AGE ESTIMATION FOR SCALD INJURY AND ITS PROBAPLE RELATION TO TESTICULAR FUNCTION IMPARTMENT

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ABSTRACT

Background and Rationale: From the medicolegal point of view, long term systemic consequences of scald injury should be identified for proper compensation and legal action, especially for scald injury involving skin full-thickness in 20% of total body surface area (TBSA) which is known to have long term grave consequences on general health. **Aim of the Study:** The current experimental study was carried out to: 1-identify the histopathological changes and TNF-α Immunoexpression of different ages of a full thickness scald injury occupying 20% of the total body TBSA; 2- study the probable relation between the scald age and the testicular function impairment. **Materials and Methods:** A total number of 40 adult male albino rats were used in the study. Histopathological examination for the scald area and testis were performed with hematoxyline & eosin stain and TNF-α Immunoexpression. Epididymal semen analysis and serum testosterone were also performed. These procedures were carried out after 2 days, 7 days, 1 month and 3 months of scald infliction. **Results:** Progressive histopathological changes were observed in the early scald ages while healing manifestations and improvement in testosterone serum levels, when compared to the other periods, started to appear after 1 and 3 months of scald infliction. **Conclusion:** It can be concluded that scald injury involving 20% of TBSA can cause long term impairment of testicular function.

Keywords: Scald; TNF-α; histopathology; Testicular function

INTRODUCTION

Worldwide the circumstances surrounding scald assault and burning fall into three main categories: domestic maltreatment, elder abuse, or conflicting business affairs **(Peck, 2012)**, where in most cases the assailant is known to the victim which allows him to be at a near distance from the victim **(Dorn et al., 2001)**.

The incidence of burn injury is not specific to any population or age group, but the forensic medicine is concerned if the occurrence of burns has been attributed to abuse, ill-treatment, neglect or torture **(Huang et al., 2008; Pollanen, 2018)**.

According to **Adeteye et al. (2011),** severity is related to burn depth and percentage of the total body surface area (TBSA), where 3rd degree is a full thickness burn destroying all the skin layers till below hair follicles, sweat glands and subcutaneous fat tissue, accordingly, it is usually not painful due to destruction of the nerve endings.

As stated by **Agay et al. (2008),** a scald burn is a type of tissue damage from any hot liquid or fluids as oils, steam and molten rubber, and however, scalding by water is a common domestic accident, especially to children and old people, who are vulnerable to many types of accidents.

Scalding burns are of three types: immersion in a hot liquid whether accidental or deliberate, splash (or spill) burns which is usually accidental, and steam burns i.e. exposure to superheated steam. Hot water is found to be the most common cause of the immersion, spill, and splash burns **(Jewo et al., 2010).**

It was proven that burn injury caused changes in the endogenous production of cytokines, adrenal and gonadal steroids, where previous studies have reported sex-related differences in the outcome following burn injury **(Bergquist et al., 2016)**.

Cytokines are important mediators in post-burn pathophysiological process **(Agay et al., 2008)**. This enhanced production of inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin 1beta (IL-1β), and prostaglandins may cause a failure of different organ systems, at least in part, due to increased apoptotic cell death and **(Gatson et al., 2009),** where tumor necrosis factor-alpha (TNF-α), as one of the proinflammatory and immunoregulatory cytokines, exhibits a surge particularly in the early stage of the wound healing process **(Bai et al., 2008)**.

The current experimental study was carried out to: 1-identify the histopathological changes and TNF-α Immunoexpression of different ages of a full thickness scald injury occupying 20% of the total body TBSA; 2- study the probable relation between the scald age and the testicular function impairment.

MATERIALS AND METHODS

MATERIALS

1. Animals:

- Experimental Design

Forty adult albino rats were used in the study. Eight albino rats formed the control group which received only regular diet and tap water to measure the basic parameters. The remaining 32 rats were subjected to a model for scald injury to be sacrificed by cervical dislocation **(Tomita et al., 2004)** and studied at 4 different scald ages: after 2 days, 7 days, 1 months and 3 months of injury infliction (8 albino rats/scald age).

This study was done after taking acceptance of Institution Review Board (IRB) of Faculty of Medicine, Zagazig University. The 40 adult male albino rats were obtained from the animal house of the Faculty of Veterinary Medicine, Zagazig University; their weights ranged between 290 and 310 gm. The guidelines stated in "The Guide for the Care and Use of Laboratory animals" **(Institute of laboratory animals resources, 1996),** were used to care for the experimental animals used in the current study.

2. Chemicals:

Sodium citrate solution (2.9-3%) and physiological saline solution (0.9%) were used for epididymal spermatozoal examination. Ketamine hydrochloride and diazepam were used in the burn model. All chemicals were obtained from El- Nasr Co., Egypt.

METHODS

1. Scald model:

Each rat was anesthetized, and the analgesic was given with intraperitoneal injection of ketamine hydrochloride (20 mg/kg BW) and diazepam (0.1 mg/kg BW). The back and flank skin of the rats was shaved. Rats were placed in supine position in a plaster cast exposing an area in their backs through an opening in the cast, then immersed in a hot water bath (100°C) for 10 seconds **(Jewo et al., 2011)**. This should produce a nonlethal full thickness injury to the skin that covers 20 % of the total body surface area which was calculated by using the formula of Lee which is: Total body surface area (TBSA) = (body weight in grams x 0.78) + 148 **(Gouma et al., 2012).** TBSA of a rat

weighing 300 grams is 382 cm ². So that 20% of TBSA equals 76.4 cm².

2. Histopathological Examination:

Light microscopic examination was performed to detect histopathological changes of the skin with scald injury and the testis. After 2 days, 7 days, 1 month and 3 months of scald infliction, the scald area in the skin was immediately dissected out and fixed in Bouin's solution **(Prophet et al., 1992),** while testicular specimens were fixed in 10% formalin saline. After fixation, specimens were embedded in paraffin blocks and processed for the preparation of sections in 5 µ thickness. These sections were subjected to:

1- **Hematoxylin and Eosin** staining according to the method described by **Wilson & Gamble (2002).**

2- **TNF-α Immunohistochemical** detection according to the method described by **Carreiraa et al. (2012).** Staining was considered positive if the tissue demonstrated brown staining.

3. Epididymal Sperm Analysis:

Spermatozoa collection was done as described by **Klinefelter et al. (1991).** Epididymal content was obtained by cutting the tail of epididymis and squeezing it gently to get the fresh undiluted semen in a clean Petri dish to perform the following examinations:

1- **Sperm motility:**

Sperm motility was estimated by mixing an undiluted semen droplet to a drop of sodium citrate solution 2.9-3% on warm slide, several fields were inspected under light microscope, then the incidence of progressive motile sperms were counted and recorded **(Tardif et al., 1999)**.

2- **Sperm count:**

Sperm cell concentration was determined by withdrawing undiluted semen up to the mark 0.1 and a hemocytometer pipette was filled up to the level 101 by normal saline, then stained with eosin, and shook vigorously. A drop of diluted semen was spread between the haemocytometer chambers after placing over the counting chamber cover slide. Using the high power lens of light microscope (40x), the sperms in 5 large squares (80 small squares) were counted. The sperm cell concentration is calculated by multiplying the number of sperms by 100 (depth) and 1000 (dilution) **(Blazak et al., 1993)**.

3- **Sperm abnormal forms:**

Two equal drops of epididymal content and eosin-nigrosin stain were mixed, and spread on clean and grease free slides. Two hundred sperms were randomly examined for each rat samples under the light microscope high power lens to record both the percentages of abnormal sperms and the abnormal forms. Moraes's classification was used to describe the sperm abnormal forms observed in the current study **(Moraes et al., 2008),** which were classified mainly into: sperms with deformed or absent tails (tailless sperm) and sperms with abnormal heads.

4. Biochemical Measurement for Testosterone Hormone:

Venous blood samples were collected from the retro-orbital plexus of the animals by capillary glass tubes using light ether anaesthesia according to procedure described by **Joslin (2009)**. According to the, manufacturer's guidelines, for each animal, not less than 2 ml of blood was collected into a glass tube for the quantitative measurement of testosterone in rat and mouse by ELISA.

5. Statistical Analysis:

For statistical analysis, SPSS 13.0 for windows programme was used. Data was represented in terms of means ± SD. The differences were compared for statistical

significance by ANOVA. Statistical difference between groups was calculated by LSD. Significant difference in the percentage of sperm motility and sperm abnormal forms was calculated by Chi-Square for Linear Trend test. Difference was considered significant at $p \leq 0.05$.

RESULTS

- Histopathological Examination

Skin Examination (Table-1)

In H & E skin sections ***(Figure-1)***, control group showed intact dermal epidermal layers with normal adnexal structures after 2 days, 7 days, 1 month and 3 months**.** In Scald injured group, skin with scald injury revealed ulceration of the epidermis and dermal inflammatory infiltrate that invade muscles in some area by the end of the 2nd day of scalding injury. By the end of 7th, there was destruction of adnexal structures, and area of coagulative necrosis invaded by inflammatory cells.

By the end of 1st month, a decrease in the acute inflammatory response findings were observed with deposition of multiple collagen layers. By the end of 3 months, re-epithelialization was observed in the form of multiple epithelial cells islands migrating towards the wound surface from underlying dermal appendages.

For TNF-α immunohistochemical expression ***(Figure-2)***, it was negative in the dermis, endothelial cells and perivascular cells of the skin of the control group after 2 days, 7 days, 1 month and 3 months. In Scald injured group, skin showed a positive expression of TNF-α in the dermis, endothelial cells and perivascular cells after 2 days and 7 days. By the end of 1 month and 3 months, there were a recorded improvement as all skin specimens of the rats of both groups showed a negative expression of TNF-α in the dermis, endothelial cells and perivascular cells, respectively.

Testes (Table-2)

In H & E sections ***(Figure-3)***, the testes of the control group showed normal cell arrangement and the lumen is full of mature sperm cells after 2 days, 7 days, 1 month and 3 months**.** In scald injured group, microscopic examination of the testes revealed reduction of number of germ cell layers and dissociation of the germ cells from the tubular basement membrane by the end of the 2nd day of burn application**.** By the end of 7th day of burn application, there was germ cell atrophy, absence of free spermatozoa, the tubules showed scanty cells even in their basal areas which lined only by Sertoli cells. By the end of 1st month the same histopathological changes as those described by the end of the 7th day were noted. Widespread cyto-architectral disruption of the seminiferous tubules, the tubules lined only by Sertoli cells**.** By the end of 3 months microscopic examination of testes showed considerable seminiferous tubular damage**.**

For TNF-α immunohistochemical expression ***(Figure-4)***, the control group showed normal appearance of testes specimens with negative expression of TNF-α in the cytoplasm of spermatogonia, spermatocytes and the interstitial leydig cells after 2 days, 7 days, 1 month and 3 months. In Scald injured group, the testes showed a positive expression of TNF-α in the cytoplasm of spermatogonia, spermatocytes and the interstitial leydig cells by the end of the 2nd and 7th days, while by the end of 1 month and 3 months, a recorded improvement was detected with a negative expression of TNF-α in the cytoplasm of spermatogonia, spermatocytes and the interstitial leydig cells**.**

- Epididymal Sperm Analysis (Tables-2, 3, Figure-5)

Scald injury was associated with significant decreases ($p<0.05$) in the mean values of epididymal sperm analysis parameters (sperm count (106/mm3), the percent (%) of sperm motility, and abnormal forms) after 1 and 3 months of injury infliction. Abnormal sperm forms

were: coiled tailed, bent tail, 2-tailed and flat head. Scald injuries of 2 and 7 days of age were not associated with any significant changes ($p>0.05$) in the mean values of epididymal sperm analysis parameters compared to the control group mean values on one side, and between one another on the other side.

- Testosterone Measurements (Table-4)

Scald injury was associated with progressive significant decreases ($p<0.05$) in the mean values of serum testosterone after 2 & 7 days and 1 month of injury infliction, with a percentage of decrease compared to the control level by 82%, 87% and 90%, respectively. After 3 months of injury infliction, significant improvement ($p<0.05$) in the mean values of serum testosterone was recorded when compared to the control level and the mean levels recorded in the earlier periods.

Table-1: Characteristic light microscopic findings observed in skin in terms of absent (-), mild (+), moderate (++) and severe (+++) after 2 days, 7 days, 1 month and 3 months from exposure to antemortem scald injury in adult male albino rats

Light Microscopic Finding	Age of Scald Injury			
	2 Days	7 Days	1 Month	3 Months
Destruction of Adnexal Structures	+	++	+	+
Ulceration in the Epidermis	+	++	+	-
Inflammatory Infiltrate in the Dermis	++	+++	-	-
Deposition of Multiple Collagen Layers	-	-	++	+++
Re-epithelialization	-	-	+	+++
Multiple Cell Islands	-	-	++	++
Epithelial Cell Migration to the Scald	-	-	+	++
TNF-α Immunoexpression	+++	++	-	-

Table-2: Characteristic light microscopic findings observed in the testis in terms of absent (-), mild (+), moderate (++) and severe (+++) after 2 days, 7 days, 1 and 3 months from exposure to antemortem scald injury in adult male albino rats

Light Microscopic Finding	Age of Scald Injury			
	2 Days	7 Days	1 Month	3 Months
Reduction in Germ Cell Layers Number	+	++	++	+++
Dissociation of Germ Cells	+	++	++	++
Germ Cell Atrophy	-	+	++	++
Absent Free Spermatozoa	-	+	++	+
Scanty Sertoli Cells Layer	-	+	++	+
Tubular Cyto-architectral Disruption	-	-	+	++
Sperm Cell Count	-	-	++	+
Decreased Sperm Motility	-	-	++	+
Presence of Sperm Abnormal Forms	-	-	++	++
TNF-α Immunoexpression	+++	++	-	-

Table-3: Mean values of epididymal sperm analysis (sperm count in 106/mm3, sperm motility percent and percent of abnormal sperm forms) of control and scald injury groups after 2 days, 7 days, 1 month and 3 months

Testicular Function Parameters		Control Group	Scald Injury Group			
			After 2 days	After 7 days	After 1 month	After 3 months
Epididymal Sperm Analysis	Sperm Count (10^6/mm^3)	118.85 ± 2.4	117.6 ± 2.4	117.3 ± 2.1	35.8^{ab} ± 2.3	32.3^{abc} ± 2.1
	Epididymal Sperm Motility (%)	85.3 ± 1.7	82.9 ± 2.2	83.8 ± 2.1	71.3^{ab} ± 2.2	65.5^{abc} ± 2.8
	Sperm Abnormal Forms (%)	32.98 ± 2.6	36.2 ± 4.3	32.4 ± 3.3	66.3^{ab} ± 2.0	67.1^{abc} ± 2.3

Data are expressed in terms of mean ± standard deviation for each parameter, (%): percent, Significance is considered when $p \leq 0.5$, (↑): increase, (↓): decrease, a: significant difference when compared to the control group, b: significant difference when compared to scald injury group after 2 & 7 days, c: significant difference when compared to scald injury group after 1 month.

Table-4: Testosterone mean serum levels of scald injury group after 2 days, 7 days, 1 month and 3 months, and the percentage of change compared to mean serum level of control group

Parameter	Control Group	Scald Injury Group							
		After 2 days		After 7 days		After 1 month		After 3 months	
		Level	Change %	Level	Change %	Level	Change %	Level	Change %
Testosterone Serum level (ng/ml)	3.26 ± 0.26	0.59^{a} ± 0.03	↓ 82%	0.42^{ab} ± 0.02	↓ 87%	0.33^{acb} ± 0.02	↓ 90%	0.76^{abcd} ± 0.03	↓ 77%

Data are expressed in terms of mean ± standard deviation in terms of ng/ml, Change %: percent of change compared to control level, ↓ : decrease, Significance is considered when $p \leq 0.5$, (↑): increase, (↓): decrease, a: significant decrease when compared to the control group, b: significant difference when compared to scald injury group after 2 days, c: significant difference when compared to scald injury group after 7 days, d: significant difference when compared to scald injury group after 1 month.

DISCUSSION

Scald injury is the most common type of burn **(Singh et al., 2017)**, whether accidental or non-accidental as in case of deliberate self-immolation (attempt suicide) or due to assault (attempt homicide) **(Silverstein and Lack, 1987)**. Scald injury has proven to elicit an immediate response in almost all body systems due to vascular permeability changes that lead to fluid and colloid loss, and pathophysiologic changes can occur in several body systems in the proceeding days **(Jewo et al., 2011).**

In the current study, skin with scald injury showed histopathological findings in the form of ulceration of the epidermis and severe dermal

inflammatory infiltrate that invades muscle layer in some areas by the end of the 2nd day of scalding injury, and then progressed to destruction of adnexal structures, and area of coagulative necrosis invaded by inflammatory cells by the end of 7th day. Healing process was evident by deposition of multiple collagen layers were observed by the end of the first month and re-epithelialization was observed in the form of multiple epithelial cells islands migrating towards the wound surface from underlying dermal appendages by the end of 3 months. The previously described inflammatory response is supported by the TNF-α immunohistochemical expression which was strongly positive during the early age of scald injury (after 2 and 7 days), and became negative in scald injury of 1 and 3 months of age.

The previously described histopathological changes can be referred to the study of **Ipaktchi et al. (2014)** who stated that burn injury strongly stimulates dermal release of proinflammatory mediators, resulting in progressive wound inflammation and tissue edema. This can be demonstrated, microscopically, by blistering of the epidermal layers, epithelial cells with pyknotic nuclei, injured adnexal cells in the deep dermis, and destruction of the superficial dermal appendages **(Cribbs et al., 1998). Adeteye et al. (2011)**, also, stated that the microscopic examination of 3rd degree full-thickness thermal injury showed layers of immature collagen fibers amidst fibroblasts, dermal ulceration, areas of destruction of skin adnexal structures and replacement with coagulative necrosis**.**

Yongqiang et al., (2016) studied a mouse model of scald wounds in which a full-thickness scald injury was developed by exposing the dorsal skin to a 90^{o}C water for 9 seconds. It was found that the epidermis of scalded mouse skin was broken and become separated from the dermal layer. The dermal hair follicles were, severely, damaged and almost unviable. In addition, there were homogenization and coagulative necrosis of some areas in the subcutaneous adipose tissue, and damage of superficial intradermal muscle layer.

During identifying the age of the scald, it has to be noted that it is a progressive injury, as stated earlier in the current study, during the further days after infliction i.e. extending in the area and depth in the days following the accident. This 2^{ry} damage, as stated by **Winter (1975)** which occurs within 5-8 days**,** is, primarily, due to the heat coagulation of the contents of venules and capillaries with stagnation of the tissue fluid leading to inability of the surrounding tissues to provide the injured area with vital supplies of oxygen and glucose to the cells on the rim of the zone of severe and irreversible damage, and, secondarily, to loss of water vapour through the injured surface causing dehydration of the exposed dermis.

According to the results of the current study, a TNF-α immunohistochemical expression is positive in scald injury till 7 days of infliction, and becomes negative after 1 month of scald injury age. This can be explained by the fact that cytokines are mediators in the post-burn pathophysiological process and as an important pro-inflammatory cytokine, TNF-α is a key product released following a cutaneous thermal injury **(Clark et al., 1995)**. The release pattern of TNF-α in the scald area of the current study is confirmed by the work of **Kubo et al. (2014)** who stated that TNF-α gene expression (together with other cytokines) increased significantly in a biphasic pattern from

3 or 6 hours to 12 hours or 1 day (inflammatory phase) and from 3 or 5 days to 7 days (proliferative phase).

Jeschke et al. (2007) reported the strength of the inflammatory and hypermetabolic responses is determined by the burn size, in which an increase in the later is associated with increased hyper-metabolic state, persistent inflammation, catabolism and organ dysfunction. This study explains the significant testicular dysfunction detected in the scald injured albino rats of the current study, on the histopathological, epididymal semen analysis and serum testosterone levels. **Yang et al. (2011)** studied a quantitative model of thermal injury-induced acute inflammation and postulated that the initiator of the inflammatory trajectory after was the release of TNF. The same authors added that a 20 % TBSA scald injury can cause marked inflammatory response and elevation in catecholamines, where both of which are associated with increased metabolic rate. The postulation of **Yang et al. (2011)** can, also, explain the positive immunohistochemical expression of TNF-α in the cytoplasm of spermatogonia, spermatocytes and the interstitial leydig cells by the end of the 2^{nd} and 7^{th} days of scald infliction, and became negative in later scald ages (after 1 and 3 months).

In scald injured group of the current study, microscopic examination of the testes revealed reduction of number of germ cell layers and dissociation of the germ cells from the tubular basement membrane by the end of the 2^{nd} day of scald application. Histopathological changes progressed to germ cell atrophy, absence of free spermatozoa, the tubules showed scanty cells even in their basal areas which lined only by Sertoli cells by the end of the 7^{th} day, widespread cyto-architectral disruption of the seminiferous tubules and the tubules lined only by Sertoli cells by the end of 1 month**,** and considerable seminiferous tubular damage was present by the end of 3 months of scald infliction. The previous histopathological changes were accompanied by progressive significant decreases in the mean values of serum testosterone after 2 & 7 days and 1 month of injury infliction, with a percentage of decrease compared to the control level by 82%, 87% and 90%, respectively. After 3 months of injury infliction, significant improvement in the mean values of serum testosterone was recorded when compared to the control level and the mean levels recorded in the earlier periods.

These results correlated with the study of **Jewo et al. (2011)** who studied the histopathological changes and affection of testicular function in severely burned rats (after 8 and 16 weeks since scald application) and have reported that severe burns produced significant seminiferous tubular damage, atrophy of germ cell in the ad-luminal area, with tubular atrophy almost three times more than that found in the control group. Other histological changes were in the form of sloughing leaving only basal cells such as spermatogonia and Sertoli cells in many tubules. Serum testosterone showed significant progressive decline till after 16 weeks since scald application. Significant decrease in serum testosterone level recorded in the current study is supported by the results of previous researches. **Emanuele et al. (2005)**, in their study, have subjected young adult male mice to a 15% total body surface area and full thickness scald, and were sacrificed 48 hours later. Scald injury has reduced serum testosterone with an increase in hypothalamic concentrations of TNF-α (with other proinflammatory cytokines).

Fadeyibi et al. (2010) reported that a significant fall in serum levels of testosterone, luteinising hormone (LH) and

follicle-stimulating hormone (FSH), and the fall correlated with burn size. Also, **Bergquist et al. (2016)** have reported that burn injury has altered endogenous steroid biosynthesis, with decreased testosterone concentrations and elevated estrone concentrations, during the first 21 days after the burn injury.

CONCLUSION

According to the finding of the current study, identifying the age of scald injury can be performed using routine histopathological examination. For TNF-α Immunoexpression, it can be used differentiating early and long term scald injury. A full-thickness scald injury involving 20% of TBSA can cause long term impairment of testicular function. Further studies for longer durations are required to confirm or deny this cause-effect relationship.

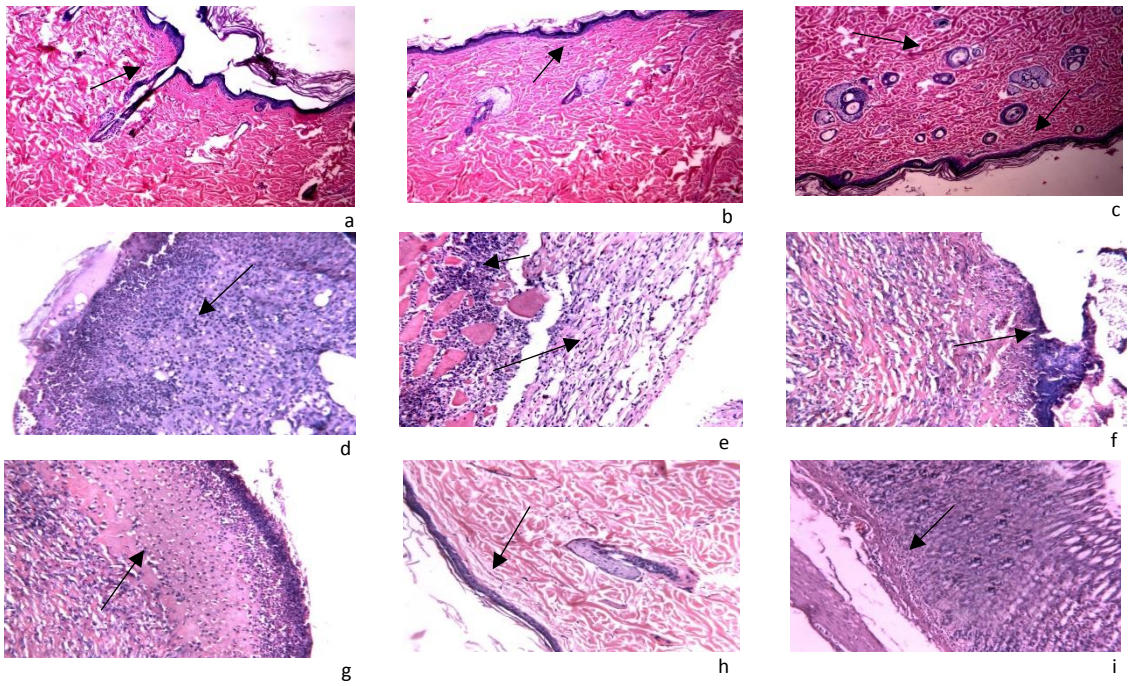

Figure-1: An H & E photomicrograph of a section in the skin of adult male albino rat. a, b, c: control group showing normal epidermis, dermis and adnexal structures, d: scald injury after 2 days showing heavy dermal inflammatory infiltrate, e: scald injury after 2 days showing destruction of epidermis and dermis heavy dermal inflammatory infiltrate invading muscle layer, f: ulceration of the epidermis and dermis, g: scald injury after 7 days showing coagulative necrosis, h: scald injury after 1 month showing destruction of skin adnexa and deposition of multiple layers of collagen, i: scald injury after 1 month showing re-epithelialization with multiple islands of epithelial cells migrating to the wound surface ( ) (x 200).

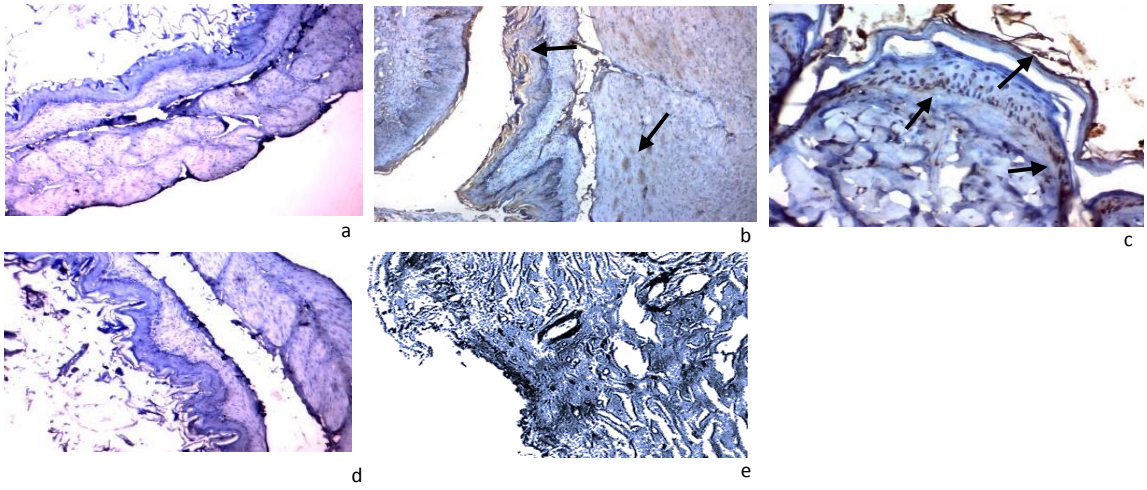

Figure-2: A DAB photomicrograph of a section in the skin of adult male albino rat showing the brown TNF-α Immunoexpression (⟶: a: control group showing negative expression (x 200), b: scald injury after 2 days showing positive expression (x 200), c: scald injury after 7 days showing positive expression (x 400), d: scald injury after 1 month showing negative expression (x 200), e: scald injury after 3 months showing negative expression (x 200).

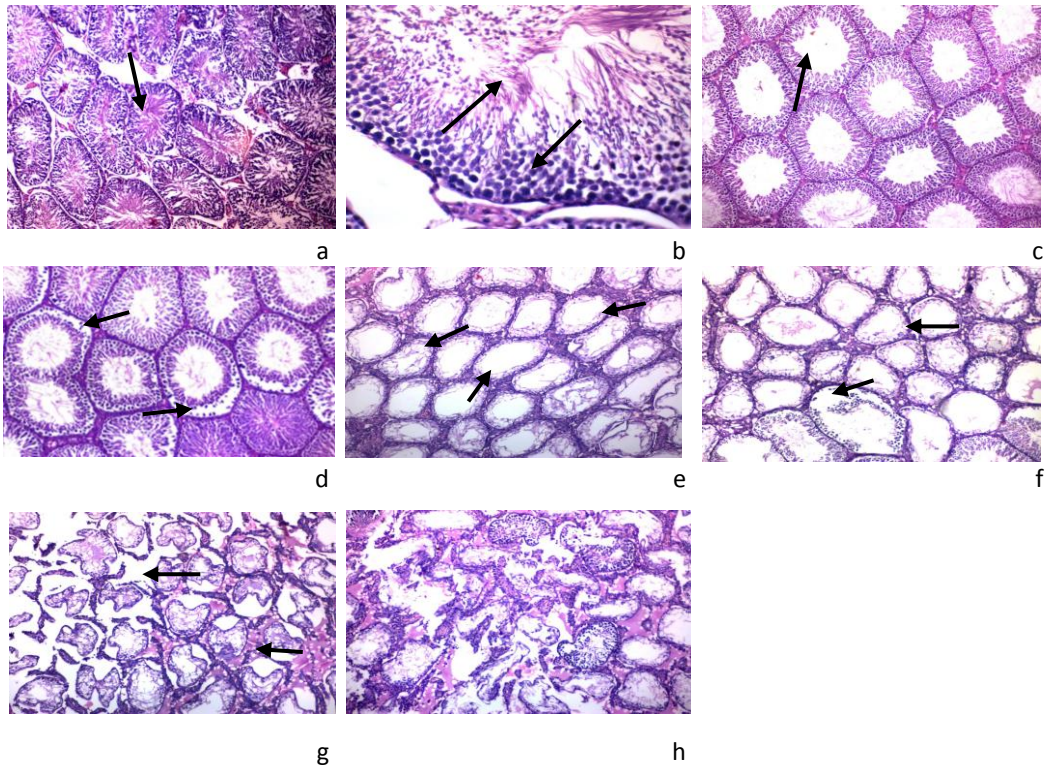

Figure-3: An H & E photomicrograph of a section in the testis of adult male albino rat. a, b: control group showing normal testicular tissue with normal cell arrangement and the lumen is full of mature sperm cells, c, d: scald injury after 2 days showing reduction of the number of germ cell layers, most tubules show no free mature spermatozoa in their lumen and dissociation of the germ cells from the tubular basement membrane, e, f: scald injury after 7 days showing germ cell atrophy and absence of free spermatozoa. The tubules lined only by Sertoli cells, g: scald injury after 1 month showing marked germ cell loss. The tubules show scanty cells even in their basal area. Some tubules show sloughing of the cells in their lumen, h: scald injury after 3 month showing considerable seminiferous tubular damage ( ) (x 200).

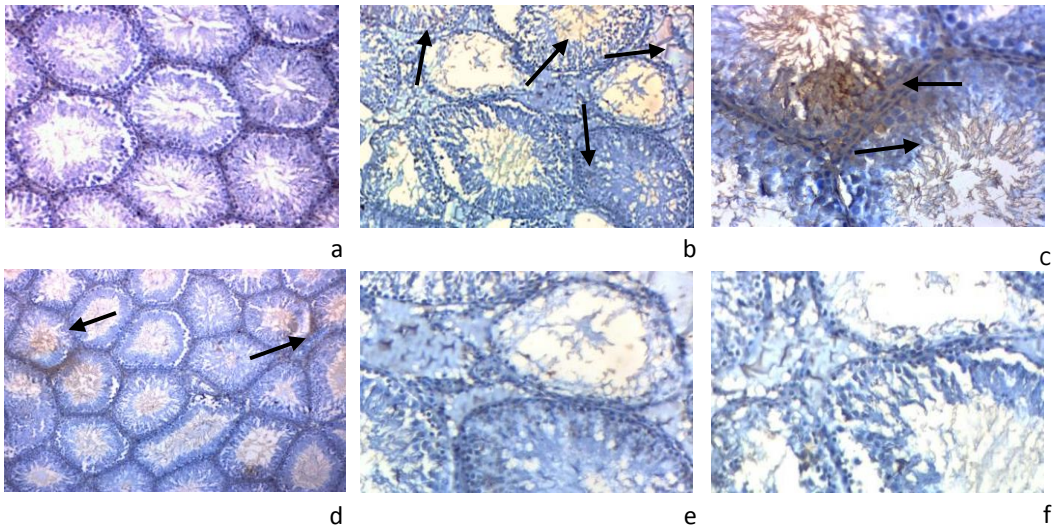

Figure-4: A DAB photomicrograph of a section in the testis of adult male albino rat showing the brown TNF-α Immunoexpression (⟶). a: control group showing negative expression (x 200), b: scald injury after 2 days showing positive expression (x 200), c: scald injury after 2 days showing positive expression (x 400), d: scald injury after 7 days showing positive expression (x 200), e: scald injury after 1 month showing negative expression (x 400), f: scald injury after 3 months showing negative expression (x 400).

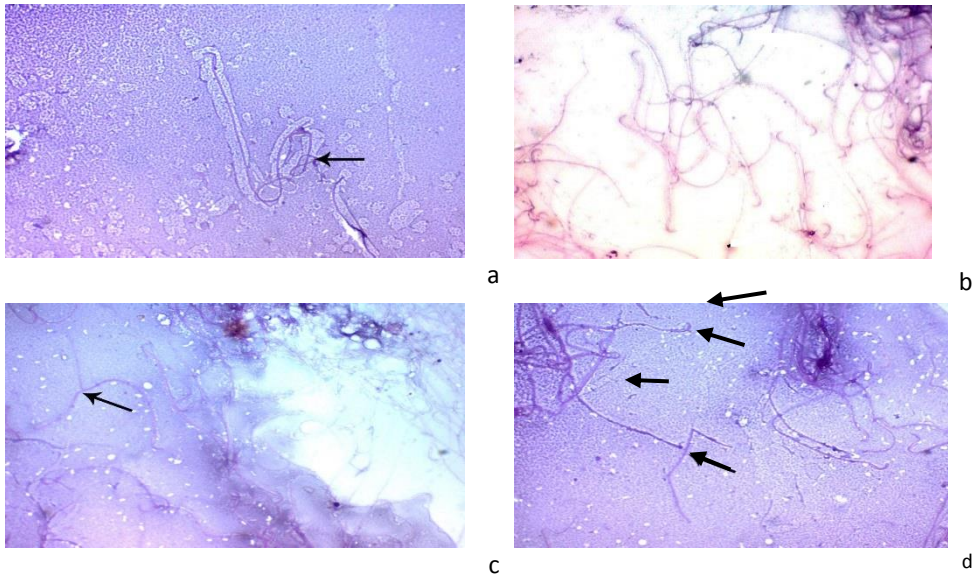

Figure-5: A Nigrosin and Eosin photomicrograph of sperm abnormal forms detected in scald injury group after 1 and 3 months. a: showing coiled tail sperm b: bent tail sperm, c: 2-tailed sperm, d: flat head and bent tail sperm (→), (x400).

REFERENCES

Adeteye OV, Yama OE and Gbotolorun SC (2011): Third degree burns in female Wister rats: The corollary on estrous cycle and ovarian histo-architectural organization. International journal of medicine and medical science. 3: pp 256-61.

Agay D, Sanchez M, Claeyssen R, et al., (2008): Interleukin-6, TNF-alpha and.interleukin-1 beta levels in blood and tissue in severely burned rats. European Cytokine Network. 19: pp 1-7.

Bai R, Wan L, and Shi M (2008): The time-dependent expressions of IL-1β, COX-2, MCP-1 mRNA in skin wounds of rabbits. Forensic

Science International, 175(2–3): pp 193-7.

Bergquist M, Huss F, Fredén F, et al. (2016): Altered adrenal and gonadal steroids biosynthesis in patients with burn injury. Clinical Mass Spectrometry. 1: pp 19-26.

Blazak WF, Treinen KA and Juniewicz PE (1993): Application of Testicular Sperm Head Counts in the Assessment of Male Reproductive Toxicity. In: Methods in Toxicology, Vol. 3, Part A, Male Reproductive Toxicology. Chapin R. E. and Heindel J. J. (eds). Academic Press, Inc., San Diego, pp. 86–94.

Carreiraa RP, Santanaa I, Piresa MA, et al., (2012): Localization of tumor necrosis factor in the canine testis, epididymis and spermatozoa. Theriogenology. 77: pp. 1540-8.

Clark RA, Nielsen LD, Welch MP and McPherson JM (1995): Collagen matrices attenuate the collagen-synthetic response of cultured fibroblasts to TGFbeta. Journal of Cellular Science. 108(3): p 1251.

Cribbs RK, Luquette MH and Besner GE (1998): A Standardized Model of Partial Thickness Scald Burns in Mice. J Surg Res. 80: pp. 69-74.

Dorn TW, Still JM, Law E, and Still R (2001): Assault by burning – a retrospective review with focus on legal outcomes. J Burn Care Rehabil. 22(5):334–6.

Emanuele NV, LaPaglia N, Kovacs EJ and Emanuele MA (2005): The impact of burn injury and ethanol on the cytokine network of the mouse hypothalamus: Reproductive implications. Cytokine. 30(3):pp 109-15,

Fadeyibi IO, Jewo PI, Saalu L, et al., (2010): Burn severity and post-burn infertility in men. Burns. 36: pp 367-71.

Gatson J, Maass D, Simpkins J, et al. (2009): Estrogen treatment following severe burn injury reduces brain inflammation and apoptotic signaling. J Neuroinflammation. 6: p 30.

Gouma E, Simos Y, Verginadis I, et al. (2012): A simple procedure for estimation of total body surface area and determination of a new value of Meeh's constant in rats. Laboratory Animals. 46(1): pp 40–5.

Huang SB, Chang WH, Huang CH and Tsai CH (2008): Management of elderly burn patients. Int J Gerontol. 2(3): pp 91–7.

Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996): Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C, http://www.nap.edu/openbook.php. pp 21-55.

Ipaktchi K, Mattar A, Niederbichler A, et al. (2014): Attenuating Burn Wound Inflammatory Signaling Reduces Systemic Inflammation and Acute Lung Injury. Journal of immunology. 177: pp. 8065-71.

Jeschke MG, Mlcak RP, Finnerty CC et al. (2007): Burn size determines the inflammatory and hypermetabolic response. Crit Care. 11: R90.

Jewo PI, Duru FI, Fadeyibi IO, et al. (2010): The protective role of ascorbic acid in burn-induced

testicular damage in rat. Burns. 38: pp 113-9.

Jewo PI, Duru FI, Osinubi AA, et al. (2011): Histological Changes and Testicular Dysfunction in Severely Burned Rats. Burns. 4: pp 227-33.

Joslin L (2009): Blood Collection: technique in exotic small mammals. Journal of exotic Pet Medicine. 18(2): pp 117-9.

Klinefelter GR, Gray LE and Suare JD (1991): The method of sperm collection significantly influences sperm motion parameters following ethane dimethanesulphonate administration in the rat. Reprod Toxicol. 5: pp 39–44.

Kubo H, Hayashi T, Ago K, et al. (2014): Temporal expression of wound healing-related genes in skin burn injury. Legal Medicine. 16(1): pp 8-13,

Moraes AJ, Pereira RM, Cocuzza M, et al. (2008): Minor sperm abnormalities in young male post-pubertal patients with juvenile dermatomyositis. Braz J Med Biol Res. 41(12): pp 1142-7.

Peck MD (2012): Epidemiology of burns throughout the World. Part II. Intentional burns in adults. Burns. 38(5): pp630–7.

Pollanen MS (2018): The pathology of torture. Forensic Science International. 284: pp 85-96.

Prophet, EB, Mills B and Arrington JB (1992): In Sobin L.S. (ed) Laboratory Methods in Histology. Washington DC: Armed forces Institute of pathology.

Silverstein P and Lack BO (1987): Epidemiology and prevention. The Art and Science of Burn Care. Rockville. Md: Aspen Publishing. p. 11-9.

Singh RK, Mishra KB, Maurya RK, et al. (2017): Medico-social aspects of burn injuries. Asian Pac. J. Health Sci. 4(4): pp 94-97.

Tardif S, Laforest JP, Comier N et al. (1999): The importance of porcine sperm parameters on fertility in vivo. Theriogenology. 52: pp 447–59.

Tomita, Y.; Nihira, M; Ohno, Y. and Sato, S. (2004): Ultrastructural changes during in situ early postmortem autolysis in kidney, pancreas, liver, heart and skeletal muscle of rats. Legal Medicine (Tokyo). 6(1): pp 25-31.

Wilson I and Gamble M (2002): The hematoxylins and eosins, In: Theory and practice of histological techniques, Bancroft JD and Gamble M (eds.), 5th ed., Churchill Livingston, Elservier Science Limited, London, UK, pp. 125-38.

Winter GD (1975): Histological aspects of burn wound healing. Burns. 1(3): pp 191-6.

Yang Q, Berthiaume F and Androulakis P (2011): A quantitative model of thermal injury-induced acute inflammation. Mathematical Biosciences. 229(2): pp 135-48.

Yongqiang X, Fang Z and Zhaofan X (2016): A Mouse Model of Scald Wounds. Austin J Emergency & Crit Care Med. 3(2): pp 1047-53.

الملخص العربي

تقدير عمر إصابات الحروق لدراسة التأثير المحتمل على وظيفة الخصية لذكور الجرذان البيضاء

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الخلفية: من وجهة النظر الطبية الشرعية ، يجب تحديد العواقب طويلة الأمد لإصابات الحروق بالسوائل الساخنة من أجل تحديد التعويض المناسب والإجراء القانوني ، خاصةً لإصابة الحرارية التي تتضمن سمك كامل للجلد في 20٪ من إجمالي مساحة سطح الجسم. وهو المعروف أن لها عواقب خطيرة على المدى الطويل على الصحة العامة. **الهدف من الدراسة:** كان الهدف من هذا العمل التجريبي هو التعرف على التغيرات النسيجية والتناسلية المناعية لتحديد عمر الاصابات الحرارية التي تشغل 20٪ من إجمالي مساحة سطح الجسم. الى جانب ذلك ، تم دراسة التأثير طويل المدى لهذا الاصابة على الوظائف التناسلية لذكر الجرذان البيضاء. **المواد والطرق:** تم استخدام عدد 40 من ذكور الجرذان البيضاء في الدراسة. أجريت فحص فى نهاية اليوم الثاني، اليوم السابع، الشهر الأول، والشهر الثالث من الاصابة تم ذبح الفئران لاخذ جزء الجلد المصاب بالحرق والخصيتين لعمل دراسة هيستوباثولوجية باستخدام المجهرالضوئي ، ودراسة حيوية وعدد الحيوانات المنوية، وقياس مستوي هرمون التستوستيرون. وتم عمل دراسة هيستوكيمائية مناعية لقطاعات من الجلد والخصية للكشف عن البروتين عامل نخر الورم (تي-إن-إيه). **النتائج:** لوحظت تغيرات نسيجية تقدمية في حين بدأت مظاهر الشفاء والتحسن تظهر بعد 1 و 3 أشهر من الاصابة. **الاستنتاج:** يمكن استخدام التغييرات الهستوباثولوجية والهيستوكيمائية المناعية للإصابة في تحديد عمر حرق يشمل 20٪ من إجمالي مساحة سطح الجسم والذي قد يتسبب ذلك في حدوث اختلال علي المدي البعيد في الوظيفة التناسلية للذكور.