hair follicles (H&E 400)

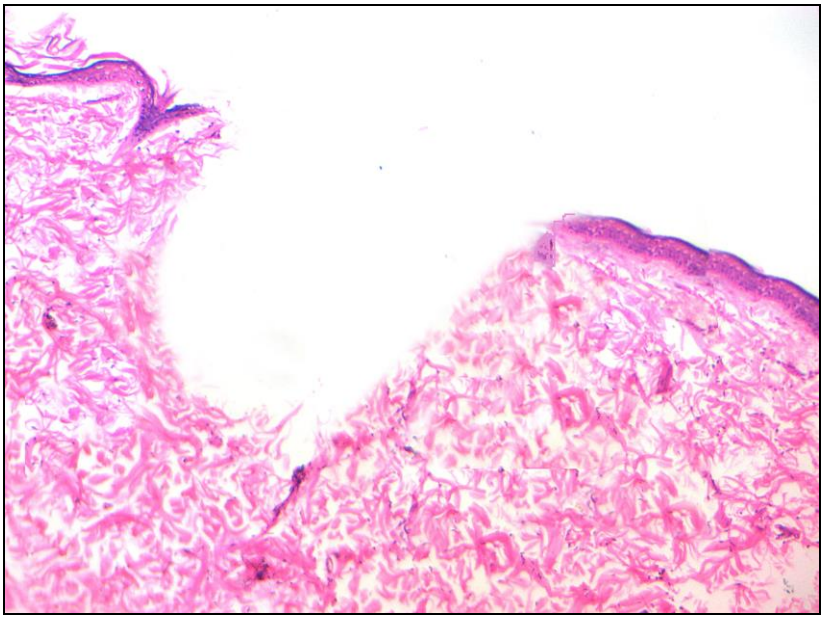

Figure (4): A photomicrograph of rat skin wound at 0.5 hours after wound infliction showing thin epidermis consists of epidermal cells with underlying dermis (H& E 400)

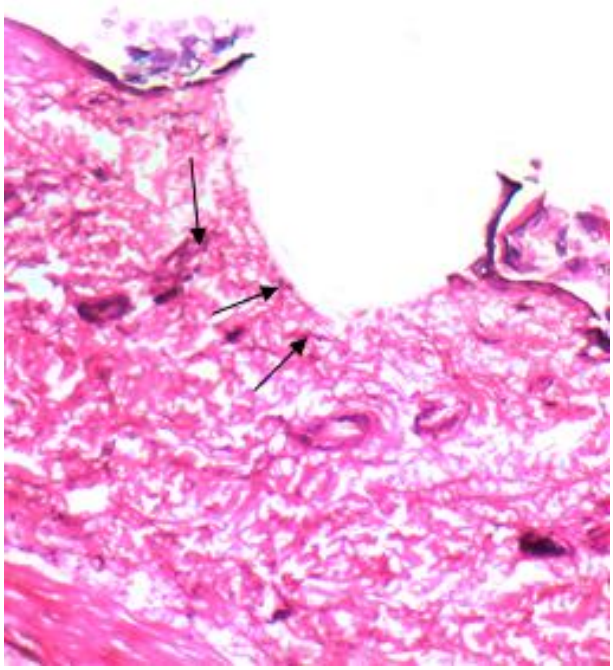

Figure (5): A photomicrograph of rat skin wound at 3 hours after wound infliction showing mild hyperplasia of epidermis with mild aggregation of inflammatory cells in the dermis around wound margins (arrow) (H& E 400)

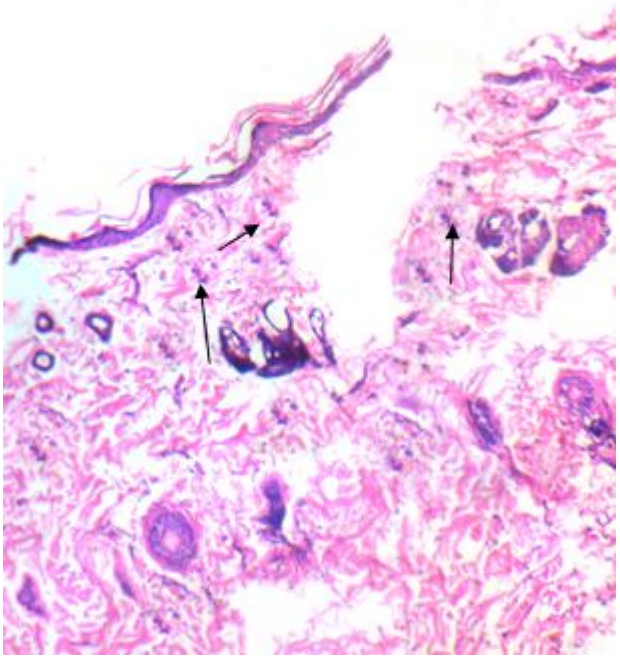

Figure (6): A photomicrograph of rat skin wound at 6 hours after wound infliction showing increased aggregation of inflammatory cells in the dermis (arrow) (H & E 400)

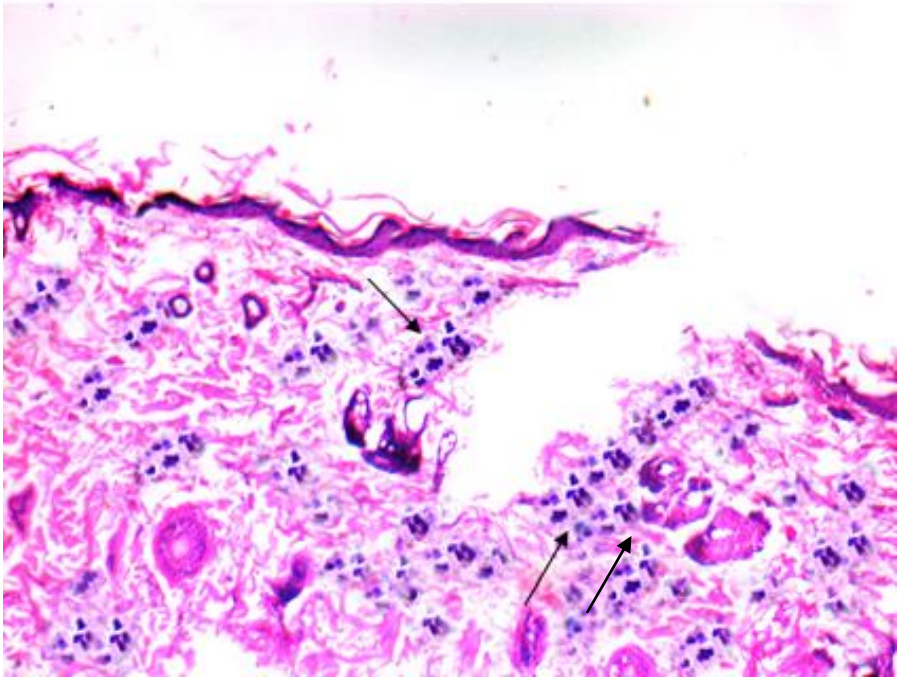

Figure (7): A photomicrograph of rat skin wound at 24 hours after wound infliction showing heavy aggregation of inflammatory cells in the dermis (arrow) (H& E 400)

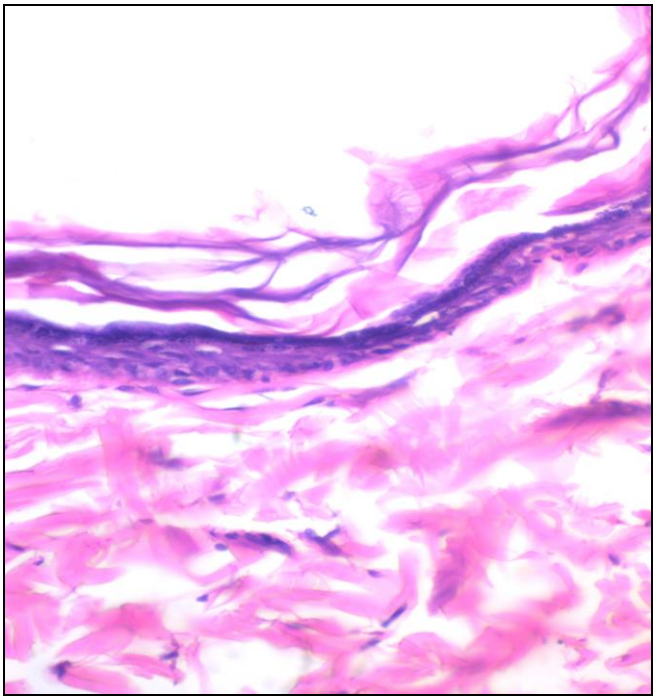

Figure (8): A photomicrograph of intact skin wound at 6 hours postmortem showing loss of cell integrity, hazy appearance of epithelial cells. Increased eosinophilia(H&E 400)

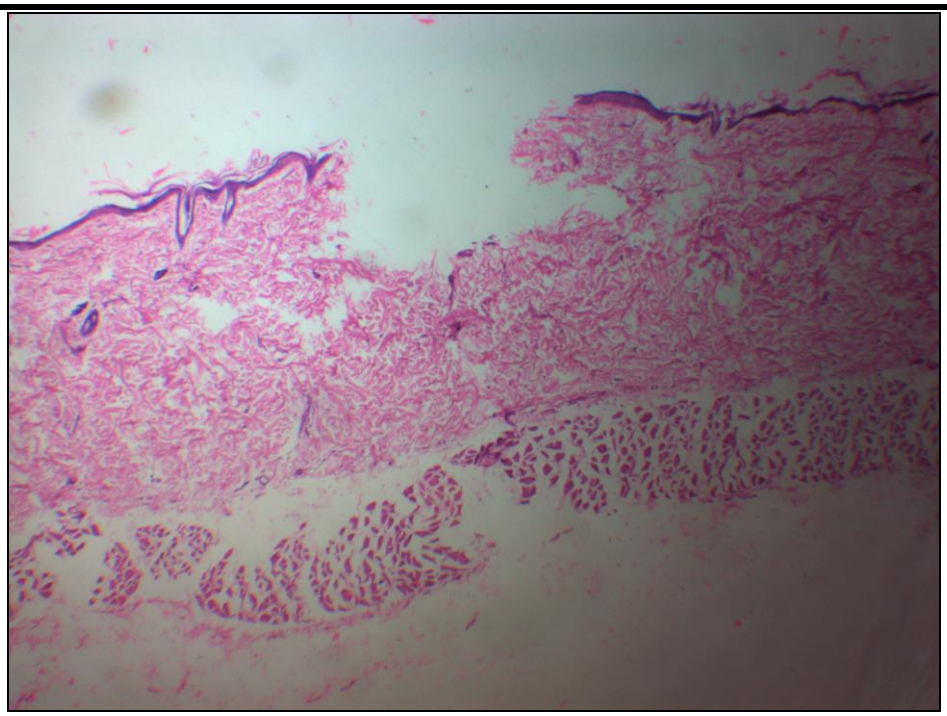

Figure (9): A photomicrograph of skin wound at 6 hours postmortem showing hazy appearance of epithelial cells and increased eosinophilia (H & E 400)

DISCUSSION

The determination of wound age or wound vitality is very important in forensic practice. Analysis of wound vitality markers, such as the inflammatory reaction mediators is essential for diagnosing the vitality of the lesions and to establish lesion chronology, pre and post-mortem to correctly evaluate the relationship between death and any wounds **(Grellner and Burkhard, 2007).** With the development of chemical analyses, the scientific field of wound age determination has advanced progressively. It has been demonstrated that collagens, cytokines, and growth factors are good markers for the determination of wound vitality or age **(Braiman-Wiksman et al., 2007, Cecchi et al., 2012).** Local quantitative analysis of inflammatory mediators has an advantage as many diseases, mainly systemic, may influence the levels of inflammatory mediators in the blood (**Balk and Bone**, **1989)**. Also, **Carvalho, et al., (2008, 2012)** found in that there is no correlation between wound exudates levels of IL- 6, IL-1β and other cytokines and their serum levels in patients undergoing surgical procedure which emphasizes the importance of determining site specific release. Therefore, local determination of the mediators in the skin wound prevents theoretically possible falsifications.

It would be ideal to conduct this research on healthy human subjects. However, because of the ethical restrictions it is difficult to do this. Therefore, the best available alternatives were to conduct experiments concerning wound age estimate on animal skin.

In this study, estimation of IL-1 β, IL-6 and Fn levels in the skin of rats was analyzed as indicators of wound age, to differentiate between AM and PM wounds and as a trial for rough determination of the time elapsed since infliction of wounds. Moreover, histopathological examination of specimens was done to detect vital reactions following skin wound. The results revealed time dependent significant increase in IL-1 β, IL6 and Fn levels at 0.5, 3, 6, 24 hours after wound infliction when compared with that of AM control skin samples. This was accompanied by progressive increase in inflammatory cell infiltrations in AM wound sections**.** In line with the result of this work, **Rizk, et al., (2013)** reported that the mean

tissue extract fluid levels of IL-1β, IL-6 and TNF-α estimated in AM specimens were significantly higher as compared to their levels estimated in the three PM specimens.

Harvima et al., (1994) study explained that many factors may contribute to the quantitative increase of cytokines. The increase of IL-6 firstly by local release from preformed stores Keratinocytes, mast cells, sweat glands and partly macrophages. Few hours later; the induction of acute phase response to inflammation resulted in IL-6 production by a variety of cells such as leukocytes, monocytes, macrophages, lymphocytes and endothelial cells in response to inflammatory tissue injury. In addition, the transudation with blood components especially soluble cytokines. Our histopathological analyses lightened this theory as examination of AM inflicted wound sections revealed progressive aggregation of inflammatory cells in the dermis of different sections of skin wounds at 3, 6 and 24 hours after infliction of the incision. In line with these finding, **Sheta, et al., (2009)** reported increased thickness of the epithelium at the wound edge and increased cellular infiltration into underlying dermis 24 hours post infliction of the incision.

Grellner, (2002) demonstrated an increase of IL-6 after one hour of wound infliction. He also noticed the persistence of this increase up to 5 hs and its reappearance in few days after. This difference in pattern of increase of wound IL-6 in comparison to our results could be referred to the different method used for assay. **Grellner, (2002**) have used immunohistochemical method for assaying IL-6 that detects mainly cytokines bound on the cell surfaces.

Bai et al.(2008) reported time-dependent expression of IL-1β, COX-2, MCP-1 mRNA after incised wounds in rabbit skin using real-time fluorescent quantitative PCR and reported that expression of IL-1β mRNA showed significant increase and appeared at less than 0.5 h ($p < 0.01$), and the peak level at 2 hours in comparison with control group. **Takamiya et al. (2008 & 2009)** performed quantification of IL-2, 4, 6, 8 and 10, IFN-γ and TNF-α in human dermal wounds for wound age estimation and found that among the cytokines analyzed IL 6, IL 8, IFN-γ, and TNF-α were strongly expressed and suggested that multiplex cytokine analysis at the wound site can be useful in daily forensic practice for wound age estimation.

The present study revealed the significantly higher levels of the estimated cytokines in AM specimens compared to PM specimen. Similar findings were reported in an earlier study; **Sheta** et al., (2009) found that Fn is the first mediator to increase, in 30 minutes, after wound infliction then, after 3 and 6 hours both Fn and IL-6 were increased. At 24 hours of wounding LXA4 increases to join Fn and IL-6.

This result could be explained by the fact that the transudation of the tissue with blood components (soluble cytokines in the serum and additional release from cellular elements such as monocytes) may contribute to the rapid quantitative increase of proinflammatory mediators and the negative results in PM wound point to the fact that intact circulation is required and that sole passive transudation occurring in PM injury is not sufficient to raise tissue cytokine levels, (**Grellner et al.,2000)**.

Regarding postmortem inflicted wounds IL-1, IL-6 and Fn showed insignificant increase 6 hours after wounding compared to control skin samples. However, some increase was noticed which can be explained by passive transudation of blood components from injured vessels as **(Grellner et al 1998)** explained. Therefore, it is apparent that intact circulation is required and that sole passive transudation that may occur postmortem is not sufficient to elevate the level as the vital wound.

The increase in Fn level was progressive to a maximum at 24 hours. In parallel with this; **Betz et al., (1992**) reported that the early appearance of Fn in wounds may be due to the rapid accumulation of this extra-cellular matrix glycoprotein in the dermis soon after the injury. This helps in migration of leukocytes in the inflammatory phase of skin wound healing. Also, Fn helps in interaction between leukocytes and

endothelial cells which is the most important event in inflammation. Furthermore, it is probably derived from damaged blood vessels. All these criteria may explain the early detection of Fn rendering it a good marker for wound vitality and wound age determination. In another trial for estimation of wound age in early skin injuries**, Van de Goot et al (2014**) develop a wound age probability scoring system that could be used in forensic autopsies to improve wound age estimation in early skin injuries based on the immunohistochemical (IHC) expression levels of Fibronectin, CD62p and Factor VIII in wound hemorrhage. Tissue slides were stained for Fn, CD62p and Factor VIII and were subsequently scored for staining intensity (IHC score). The probabilities that a wound was non vital in case of an IHC score 0 (negative) were 87%, in case of few minutes old were 82/90% for Fn and in case of an IHC score 3 (strong positive), the probabilities that a wound was 15-30 minutes old were 65% for Fn.

. **Ortiz-Rey, et al., (2002) and Fieguth, et al., (2003)** reported that fibronectin-positive reactions was detected in cases that had longer survival times but not detected in immediately fatal human skin wounds or PM cases, although extensive lacerations in their examined samples after massive injuries may lead to a more intensive wound reaction than an incision under sterile conditions in our study. Furthermore**, Liu et al., (2006**) reported that fibronectins have peculiar of anti-autolysis characteristic which maintain its level PM**.** For that reason assay of Fn may solve some problems in forensic wound age determination. Our study confirmed the findings of the above-mentioned articles.

CONCLUSION

We concluded that the combined assay of IL-1β, IL-6 and Fn may be a useful tool in determination of the duration lapsed since AM wound infliction. Moreover, this pattern of time dependent increase of the three parameters may be also useful in age determination of multiple inflicted wounds at variable intervals in the same victim. We can also conclude that vital reactions are essential for release of the assayed parameters. This can be documented by the lack of significant increase of these parameters in postmortem-inflicted wounds.

RECOMMENDATIONS

Further studies regarding these parameters as well as other inflammatory parameters for determining wound age and vitality are recommended.

REFERENCES

Bai, R.; Wan, L. and Shi, M. (2008): The time-dependent expressions of IL-1beta, COX-2, MCP-1 mRNA in skin wounds of rabbits. Forensic Sci Int.; 175 (2-3):193-197.

Balk, R.A. and Bone, R.C. (1989): The septic syndrome: definition and clinical implications. Crit Care Clin.; 5 (1): 1– 8.

Bancroft, J. and Gamble, A. (2002): Theory and Practice of Histological Techniques. 5th ed, Churchil, Living stone, New York and London: 165-180.

Betz, P. Nerlich, A.; Wilske, J. et al. (1992): Immunohistochemical localization of fibronectin as a tool for the age determination of human skin wounds, Int J Legal Med; 105: 21–6.

Braiman-Wiksman L.; Solomonik I.; Spira R. and Tennenbaum T (2007)**:** Novel insights into wound healing sequence of events. Toxicol Pathol.,; 35(6):767-79.

Carvalho, B.; Clark, D.J. and Angst, M.S. (2008): Local and systemic release of cytokines, nerve growth factor, prostaglandin E2, and substance P in incisional wounds and serum following cesarean delivery. J Pain.; 9(7):650-7.

Carvalho, B.; Clark, D.J.; Yeomans, D. and Angst, M.S. (2012): Collecting and measuring wound exudates biochemical mediators in surgical wounds. J Vis Exp.;(68): 156-9.

Cecchi R.; Aromatario M.; Frati P.; Lucidi D. and Ciallella C. **(2012):** Death due to crush injuries in a compactor truck: vitality assessment by immunohistochemistry. Int J Legal Med.;126(6):957-960.

Dinarello, C.A. (1992): ELISA kits based on monoclonal antibodies do not measure total IL-1β synthesis. J Immunol Methods; 148: 255–9.

F**ieguth, A.; Feldbrügge, H.; Gerich, T.et al. (2003):** The time-dependent expression of fibronectin, MRP8, MRP14 and defensin in surgically treated human skin wounds. Forensic Sci Int; 131 (2-3): 156-61

Gaines-Das, R.E. and Poole, S. (1993): The international standard for interleukin-6—evaluation in an international collaborative study. J Immunol Methods., 160: 147–53.

Grellner, W. (2002); Time-dependent immunohistochemical detection of proinflammatory cytokines (IL-1beta, IL-6, TNF-alpha) in human skin wounds. Forensic Sci Int.;130(2-3):90-6.

Grellner, W. and Burkhard, M. (2007): Demands on scientific studies: vitality of wounds and wound age estimation. *Forensic Sci Int.*, 165(2-3), 150-154.

Grellner, W.; Dimmeler, S. and Madea, B. (1998): Immunohistochemical detection of fibronectin in postmortem incised wounds of porcine skin. Forensic Sci Int; 97 (2-3): 109–116.

Grellner, W.; Georg, T. and Wilske, J. (2000): Quantitative analysis of proinflammatory cytokines (IL-1beta, IL-6, TNF-alpha) in human skin wounds. Forensic Sci Int.; 113(3):251-264

Grinnell, F., Billingham, R.E., and Burgess, L. (1981): Distribution of fibronectin during wound healing in vivo. J. Invest. Dermatol. 76, 181-189

Harvima, T.; Horsmanheimo, L. and Naukkarinen, A. (1994) Mast cell proteinases and cytokines in skin inflammation. Arch Dermatol; 287: 61–67.

Ishida Y.; Kondo T.; Kimura A.; et al., (2006) Absence of IL-1 receptor antagonist impaired wound healing along with aberrant NF-κB activation and a reciprocal suppression of TGF-β signal pathway. J Immunol, 176, (9): 5598- 56016

Kondo, T. (2007): Timing of skin wounds. *Legal Medicine*, 9 (2): 109-114.

Liu, N.; Chen, Y. and Huang, X. (2006): Fibronectin EIIIA splicing variant: A useful contribution to forensic wounding interval estimation. Forensic Sci Int; 162 (1-3): 178-82.

Madea, B. and Grellner, W. (2003): Vitality and supravitality in forensic medicine, Teil 2, Rechtsmed(13) 32–48.

Nickoloff, B.J.; Kunkel, L,; Burdick, M. and Strieter, R.M. (1995): Severe combined immunodeficiency mouse and human psoriatic skin chimeras. Am. J. Pathol.; 146 (3): 580–588.

Nishio, N;, Okawa, Y.; Sakurai, H. and Isobe, K. (2008): Neutrophil depletion delays wound repair in aged mice. Age (Dordr), 30 (1):11-19

Oehmichen, M. (2004): Vitality and time course of wounds. *Forensic Sci Int.*, 144 (2- 3): 221-31.

Ohshima, T., (2000). Forensic wound examination. *Forensic Sci Int.*, 113(1-3): 153-164.

Ortiz-Rey, J.A.; Suàrez-Peñaranda, J.M.; Da Silva, E.et al. (2002): Immunohistochemical detection of fibronectin and tenascin in incised human skin injuries. Forensic Sci Int; 126 (2): 118–122.

Prostmann, T. and Tiessing, T. (1992): Enzym**e** Immuno Assay Techneque. An Overview. J Immunol Methods; 150: 5-21.

Rizk, AH.; El-ShishtawyM M and Al-Kholy, A. F.(2013): Tissue Extract Fluid Cytokine Levels as Markers for Wound Vitality: An Experimental Comparative study. J. Ameri. Sci.; 9(1): 188-193.

Sheta, A.; Soliman, S. and Megahed, S. (2009): Antemortem And Postmortem Estimation Of Rat Wounds Biology Essay. Bull. Alex. Fac. Med.; 45 (1):241- 251

Singer, J. and Clark, R.A. (1999): Cutaneous wound Healing. N Engl. J Med.; 341: 738- 46.

Sue, E.; Huether, R.N.; Kathryn, L. and McCance, R.N. (2013): ch (5), innat immunity: inflammation and wound healing 282-290. ‹

Takamiya, M.; Fujita, S.; Saigusa, K. and Aoki, Y. (2008): Simultaneous detection of eight cytokines in human dermal wounds with a multiplex bead-based immunoassay for wound age estimation. Int J Legal Med.; 122 (2):143-8.

Takamiya, M.; Biwasaka, H.; Saigusa, K.; et al., (2009): Wound age estimation by

simultaneous detection of 9 cytokines in human dermal wounds with a multiplex bead-based immunoassay: an estimative method using outsourced examinations. Leg Med (Tokyo); 11 (4): 186-90

Van de Goot, F.R.; Korkmaz, H.I.; Fronczek, J.; Witte B. et al (2014): A new method to determine wound age in early vital skin injuries: A probability scoring system using expression levels of Fibronectin, CD62p and Factor VIII in wound hemorrhage. Forensic Sci Int.; 28 (244C):128-135.

Zubair, M.; Malik, A. and Ahmad, J. (2012): Plasma adiponectin, IL-6, hsCRP, and TNF-α levels in subject with diabetic foot and their correlation with clinical variables in a North Indian tertiary care hospital. Indian J Endocrinol Metab. 16 (5): 769-76

دراسة الجروح قبل وبعد الوفاة : مستوى السيتوكينات كعلامات لتحديد عمر وحيوية الجروح في الجرذان البيضاء

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المقدمة: إن تحديد عمر وحيوية الجرح لهما دور مهم في ممارسة الطب الشرعي.
الاهداف: تم تعيين إنترلوكين -١ بيتا وإنترلوكين -٦ وفيبرونيكتين من مستخلص عينات الجلد فى ذكور الجرذان البيضاء البالغة وكذلك تم الفحص الهستوباثولوجي للتمييز بين الجروح قبل وبعد الوفاة وتحديد عمر الجرح.
الأساليب: تم تقسيم إثنين وأربعين من الجرذان إلى سبع مجموعات . تم أخذ العينات من المجموعة الضابطة قبل الوفاة (المجموعة الأولى) والمجموعة الضابطة بعد الوفاة (المجموعة الثانية) من جلد سليم من ظهور الجرذان. وتم أخذ عينات الجروح بعد ٣٠ دقيقة (المجموعة الثالثة) و٣ ساعات (المجموعة الرابعة) و٦ ساعات (المجموعة الخامسة) و٢٤ ساعة (المجموعة السادسة) بعد إلحاق جروح قطعية. المجموعة السابعة: أخذت العينات من جرح واقع بعد الوفاة و قد أخذت العينات للتحليل الكيميائى والهستوباثولوجي تحت تأثير المخدر لكل المجموعات المختبرة قبل الوفاة .
النتائج: لقد أوضحت النتائج حدوث زيادة ذات دلالة إحصائية فى إنترلوكين-١ بيتا وإنترلوكين -٦ وفيبرونيكتين عند .نصف ساعة و٣ و٦ و٢٤ ساعة مقارنة بمجموعته الضابطة وتبين أن هذه الزيادة معتمدة على الوقت. بينما العينات التي أخذت من الجروح الواقعة بعد الوفاة أوضحت تغير ليس له دلالة إحصائية فى نفس المعاملات السابقة مقارنة بمستوى مجموعته الضابطة. كما تبين من الفحص الهستوباثولوجي ظهور علامات حيوية فى الجروح المحدثة قبل الوفاة والتي كانت غائبة فى الجروح المحدثة بعد الوفاة.
االخلاصة: استنتجنا من الدراسة أن قياس مستوى كلٍ من إنترلوكين-١ بيتا وإنترلوكين -٦ وفيبرونيكتين قد يكون أداة مفيدة في تحديد عمر الجروح المحدثة قبل الوفاة. استنتجنا أيضاً أن التفاعلات الحيوية التي ظهرت فى قطاعات الجروح المحدثة قبل الوفاة ضرورية لإطلاق هذه المعاملات.
التوصيات: إجراء مزيد من الدراسات على هذه المعاملات والمعاملات الالتهابية الأخرى لتحديد عمر وحيوية الجروح