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# Role of Inducible Nitric Oxide Synthase and Interleukin-6 Proteins Expression in Estimation of Skin Burn Age and Vitality: Immunohistochemical Study in Rat

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**Abstract** Estimation of age and vitality of burn injury both in the living and dead is essential in forensic practice. Nitric oxide and interleukin-6 (IL-6) play an important role in skin burn healing. In this immunohistochemical study, the expression of inducible nitric oxide synthase (iNOS) and IL-6 proteins during skin burn injury healing in rats was studied for purposes of burn dating and to differentiate between ante-mortem and post-mortem burn. Ante-mortem full- thickness skin burns were created on forty five rats with a heated soldering iron applied for three seconds. Normal and burnt skin samples were taken at 1, 3, 5, 7, 9, 11, 13, 15 and 21 days following burn induction (5 rats for each stage). Post-mortem burn was inflicted 6h. after scarification in another five rats. There was a statistically significant difference in both iNOS and IL-6 expression between the different studied time intervals of the ante-mortem burn. Expression of both iNOS and IL-6 decreased remarkably in the post-mortem burn with a statistically significant difference from all the studied ante-mortem intervals. A statistically significant positive association between the two markers was found; both increased gradually in the inflammatory and early proliferation stages and started to decrease in late proliferative and remodeling stages while reaching the minimum in the post-mortem burn. These results indicate that both iNOS and IL-6 expression in ante-mortem burnt skin was time dependent and significantly differed from post-mortem burn. Further research on humans is recommended.

**Keywords** **Keywords;** Skin burn aging; skin burn vitality; inducible nitric oxide synthase; interleukin-6; immunohistochemistery; rat**.**

## Introduction

Wound examination is an essential issue in forensic practice. Determination of wound age and vitality is a classic but still popular and pivotal issue in forensic pathology to evaluate accurately its causal relationship to death *(Kubo, 2014).*

Wound healing is a dynamic process consisting of three overlapping phases: the inflammatory phase; including coagulation and inflammation, the proliferative phase; consisting of angiogenesis, the formation of granulation tissue, and re-epithelialization, and lastly the maturation phase; involving matrix formation and tissue remodeling *(Mendonça and Coutinho-Netto, 2009).*

There are many studies investigated determination of mechanically induced skin wounds by sharp or blunt objects using either animal experiments or samples from cadavers *(Takamiya et al., 2002; Kondo, 2007; Kondo and Ishida, 2010*). Despite burn injuries are considered a major cause of disability and death *(Alsarhan et al., 2013);* limited information is available on the determination of skin burn injury.

To study the age and vitality of burn injury, inflammatory mediators or cells and matrix proteins in injured tissue could be analyzed *(Dong et al., 2015).*Nitric oxide (NO) plays a crucial role as a signal molecule in the healing process of skin burn *(Lakshmi et al., 2011).*It is synthesized during the conversion of L-arginine to L-citrulline by the action of nitric oxide synthase (NOS) family of enzymes (*Speranza et al., 2012).*

Three NOS isoforms have been identified; two are constitutively expressed in cells including neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS). The third isoform is inducible (iNOS) and is activated in response to various stimuli such as cytokines, endotoxins and physiopathological conditions *(Förstermannand Sessa, 2012).*

Interleukin-6 (IL-6) is a multi-functional cytokine released by a variety of cells including macrophages, T cells, fibroblasts, keratinocytes and endothelial cells. It could regulate the inflammatory response of wound healing process *(Abali et al., 2013).*

Dating of an injury depending on the subjective naked eye evaluation is highly variable. Therefore, it is important to study the injuries microscopically. Immunohistochemical staining supports the histological findings and makes observations and interpretation more objective *(Kumar et al., 2011)*

The aim of this study was to investigate the expression of iNOS and IL-6 proteins during skin burn injury healing in rats by immunohistochemistry for its forensic application in determination of skin burn age in addition to their possible role in differentiating between ante-mortem and post-mortem burn.

## Materials and Methods

### Ethical Considerations

Experimental procedures were performed according to the guidelines for the care and use of laboratory animals approved by the Ethical Committee of Faculty of Medicine, Tanta University, Egypt; fewer numbers of animals estimated to affair valid results were used and animal painless procedures were conducted with appropriate sedation to avoid pain and stress. This was in accordance with the National Institutes of Health guide for the care and use of Laboratory animals *(Clark et al., 1997).*

### Animals

This study was conducted on fifty adult male albino rats, their weight ranged from 150-200 g. During the study, the animals were kept in wire mesh cages with ad-libitum access to water. The room temperature was about 22-24 $^{\circ}$C and the animals were exposed to 12:12 hours light dark cycles. Animals were allowed a two week pre-experimentation period to be acclimatized prior to thermal injury.

### Experimental design

The animals were anesthetized with diethyl ether inhalation in a glass cage. Once anesthetized, the hair on the dorsum of each rat was removed by a sterile shaving razor to reveal a bare region with a diameter of 2 x 2 cm. Thereafter and according to *Nagata et al. (1999)* full-thickness skin burns were made on rats with a heated soldering iron applied for three seconds. The induced burn injuries in all rats were washed with running water for 1 min followed by intra-peritoneal injection of 25mg/kg of acetaminophen in a volume of 10mg/ml (Acetaminophen, Perfalgan®, BMS Pharmaceutical, Italy) to overcome pain according to *Im et al. (2012)*. They were also injected 1–1.5 ml isotonic saline intra-peritoneal for fluid resuscitation. The animals stopped experiencing any pain by about 12 h. after the burn infliction which was evident by their return to normal behavior.

At 1, 3,5,7,9,11,13,15 and 21 days following the burn, a total of 5 rats for each interval were sacrificed by cervical decapitation after being anesthetized by diethyl ether inhalation. These ante-mortem intervals covered the three stages of healing process; inflammatory stage (12hours-2 days), proliferation stage (3-14 days) and the remodeling stage (15-28 days).The remaining five rats encountered post-mortem burn which was inflicted 6h after scarification and the samples were taken immediately from them.

Sample preparation and immunohistochemistry staining for iNOS and IL-6

Skin samples were taken from both the center and periphery of the burn and from adjacent non-burned shaved skin of the same animal (served as a control). The dissected tissues were immersed in 10% formaldehyde solution, with a volume ten times the volume of the tissues. Then, they were embedded in paraffin and cut into 5-µm sections for staining with hematoxylin and eosin *(Bancroft and Gamble, 2008)* followed by immunohistochemical staining for iNOS and IL-6.

Immunohistochemistry is readily available and widely used technique in diagnostic and research laboratories *(Duraiyan et al., 2012).*In this technique, sections of skin samples were dewaxed in xylene and rehydrated in graded ethanol solutions. Endogenous peroxidase activity was blocked by 3% hydrogen peroxides. The specimens were permeabilized in phosphate buffered saline (PBS) for 10 minutes, blocked in 20% normal goat serum in 0.01M PBS, and subjected to antigen retrieval in citrate buffered solution at 92 °C for 15 minutes. After being washed in PBS, the slides were incubated with the antibody. After washing in PBS, the tissues were incubated by use of biotin-conjugated secondary antibody for one hour. Then the slides were incubated in streptavidin-biotin horseradish peroxidase complex. Immunoreactivity was visualized by exposing the specimens to diaminobenzidine tetra- hydrochloride (DAB). The sections were counter stained with hematoxylin and then rinsed and mounted. Primary antibodies used were iNOS, Rabbit Polyclonal Antibody (RB-9242-R7, Ready-to-Use: Thermo Fisher Scientific Anatomical Pathology, Fremont, CA, USA and αIL-6 polyclonal rabbit antibody (Cat-No: ab662 Abcam, Cambridge, UK).

Semi-quantitative evaluation of iNOS and IL-6 staining

To evaluate iNOS staining, only cells with evidence of cytoplasmic staining were considered positive. The number of iNOS-positive cells was determined by evaluation of 4fields for each slide for each rat per each group at a magnification of x100 and the results were given as the mean± SD *(Muna et al, 2002).*Regarding IL-6 staining; the reaction was evaluated in relation to its intensity and its area percentage. IL-6 staining intensity was scored as weak ( score 1), moderate ( score 2) or strong ( score 3), while its area percentage was scored positive if more than 50 % of the slide was expressing IL-6 staining in the cytoplasm*(Andrej et al., 2015).*

Statistical analysis

Data were analyzed using statistical package for social sciences (SPSS) version 20. The data were tested for normality and homogeneity of variance. One way analysis of variance (ANOVA) and Kruskal-Wallis tests were used to analyze iNOS and IL-6 expression in the different studied groups respectively. Mean rank of scores of IL-6 expression was calculated by ranking all sample data from the smallest to the largest and according to its position in the combined data set the rank was assigned, then the mean of theses

ranks was calculated. In addition, Spearmen's rank correlation coefficient was used to investigate the association between the studied two markers. The significance was declared at a P value of less than 0.05.

## Results

**Histopathological examination:**

Examination of H&E stained sections at the normal skin revealed its characteristic epidermis and dermis (Fig. 1a) while at the burned area revealed deep second degree burns involving most of the dermis. During the inflammatory stage, the most prominent changes were infiltration of neutrophils and extensive edema and necrosis (Fig. 2a).

In the proliferation stage; by the day3, macroscopically, crust was formed from the necrotic tissues and microscopically this crust was rejected from the underlying viable tissues along with the zone of neutrophils infiltration. By the day5, neutrophils were replaced largely by macrophages with the early formation of granulation tissue and new blood vessels. By the day 7, the neovascularization reaches its peak with started scab formation (Fig. 3a). By the days 9, 11 and till 14; decrease in the neovascularization with started deposition of few collagen fibers from accompanied fibroblasts was noticed. The edema fluid started to decrease with increased infiltration with histiocytes, lymphocytes and plasma cells.

In the remodeling stage (14-28 days), the number of inflammatory cells decreased or even disappeared and collagen accumulation and fibroblast proliferation was increased till all the burnt area started to be replaced by collagen at the end of the stage (Fig. 4a).

At the post-mortem stage, the changes were less prominent in the form of very few polymorphnuclear cells infiltrating the dermis with transudate fluid which was seen at the site of burn (Fig. 5a).

**Immunohistochemical results of iNOS expression**

The iNOS protein expression was negative in the normal skin (control) at all the studied stages (Fig. 1b).In the burned skin, immune-reactivity for iNOS was detected as brown-yellow staining limited to the cytoplasm of keratinocytes, fibroblasts, endothelial cells, inflammatory cells, sweat glands and hair follicles.

Time dependent changes of iNOS protein expression in the ante-mortem burn

On day 1, the burned tissue showed iNOS positive staining in the neutrophils, keratinocytes and sweat glands (Fig. 2b).On days 3 and 5, iNOS expression steadily increased and reached the peak at day 7. Thereafter, the number of positive cells declined from day 9 till the end of the proliferative stage. The iNOS protein expression was observed in the inflammatory cells, fibroblasts and endothelial cells of the granulation tissue (Fig. 3b).

Compared to the previous stages, lower rates of iNOS protein expression were detected during the remodeling stage of burn healing. It was observed mainly in the macrophages whereas in the keratinocytes, iNOS expression was very minimal. Positive iNOS expression was also detected in few histiocytes and fibroblasts (Fig. 4b).

Table (1) shows a statistically significant difference between the mean iNOS expression during the various studied stages at the different time intervals. Post hoc LSD test revealed significant differences between all the studied time intervals except day 1 versus day 11, Day 3 versus day 9, and day 5 versus day 9 ( p values = 0.708,0.264, and 0.455 respectively).

iNOS protein expression in the post-mortem burn

iNOS protein expression declined remarkably in all the cells including polymorphnuclear cells, keratinocytes and sweat glands(Fig. 5b).The mean iNOS positive staining was the lowest (4.20±0.84) with a statistically significant difference from all the studied ante-mortem intervals (Table 1).

**Immunohistochemical results of IL-6 protein expression**

The samples taken from normal skin adjacent to the burn were completely negative for IL-6 expression during all the studied intervals in the various stages (Fig. 1c).Burn injury caused elevation in the expression of IL-6 in samples collected from the burn injury site throughout the various studied time-dependent intervals. It was expressed mainly in the cytoplasm of the inflammatory cells and granulation tissue fibroblastic and vascular endothelial cells.

*Time dependent changes of IL-6 protein expression in the ante-mortem burn*

On day 1, all the samples were positive for IL-6 expression with most of the samples (80%) showed scores 2 and 3 (Fig. 2c).On day 3, all the samples were positive for IL-6 expression with 100% of the cases were either score 2 or 3. This positive expression remained with higher scores on day 5.

On the day 7, still all the samples were positive but the intensity was varied with 80% were of score 2 and 3. From day 9, negative samples started to appear with one sample negative at day 9 and 11 while at day 13 there were 2 negative samples for IL-6 expression and no cases showed the intensity of score 3 (Fig. 3c).Looking at the proliferative stage in collectively, the expression was seen in 87% (26/30) of the samples with the peak intensity seen at day 5.

On day 15, 60% of the samples were positive for IL-6 expression that decreased to 40% on day 21. So, collectively during the remodeling stage, half of the cases were positive and the expression decreased gradually with the time (Fig. 4c).

A statistically significant difference was found between the mean ranks of IL-6 expressions in the various studied stages at the different time intervals of burn healing. Post hoc test revealed non-significant differences between day 1 and day 11, day 3 and day 9, day 5 and day 7, day 11 and day 13 (p values = 0.067, 0.875, 0.806, and 0.671 respectively) as shown in table (2).

IL-6 protein expression in the post-mortem burn

Only 1 sample was positive for IL-6 expression with score1 while 4 samples (80%) were

negative (Fig. 5c), with a statistically significant difference from all the studied ante-mortem intervals (Table 2).

Spearman's rank correlation between iNOS and IL-6 expressions during the studied stages of burn healing revealed a statistically significant positive association (r= 0.74, p value < 0.001) between the two markers. Both increased gradually in inflammatory and early proliferation stages and started to decrease gradually in late proliferative stage and remodeling stage while reaching the minimum at the postmortem stage.

**Table 1: ANOVA test for comparison of inducible nitric oxide synthase expression during the various studied stages at the different time intervals of burn healing**

| Stages and time intervals | | iNOS expression (Mean± SD) |
|---|---|---|
| **Inflammatory stage (n=5)** | **Day 1(n=5)** | 25±1.8 |
| **Proliferation stage (n=30)** | **Day3(n=5)** | 29±1.5 |
| | **Day5(n=5)** | 32±7.4 |
| | **Day 7(n=5)** | 39±5.5 |
| | **Day 9(n=5)** | 30±3.7 |
| | **Day11(n=5)** | 24±3.1 |
| | **Day13(n=5)** | 16± 3.6 |
| **Remodeling stage (n=10)** | **Day15(n=5)** | 12±2.8 |
| | **Day21(n=5)** | 8± 1.58 |
| **Post-mortem stage (n=5)** | **6 h. post-mortem (n=5)** | 4.20±0.84 |
| | | **One way ANOVA test**<br>F = 104.495<br>P value < 0.001*<br>**Footnote** |

*iNOS; inducible nitric oxide synthase, n; number, *; significant at p< 0.05, Footnote: Post hoc (LSD) test revealed significant differences between mean iNOS expression in all the studied time intervals except; Day 1 versus day 11: p value = 0.708, day 3 versus day 9: p value = 0.264, day 5 versus day 9: p value = 0.455.*

**Table 2: Kruskal -Wallis test for comparison of interleukin-6 protein expression during the various studied stages at the different time intervals of burn healing.**

| Stages and time intervals | | IL-6 expression (Mean rank) |
|---|---|---|
| **Inflammatory stage (n=5)** | **Day 1(n=5)** | 21.00 |
| **Proliferation stage (n=30)** | **Day3(n=5)** | 32.30 |
| | **Day5(n=5)** | 42.20 |
| | **Day 7(n=5)** | 40.50 |
| | **Day 9(n=5)** | 34.60 |
| | **Day11(n=5)** | 24.00 |
| | **Day13(n=5)** | 21.00 |
| **Remodeling stage (n=10)** | **Day15(n=5)** | 15.60 |
| | **Day21(n=5)** | 11.60 |
| **Post-mortem stage (n=5)** | **6 h. post-mortem (n=5)** | 7.20 |
| | | **Kruskal- Wallis test**<br>$X^2$ = 36.208<br>P value < 0.001*<br>**Footnote** |

*IL-6; interleukin-6, n; number, *; significant at p< 0.05, Footnote: Post hoc test revealed differences between mean ranks of IL-6 expressions in all the studied time intervals except: Day 1 versus day 11; p value = 0.067, day 3 versus day 9; p value = 0.875, day 5 versus day 7; p value = 0.806, and day 11 versus day 13; p value = 0.671.*

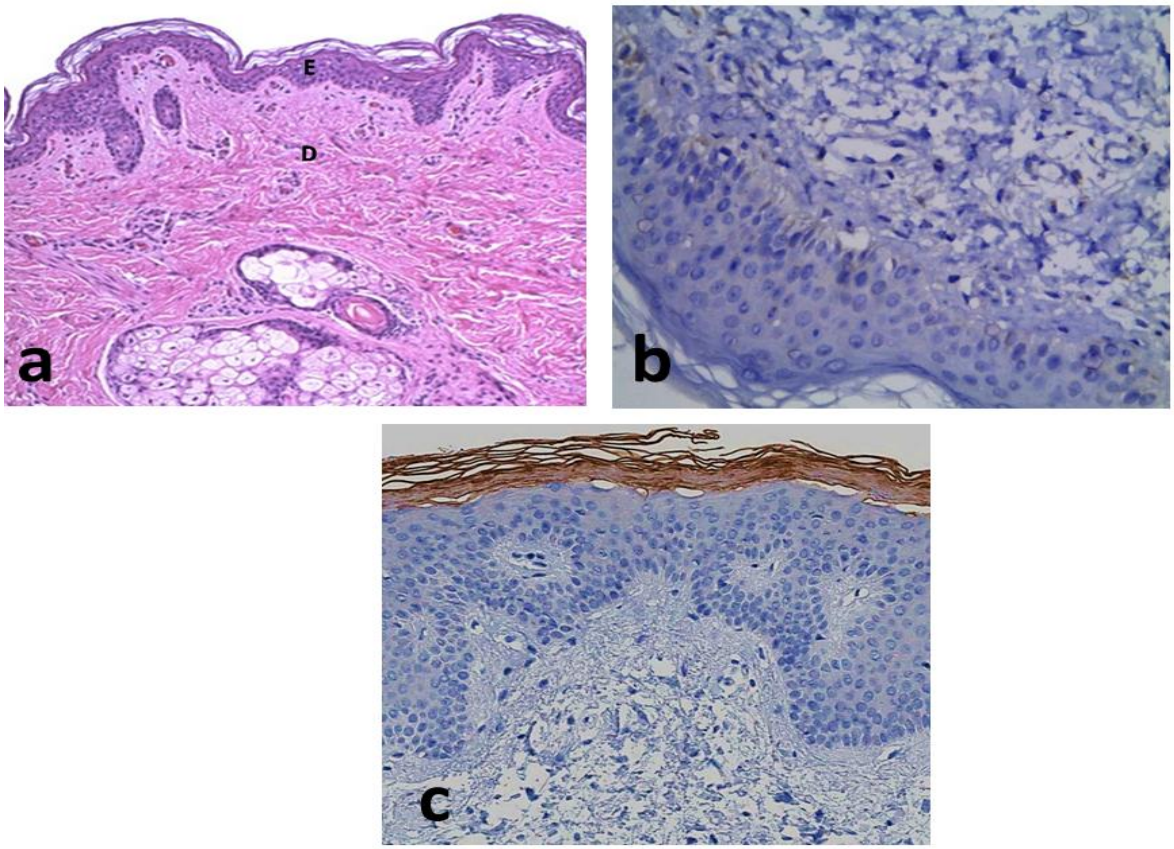


**Figure 1: Control (non-burned) skin showing a; H&E examination showing normal appearance of the epidermis (E) and dermis (D) (x100). b; negative iNOS expression (x400).c; negative expression of IL-6 (x400).**

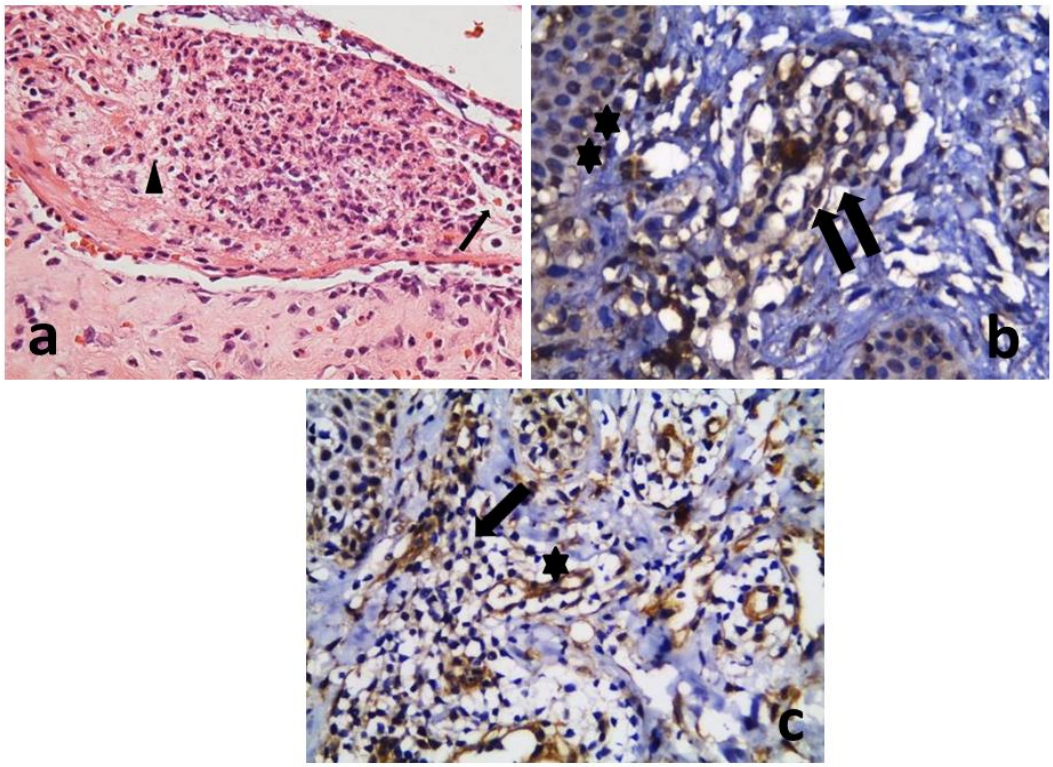


**Figure 2: Burned skin during inflammatory stage (24 h after burn) showing a; H&E examination showing acute inflammation, extensive edema (arrow) and necrosis (arrow head) (x100). b; positive iNOS expression in the cytoplasm of keratinocytes (stars) and inflammatory cells (arrows) ( x400) .c; score 3 expression of IL-6 in the inflammatory (arrow) and endothelial cells (star) (cx400).**

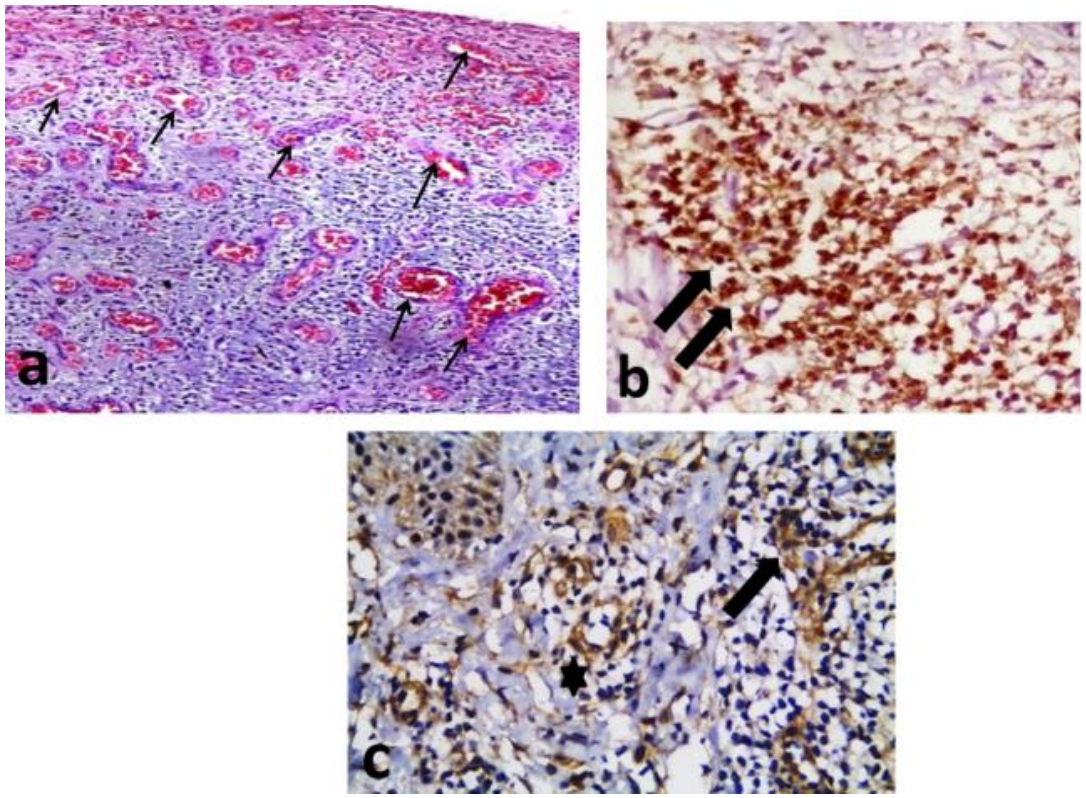


**Figure (3): Burned skin during proliferation stage (at day 7) showing a; H&E examination showing, extensive acute inflammation, neovascularization (arrows) (x100).b; positive iNOS expression in the cytoplasm of inflammatory cells (arrows) (x400). c; score 3 expression of IL-6 in the inflammatory ( arrow) and endothelial cells (star) (x400).**

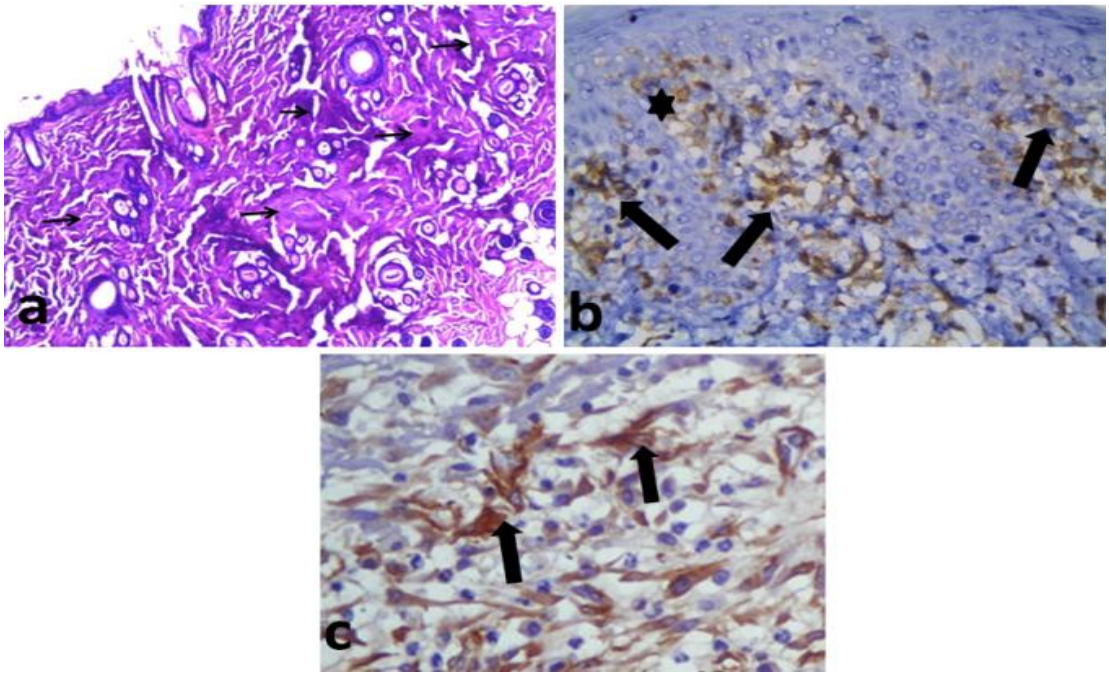


**Figure (4): Burned skin during remodeling stage (at day 15) showing a; H&E examination showing, dense collagen deposition (arrows) (ax100).b; positive iNOS expression in the few histiocytes and fibroblasts (arrows) and very minimal in the keratinocytes (star) (x400).c; score 2 expression of IL-6 in the fibroblasts (arrows) (x400).**

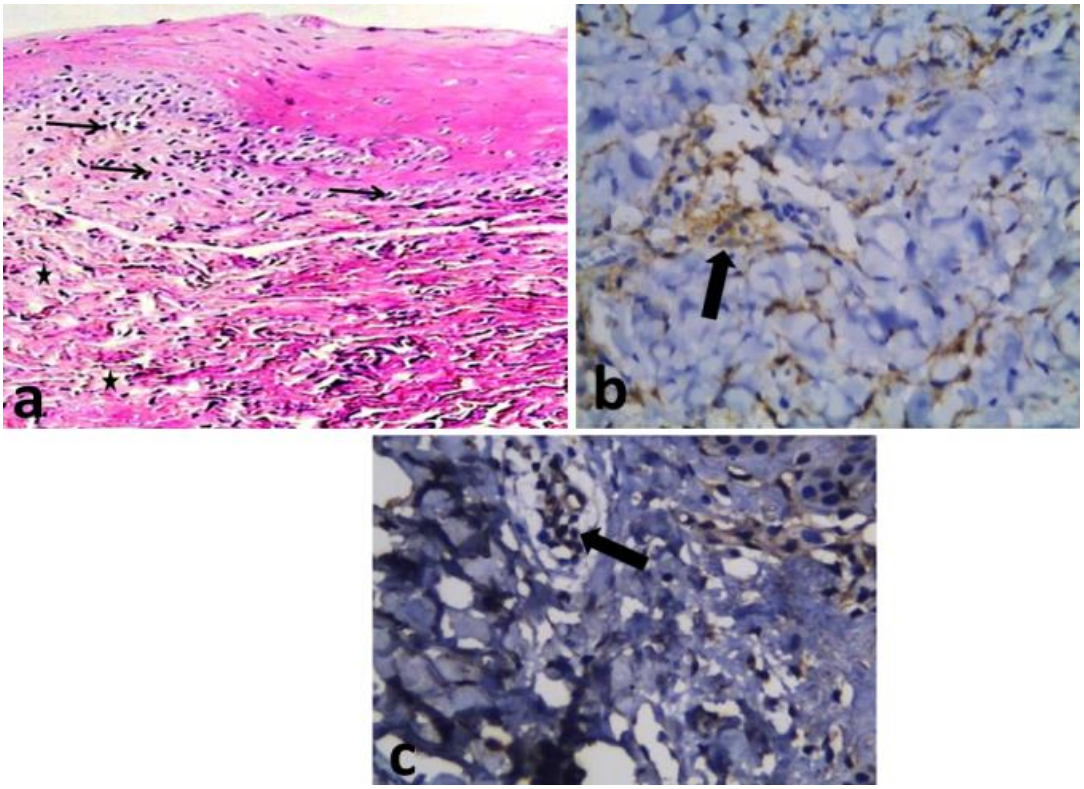


**Figure (5): Burned skin during post-mortem stage showing a; H&E examination showing mild changes in both epidermis and dermis with few polymorphnuclear leucocytes infiltrating the dermis (arrows) and transudate fluid (stars) (x100).b; positive cells for iNOS expression in the cytoplasm of inflammatory cells (arrow) (x400).c; score 1 expression of IL-6 (arrow) (x400).**

## Discussion

Determination of the age and vitality of an injury both in the living and dead is a crucial medico-legal issue in the field of crime investigation. It helps to fix the responsibility and to decide whether the injury date corresponds to the time of the crime according to the prosecution theory. However, it remains one of the most difficult aspects within forensic contexts (Kondo and Ishida, 2010).

To the best of our knowledge, this study is the first to investigate iNOS and IL-6 as biochemical markers for dating burn injury and to differentiate ante-mortem from post-mortem burn by using immunohistochemical techniques.

The present study was conducted on fifty adult male albino rats in which ante-mortem dry burn was inflicted in 45 of them while the remaining five rats were burned 6h after being sacrificed to study the post-mortem burn. All rats used in the study survived. In addition, no wound infection or other complications were found during the healing process of the burn injuries.

In the ante-mortem burned rats, our results indicate that iNOS expression was time dependent. On day 1, it was high with a mean of 25±1.8 compared to its negative expression in the normal skin. The number of iNOS positive cells progressively increased in days 3 and 5 and peaked at the day 7 with a mean of 39±5.5. Then started to decline and reached the lowest level with the end of the experimental period. Additionally, iNOS protein was not only detected in the cytoplasm of keratinocytes, fibroblasts, endothelial cells and inflammatory cells but also in sweat glands and hair follicles in the burnt skin. This pattern of changes in iNOS expression coincided with that obtained by Zhao et al. (2005) who studied the time dependent changes of iNOS and eNOS protein expression in mice cutaneous incised wound healing.

The observed cellular distributions of iNOS expression suggests that nitric oxide plays an essential role in all the sequences of wound healing; affecting inflammatory phase, adjusting cell proliferation, differentiation and apoptosis, forming granulation tissue and neo-vascularization and to some extent in tissue remodeling. The role of NO in the process of healing of burn injuries was proved by Wallace (2005) and Lakshmi et al. (2011).Additionally, Oliveira et al. (2004) found higher NO content in the skin of burnt rat compared to its levels in the plasma and visceral organs suggesting that burnt tissue may be an important site for the production of NO. Under normal conditions iNOS is not usually active; it is activated in thermal injury due to the effect of pro-inflammatory agents in affected tissues (Alsarhan et al., 2013).Moreover, Filippou et al. (2007) proved the role of iNOS in the discontinuous synthesis of high amounts of NO in the burnt patients.

The present study also revealed that IL-6 expression in the ante-mortem burned rats was time dependent. It started at day 1 and peaked with highest intensity at day 3 and remained high on day 5 as well. The positive IL-6 expression and its intensity decreased gradually from day 7 till the end of the studied period (day 21) where only 40% of the studied samples were positive with a statistically significant difference between all the studied time intervals.

Up regulation of IL-6 was observed at wound sites during skin wound healing in mice (Satoand Ohshima, 2000).Additionally, Sasaki et al. (2011) suggested positive association between IL- 6 and burn-induced immune-inflammatory response during the burn healing process. Increased levels of IL-6 within hours of thermal trauma have been reported by Modi et al. (2014).In addition, Guo et al. (1990) reported increased serum levels of IL-6 over a 3-week interval with peak concentrations reached during the first week after injury in a population of burn patients.

In the process of healing;IL-6can induce the expression of several chemokines which are chemotactic for neutrophils, monocytes and macrophages(Fenton et al., 2002).Moreover, IL-6 could promote granulation tissue formation by enhancing collagen deposition by induction of transforming growth factor- B1 (TGF-B1) gene expression(Lin et al., 2003).IL-6 is also involved in the process of neo-vascularization and angiogenesis through induction of vascular endothelial growth factor expression(Tzeng et al., 2012).It is worth to mention that Lakshmi et al.(2011) in their trial of using low molecular weight heparin in the treatment of burn patients proved the role of iNOS and IL-6 in the process of burn healing.

Burning of the body to try to conceal the homicide can cause confusion as regard the manner and cause of death. Additionally, the person might have had died due to some disease or sudden assault or injury and suffered burns later on (Tümer et al., 2012). Unfortunately, Bohnert et al. (2003) reported many problems associated with the diagnosis of vitality in burned bodies. So, recent research has focused on improved methods for distinguishing between ante-mortem and specifically early post-mortem injury by analyzing damaged tissue. In this regard, the 6h post-mortem burn model in this study revealed that iNOS expression in the burnt skin was low and only in polymorphnuclear leucocytes with significant difference from the control skin and all the studied ante-mortem intervals. Similarly, IL-6 expression was weakly positive (score 1) in only 20% of the studied samples. Thus both markers shall help in diagnosis of burn vitality. Ali (1988) studied the behavior of white blood cells in post-mortem wounds and reported that they may be motile for more than 12 h. after death and can still aggregates around chemotactic materials.

In conclusion, the current results indicate that both iNOS and IL-6 expression in ante-mortem burnt skin was time dependent and significantly differed from post-mortem burn. Further research on human is highly recommended to support the use of these markers as objective methods for burn injury dating and vitality determination.

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## الملخص العربى

# دور التعبير المناعى لبروتينات أكسيد النيتريك المستحث و الإنترلوكين –6 فى تقدير عمر وحيوية حروق الجلد: دراسة هستوكيميائية مناعية فى الجرزان

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إن تقدير عمر وحيوية الحروق فى كل من الاحياء والأموات أمر ضرورى فى ممارسة الطب الشرعى. يلعب كل من أكسيد النيتريك و الإنترلوكين– 6 دورا هاما فى إلتئام حروق الجلد. فى هذه الدراسة الهستوكيميائية المناعية تم دراسة التعبير المناعى لبروتينات أكسيد النيتريك المستحث و الإنترلوكين –6 أثناء إلتئام حروق الجلد فى الجرزان بهدف معرفة عمر الحرق وكذلك للتفرقة بين الحروق الحيوية والغير حيوية. تم إحداث حروق ما قبل الوفاة فى خمسة وأربعين من الجرزان عن طريق سبيكة لحام ساخنة وضعت على الجلد لمدة ثلاثة ثوانى. ثم أخذت عينات من الجلد الطبيعى والمحروق فى الأيام 1، 3، 5، 7، 9، 11، 13، 15، 21 التالية لحدوث الحروق (خمس جرزان فى كل مرحلة). أيضا تم إحداث حرقا بعد الوفاة بست ساعات فى خمسة جرزان أخرى. كان هناك فرق ذو دلالة إحصائية فى كل من التعبير المناعى لبروتينات أكسيد النيتريك المستحث و الإنترلوكين –6 بين كل الأزمنة محل الدراسة فى الحروق الحيوية. وبالنسبة للحروق الغير حيوية فقد قل التعبير لبروتينات أكسيد النيتريك المستحث و الإنترلوكين – 6 بشكل ملحوظ مع وجود فرق ذو دلالة إحصائية عن كل المراحل الوقتية فى الجروح الحيوية. وقد كان هناك إرتباط طردى بين التعبير المناعى لبروتين أكسيد النيتريك المستحث و الإنترلوكين –6 ، فكلاهما زاد تدريجيا فى مرحلة الالتهاب والمرحلة الأولى من التكاثر ثم بدأت مستوياتها تقل عند نهاية مرحلة التكاثر وفى خلال مرحلة إعادة بناء الجلد ووصلت للحد الأدنى فى الحروق الغير حيوية. تشير هذه النتائج إلى أن التغييرات فى التعبير المناعى لبروتينات أكسيد النيتريك المستحث و الإنترلوكين – 6 فى الحروق الحيوية كانت معتمدة على وقت الالتئام واختلفت بشكل ملحوظ إحصائيا عن الحروق الغير حيوية. ينصح بإجراء المزيد من البحوث على البشر.

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