WOUND AGE ESTIMATION: PRO-INFLAMMATORY CYTOKINES VERSUS IMMUNO-HISTOCHEMISTRY

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Abstract:

Rationale and background: In forensic medical investigations, it is an important matter to determine whether a wound found on autopsy was inflicted before or after death and, if inflicted ante-mortem, how long before death it was sustained. **Aim:** This work was to compare the biochemical inflammatory cytokines (IL1-β and IL6) and immunohistochemical (TGF-α) techniques as tools for determining wound aging in cadavers and their relation to other factors related to the wound. **Subjects and methods:** Specimens of skin and subcutaneous tissue were taken from 50 cadavers with wounds other than firearms and with known postmortem intervals. They were 11 females (22%) and 39 males (78%), and their mean age was 30.3±19.88 years, with a minimum age of 4 months and a maximum of 82 years. Wound specimens were taken from every case to estimate IL1-β and IL6 by ELISA technique and evaluate TGF-α by immuno-histochemistry. **Results:** Significant correlations between the age of the victim and the value of both IL6 and IL1-β were reported. Moreover, no significant difference between males and females concerning the cytokines IL1-β and Cytokines IL6 was found. There was no significant correlation between the time between injury and death and both IL6 and IL1-β values, but there was a significant negative correlation between the time between death and autopsy and the value of IL1-β. However, a similar correlation was not detected concerning IL6. So, TGF-α and IL6 were independent predictors for wound age determination, while IL1-β was a dependent one.

In this study, the estimation of the wound age, the time between injury and death, and the time between death and autopsy could be calculated using specific regression equations. **Conclusion and recommendations:** The quantitative analysis of pro-inflammatory cytokines in wound extracts can contribute to determining vitality and wound age.

INTRODUCTION

In forensic medical investigations, it is crucial to identify whether an autopsy wound was inflicted before or after death, and if so, how long before death it was sustained. Biological variability provides a large margin of uncertainty, allowing for a range of probability but never an exact time span. The alterations will also differ depending on the extent of the wound, the kind of lesion (bruise, abrasion, incision, or laceration), the tissue (epidermal or mesodermal), the presence of infection, and the victim's age and condition **(Saukko & Knight, 2004)**.

Antibodies have evolved into a potent diagnostic tool to identify specific antigens in situ. Immunohistochemistry is becoming more critical in pathology and the diagnostic pathology of several organ systems due to the wide availability of immunological markers. **(Buchwalow & Bocker, 2010).**

Immunoassays are tests in which antibodies are used as reagents. Enzyme immunoassays utilize enzymes connected to one of the reactants in an immunoassay to enable quantification by color development following the addition of an appropriate substrate/chromogenic. As previously stated, ELISAs include the sequential addition and reaction of reagents to a solid phase-bound material, followed by incubation and washing processes to separate bound and free reagents. **(Crowther, 1995).**

Transforming growth factor-α (TGF-α) is a polypeptide growth factor that belongs to a family of ligands that bind the epidermal

growth factor receptor (EGFR) **(Harris et al., 2004).** It functions as a pleiotropic effector molecule embryonic development and pathological processes, including wound healing and cancer progression. The ligation of EGF and TGF- to the EGF receptor initiates the various actions of EGF and TGF- on dermal fibroblasts. **(Ellis et al., 2007)**.

The pro-inflammatory factors interleukin 1-β (IL1-β) participate in the early stage of wound healing (inflammation). They may persist or reappear in the two following periods (formation of granulation tissue and remodeling). Numerous cell types, including macrophages, release pro-inflammatory cytokines, endothelial cells, and keratinocytes **(Grellner, 2005).**

This work aimed to compare the biochemical (inflammatory cytokines IL1-β and IL6) and the immunohistochemical (TGF-α) techniques, as tools for determining the wound aging in cadavers (whether antemortem or postmortem) and their relation to other factors related to the wound.

SUBJECTS AND METHODS:

Fifty Specimens of skin and subcutaneous tissue (1×1 cm) were excised from 50 cadavers. They were obtained from the forensic autopsy room, Zenhom morgue, Ministry of Justice, Cairo. These wounds were (stab wounds, incised wounds, and lacerations) excluding firearm wounds and malignant tumors. The present study included 11 females (22%) and 39 males (78%) aged between 4 months and 82 years.

The skin samples were taken parallel to the wound margin.

These wounds had known post-infliction intervals (wound age) ranging from 0.5- 48 hours.

This post-infliction interval (wound age) was divided into two periods of time:

1) The time between injury and death ranged between 0.5 and 24 hours
2) The time between death and autopsy ranged between 1.5 and 24 hours

For every specimen, the following data were collected and tabulated: Age and gender of the cadaver, history of widespread disease, the interval between injury and death, the time between death and autopsy, type of wound, and presence of other wounds. Some exclusion criteria included firearm wounds, severe malnutrition, malignant diseases, metabolic disorders, history of receiving cytotoxic agents or corticosteroids that may influence wound healing.

The wound specimen was divided into two halves:

- The first specimen was preserved in a deep freeze at –27ºC until subsequent biochemical estimation of IL-6 & IL-1β levels using the ELISA Technique with the use of a kit supplied by Quantakine R & D system (USA) **(Growther, 2005)**.
- The second half was fixed in 10% formaldehyde solution with phosphate-buffered saline (PBS at ph 7.2) and then was embedded in paraffin blocks for the subsequent immune-histochemical study of (TGF-α) by radioimmunoassay (RIA) provided by Keith Mostov, UCSF according to **Franklin et al. (2005)**.

Statistical analysis:

The data was examined using the SPSS statistical software version 15. The information was presented quantitatively as mean SD, median, minimum, and maximum. We utilized the frequency and percentage of qualitative data. To compare groups, the non-parametric Kruskal Wallis Anova test was employed, followed by Mann Whitney "U" tests if significant. To analyze the link between different numerical variables, Spearman's non-parametric correlation was utilized. The Chi-square test was used to evaluate the association between qualitative factors. The wound age was calculated using regression models based on the collected data. The significance threshold was chosen at p less than 0.05.

RESULTS

The present study included 11 females (22%) and 39 males (78%)**.** The individual ages varied from 4 months to 82 years, with a mean of 30 years and three months 19.88. There were six instances (12%) in the first decade, 16 (32% in the second decade), 8 (16%) in the third decade, 8 (16%) in the fourth decade, 3 (6%) in the fifth decade, 5 (10%) in the sixth decade, and one (2%) in the seventh decade, 2 (4%) in the eighth decade and one (2%) in the ninth decade.

For estimating time intervals between injury and death in the studied cases; There were 20 cases (40%) in the time interval A (up to 30 minutes), 13 cases (26%) in the time interval B (31-60 minutes), 10 (20%) in the time interval

C (61-120 minutes) and 7 cases (14%) in the time interval D (more than 120 minutes) **(Table 1)**.

Table (1): Time interval between injury and death

Time	Frequency	Percent
Up to 30 min	20	40.0
31—60 min	13	26.0
61—120 min	10	20.0
> 120 min	7	14.0
Total	50	100.0

Considering the type of wound in the studied cases, there were 19 cases (38%) with contused wounds, 10 cases (20%) with cut wounds, and 21 (42%) with stab wounds **(Table 2).**

Table (2): Number and percentage of different types of wounds

Type of wound	Frequency	Percent
Contused	19	38.0
Cut	10	20.0
Stab	21	42.0
Total	50	100.0

As regards the sites of wounds in the studied cases, there were 7 cases (14%) with injuries in the abdomen, 2 cases (4%) in the arms, 1 case (2%) in the back, 11 cases (22%) in the chest, 7 cases (14%) in the face, 6 cases (12%) in the forehead, 7 cases (14%) in hands, 1 case (2%) in the head, 1 case (2%) in one leg, 4 cases (8%) in the neck and 3 cases (6%) in the thighs.

The descriptive data and cytokine levels of the studied subjects are shown in **(Table 3)**:

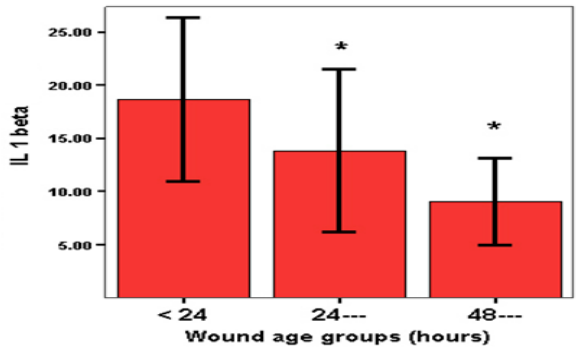

Fig. 1: showing the relation between the different wound age and the IL-1β

* Indicate statistical significant difference between group 1 (< 24 hours) and the other 2 groups regarding the IL-1β level.

Table (3): Descriptive data and cytokine levels of the studied subjects

	Age	Time between injury and death (hours)	Time between death and autopsy (hours)	IL 6	IL 1-β
N	50	50	50	50	50
Mean	30.30	1.98	24.49	30.89	15.20
Std. Deviation	19.88	4.03	8.13	17.89	7.92
Median	26.50	1.00	24.00	30.05	16.60
Minimum	0.04	.10	1.50	8.10	1.60
Maximum	82.00	24.00	48.00	88.20	29.50

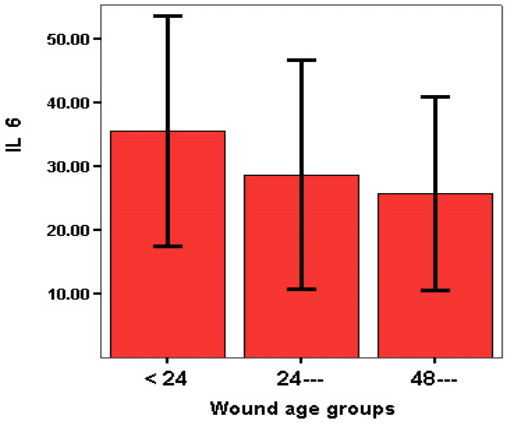

Fig 2: showing the relation between the different wound age and the IL-6 with no statistically significant difference between the different groups regarding the IL-6 levels.

<u>Immunohistochemical parameter</u>:

Concerning TGF-α; it was found (positive) in 26 cases (52%) (Fig. 4) & not found (negative) in 24 cases (48%) (Fig. 3).

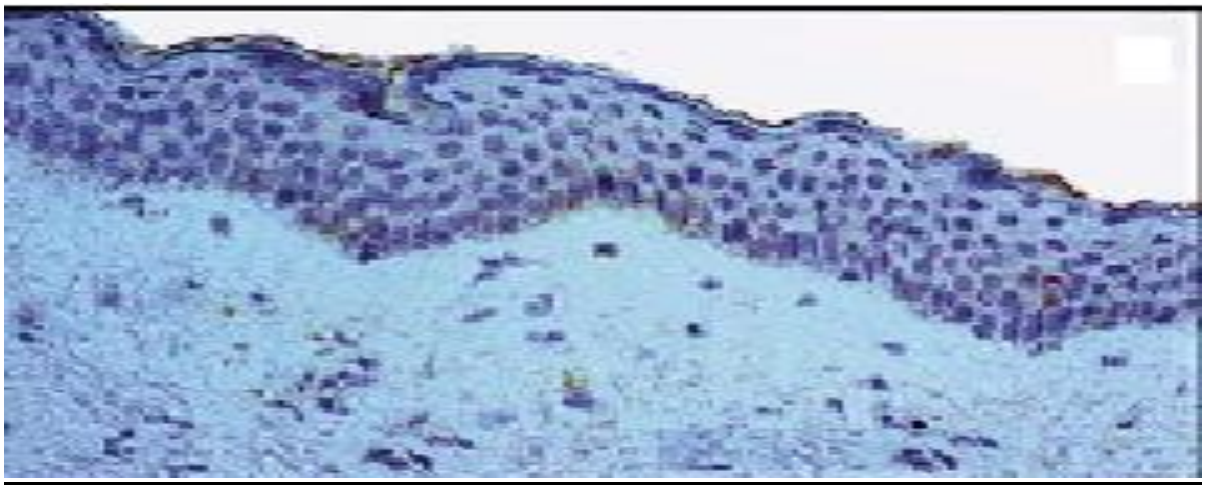

Figure (3): TGF-α: shows no immune-reaction in the median epidermis (negative reaction)

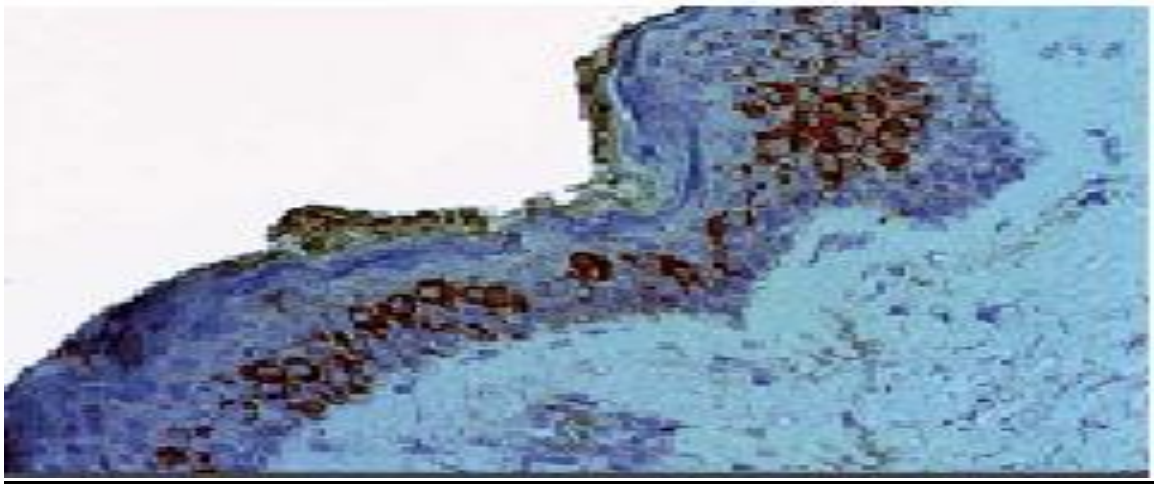

Figure (4): TGF-α: shows increase in immune-reaction in the median epidermis (positive reaction).

Sex difference and TGF-α status:

It was found that TGF-α was negative in 4 females and 20 males and was positive in 7 females and 19 males. Chi-Square There was no correlation between sex and the test results and TGF-α level **(Table 4).**

Table (4): Chi-Square test between gender and the TGF-α

		Gender		Total
		Female	Male	
TGF alpha	negative Count	4	20	24
	% within gender	36.4%	51.3%	48.0%
	positive Count	7	19	26
	% within gender	63.6%	48.7%	52.0%
Total Count		11	39	50
% within gender		100.0%	100.0%	100.0%

Relation between wound ages and TGF-a(Table5):

Table (5): TGF-a status according to the different wound ages (H)

			TGF alpha		Total
			negative	positive	
Wound Age_ Groups (in hours)	<24	Count	10	8	18
		% within TGF alpha	41.7%	30.8%	36.0%
	24	Count	13	15	28
		% within TGD alpha	54.2%	57.7%	56.0%
	48	Count	1	3	4
		% within TGF alpha	4.2%	11.5%	8.0%
Total		Count	24	26	50
		% within TGF alpha	100.0%	100.0%	100.0%

There was no association between the wound age and TGF-α **(Table 6).**

Table (6): Chi–square test between the wound age and TGF-α.

	Value	Asymp. Sif. (2-sided)
Pearson Chi-square	1.287	.525

The relation between type of wound and TGF-α was shown in **(Table 7):**

Table (7): Relation between type of wound and TGF-α

		Type of wound			Total
		Contused	Cut	Stab	
TGF alpha	negative Count	8	7	9	24
	% within Type of wound	44.4%	70.0%	40.9%	48.0%
	positive Count	10	3	13	26
	% within Type of wound	55.6%	30.0%	59.1%	52.0%
Total Count		18	10	22	50
% within Type of wound		100.0%	100.0%	100.0%	100.0%

Chi-Square analysis showed no correlation between TGF- levels and the kind of wound, including cuts, contusions, or stabbings. **(Table 8).**

Table (8): Chi-Square test between type of wound and TGF-α

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.473a	2	.290
Likelihood Ratio	2.520	2	.284
N of Valid Cases	50		

The relation between the time interval between injury and death and TGF-α was shown in **(Table 9).**

Table (9): relation between time interval between injury and death and TGF-α levels.

			TGF alpha		Total
			negative	positive	
Time between injury and death group	Up to 30	Count	18	2	20
		% Within TGF alpha	75.0%	7.7%	40.0%
	31-60	Count	6	7	13
		% Within TGF alpha	25.0%	26.9%	26.0%
	61-120	Count	0	10	10
		% Within TGF alpha	.0%	26.9%	14.0%
	>120	Count	0	7	7
		% Within alpha	.0%	26.9%	14.0%
		Count	24	26	50
Total		% Within alpha	100.0%	100.0%	100.0%

There was a significant association between time between injury and death and TGF-α ($p<0.000^{**}$) **(Table 10).**

Table (10): Chi–square test between time between injury and death and TGF-α.

	Value	Asymp. Sig. (2-sided)
Pearson Chi-Square	**29.845**	**.000**

The relation between the time interval between injury and death and cytokines level was shown in **(Table 11)**.

Table (11): Cytokines level according to time interval between injury and death.

Time between injury and death		IL 6	IL 1-β
Up to 30	N	20.00	20.00
	Mean	33.89	13.94
	Std. Deviation	21.36	6.62
	Median	32.15	16.30
	Minimum	8.10	2.40
	Maximum	88.20	24.30
31—60	N	13.00	13.00
	Mean	32.29	18.95
	Std. Deviation	13.76	7.64
	Median	33.40	20.30
	Minimum	8.50	2.90
	Maximum	54.20	27.50
61—120	N	10.00	10.00
	Mean	24.10	14.39
	Std. Deviation	14.45	8.72
	Median	21.50	17.00
	Minimum	8.60	1.60
	Maximum	52.70	24.20
> 120	N	7.00	7.00
	Mean	29.44	13.00
	Std. Deviation	19.04	9.99
	Median	28.20	10.30
	Minimum	8.60	2.70
	Maximum	56.30	29.50

Non-parametric Correlations (Spearman's rho) between the investigated parameters were presented in **(Table 12):**

Table (12): Correlations between the investigated parameters in the studied subjects

			IL 6	IL 1-β
Spearman's rho	**Age**	**Correlation Coefficient**	**-.164**	**.069**
		Sig. (2-tailed)	**.256**	**.632**
		N	**50**	**50**
	Time between injury and death (hours)	**Correlation Coefficient**	**-.152**	**.025**
		Sig. (2-tailed)	**.292**	**.863**
		N	**50**	**50**
	Time between death and autopsy (hours)	**Correlation Coefficient**	**-.230**	**-.390(**)**
		Sig. (2-tailed)	**.108**	**.005**
		N	**50**	**50**
	IL 6	**Correlation Coefficient**		**.490(**)**
		Sig. (2-tailed)		**.000**
		N		**50**
	IL 1 beta	**Correlation Coefficient**	**.490(**)**	
		Sig. (2-tailed)	**.000**	
		N	**50**	
**** Correlation is significant at the 0.01 level (2-tailed).**				

-There was a strong negative association between the time between death and autopsy. And the value of IL1-β (r= -0.390, p< 0.005**); however, a similar correlation was not detected concerning IL6.

No statistical significant difference was found between the time between injury and death and, both IL6 and IL1-β **(Table 13)**.

Table (13): Kruskal Wallis test between time interval between injury and death and, both IL6 and IL1-β.

	IL 6	IL 1-β
VALUE	**1.935**	**4.396**
P-VALUE	**.586**	**.222**
a Kruskal Wallis Test		
b Grouping Variable: Time between injury and death groups		

There was no statistical significant difference between males and females concerning the IL1-β and IL6 **(Table 14)**.

Table (14): Mann-Whitney test between gender and both investigated cytokines

	IL 6	IL 1-β
Mann-Whitney U	**210.000**	**175.000**
Z	**-.105**	**-.925**
p-value	**.916**	**.355**

There was no statistical difference between the different wounds types and the investigated cytokines **(Table 15).**

Table (15): Kruskal-Wallis test between the type of wounds and the investigated cytokines

	IL 6	IL 1-β
Value	**1.841**	**3.655**
p-value	**.398**	**.161**

Regression analysis:

Regression analysis revealed that TGF-α was an independent predictor for time interval between injury and death while, IL1-β and IL6 were dependent predictors **(Tables 16, 17 and 18)**.

a. Dependent Variable: Time between injury and death

Table (16): Model Summary

Model	R Square
1	.557

Table (17): ANOVAb

Model	F	Sig.
Regression	19.303	.000a

a. Predictors: (Constant), TGF-α, Cytokines IL 1-β, Cytokines IL 6
b. Dependent Variable: Time interval between injury and death group

Table (18): **Regression analysis between the time interval between injury and death and, IL1-β, IL6 and TGF-α**

Model	Unstandardized Coefficients		t	Sig.	95% Confidence Interval for B	
	B	Std. Error			Lower Bound	Upper Bound
1 (Constant)	**-.165**	**.423**	**-.390**	**.698**	**-1.017**	**.687**
IL 6	**-.001**	**.007**	**-.222**	**.826**	**-.015**	**.012**
IL 1-β	**-.009**	**.015**	**-.620**	**.539**	**-.038**	**.020**
TGF alpha	**1.597**	**.215**	**7.437**	**.000**	**1.165**	**2.029**

Regression analysis:

Regression analysis revealed that TGF-α and IL1-β were independent predictors for wound age while IL6 was a dependent predictor **(Tables 19, 20 and 21)**.

a. Predictors: (Constant), TGF-α, Cytokines IL 1-β, Cytokines IL 6

b. Dependent Variable: wound age

a. Dependent Variable: wound age

Regression equations were constructed to obtain the value of the unknown wound age when one or more of these cytokines is known as follows:

-Time interval between injury and death = 1.597− 0.001 * IL6 − 0.009* IL1-β − 0.165 (if TGF is + ve).

-Time between death and autopsy = 2.919− 0.011 * IL6 −0.017 *IL1-β −1.862 (if TGF is + ve).

- Wound Age =22.997 + 6.077 *1− 0.343 * IL1-β − 0.018 *IL6 (if TGF is + ve).

Table (19): Model Summary

Model	R Square
1	**.198**

Table (20): ANOVAb

Model	F	Sig.
1 Regression	**3.776**	**.017a**

Table (21): **Regression analysis between the wound age and, IL1-β, IL6 and TGF-α**

Model	Unstandardized Coefficients		t	Sig.	95% Confidence Interval for B	
	B	Std. Error			Lower Bound	Upper Bound
1 (Constant)	**22.997**	**4.851**	**4.741**	**.000**	**13.232**	**32.761**
IL 6	**-.018**	**.075**	**-.238**	**.813**	**-.168**	**.132**
IL 1-β	**-.343**	**.167**	**-2.053**	**.046**	**-.679**	**-.007**
TGF alpha	**6.077**	**2.460**	**2.470**	**.017**	**1.125**	**11.030**

DISCUSSION:

In forensic medical investigations, it is critical to ascertain whether a wound seen at autopsy was inflicted before or after death, and if so, how long before death it was received. Histochemical and immunohistochemical approaches have been extensively investigated in recent years, mostly on animals, with certain examples raising concerns about their applicability to humans. A broad range of indicators have been reported to be reliable for both differentiating antemortem and postmortem injuries as well as dating ante-mortem wounds. **(Saukko and Knight, 2004).**

Cutaneous wound healing is a complicated process with several mechanical factors influencing the quality and speed of healing. The function of mechanical forces in controlling tissue development, healing, and remodelling was discovered more than a century ago. Such pressures impact gene expression, the manufacture of growth factors and inflammatory mediators, and cellular activities such as the proliferation of numerous load-sensitive cells. **(Agha et al., 2011).**

FGFs (fibroblast growth factors) are a diverse group of polypeptide growth factors that play important roles in cell signalling, proliferation, and differentiation (Finch and Rubin, 2004). A number of growth factors and cytokines have been discovered, including transforming growth factor alpha (TGF-), interleukin-1b (IL-1b), platelet-derived growth factor-BB (PDGF-BB), and tumour necrosis factor- (TNF-) to increase keratinocyte growth factor (KGF) production. (**Wang et al., 2010).** In Forensic clinical medicine, the causal link The relation between death and any wounds must be evaluated, and forensic pathologists must always distinguish between antemortem and postmortem damage. Furthermore, when the wound is serious, determining how long it was there prior to death is critical. **(Kondo and Ishida, 2010).**

Concerning the reported demographic data and regarding the number and percentage of each gender in the studied cases, there were 39 males (78%) and 11 females (22%). This was in accordance with **(Ibrahim, 2007)** who found that the number of male victims was significantly higher (64%) than that of female victims.

Similarly, Coyne-Beasley et al. (2005) found that there is a gender discrepancy in firearm ownership reporting in terms of the quantity and kind of guns held.

Men in the United States are roughly twice as likely as women to be hospitalised with a brain injury. This masculine hegemony was reported by Olson **(2005)** to be worldwide.

Considering the number and percentage of each body-built status of the studied cases, there were 26 of cases (52%) of average built, 9 cases (18%) fatty, 3 cases (6%) muscular and 12 cases (24%) skinny.

This was in accordance with **Flower et al. (2003)** who The magnitude of the basal release was shown to be the most remarkable differential in IL-6 Even in these non-obese patients, primary biliary cirrhosis released more than adipose tissue and adipocytes. They discovered a two-fold increase in IL-6 levels across the subcutaneous adipose tissue bed in a study that included both men and women with a higher BMI and percentage body fat. They discovered significant basal release of IL-6 from abdominal subcutaneous adipose tissue in these people, but only a 30% rise in the venous sample compared to the arterial sample. Similarly, **Genton et al. (2012)** concluded that, a low fat-free mass index is an independent risk factor of mortality in elderly subjects, healthy at the time of body composition measurement.

Regarding the cause of death in the studied cases in the present work, it was found that 33 cases (66%) died from hemorrhage, one case (2%) died from cardiac tamponade, one case (2%) died from cutthroat, 2 cases (4%) died from gunshot, 13 (26%) died from brain injury, one case (2%) died from smothering and one case (2%) died from strangulation.

As regards the percentage of general diseases in the studied cases; there were 8 cases (16%) with coronary heart disease (CHD), one case (2%) with diabetes mellitus (DM), one case (2%) with hypertension, 2 cases (4%) had both (DM) and hypertension, one case (2%) with rheumatic heart disease (RHD) and 37 cases (74%) with no general disease.

In general, a wide range of natural disorders may impact the levels of pro-inflammatory cytokines (mainly systemic). The local determination of growth factors and wound mediators in skin wounds, particularly healing; The biochemical features of growth factors and their relationship to normal skin of the same person theoretically prohibit

theoretically feasible falsification healing **(Grellner et al., 2000).**

For estimating time intervals between injury and death in the studied cases; There were 20 cases (40%) in the time interval A (up to 30 minutes), 13 cases (26%) in the time interval B (31-60 minutes), 10 cases (20%) in the time interval C (61-120 minutes) and 7 cases (14%) in the time interval D (more than 120 minutes).

Considering the type of wound in the studied cases; there were 19 cases (38 %) with contused wounds, 10 cases (20%) with cut wounds and 21 (42%) with stab wounds.

As regards sites of wounds in the studied cases, there were 7 cases (14%) with injuries in the abdomen, 2 cases (4%) in arms, 1 case (2%) in the back, 11 cases (22%) with injuries in the chest, 7 cases (14%) with injuries in the face, 6 cases (12%) with injuries in forehead, 7 cases (14%) with injuries in hands, 1 case (2%) with injury in the head, 1 case (2%) with injury in a leg, 4 cases (8%) with injuries in the neck and 3 cases (6%) with injuries in the thighs.

Regarding the presence of other wounds it was found that, in 1 case (2%) there was a cut wound; in 1 case (2%) there was another contused wounds in the scalp; in 1 case (2%) there was contusion; in 4 cases (8%) few contused wounds in the scalp; 1 case (2%) with few stabs; 3 cases (6%) with few cut wounds; 1 case (2%) with inlet and exit gunshot wounds; 1 case (2%) with inlet wound; 2(4%) with multiple contusions; 4 cases (8%) with multiple contusions and fractures; 17 cases (34%) with multiple cut and stab wounds; 2(4%) with multiple cut wounds; 3 cases (6%) with multiple stabs; 1 case (2%) with trephine; 1 case (2%) had two cut wounds and rope mark and 7 cases (14%) without any other injuries .

Regarding the age of victims in this study, It varied from 4 months to 82 years, with a mean of 30.3 ± 19.88 years.

Similarly, **Kondo et al. (2002**) detected, in their study, chemokines in human skin wounds on individuals with ages at death ranged from 7 to 77 years (with a mean age of 48.8 years).

On the same ground**, Belmont et al. (2012)** There were 29.624 unique combat wounds in 7.877 combat casualties among the 1.992.232 military service men serving in Iraq and Afghanistan. The combat casualty group had a mean age of 26.0 years. The majority of battle casualties were men. (98.8%).

These findings were nearly similar to those of the study of **(Pang and Skikui (1995)** where the average of the victims' age was found to be 26.9 years. Moreover, there were 6 cases (12%) in the first decade, 16 (32%) in the second decade, 8 (16%) in the third decade, 8 (16%) in the fourth decade, 3 (6%) in the fifth decade, 5 (10%) in the sixth decade, one (2%) in the seventh decade, 2 (4%) in the eighth decade and one (2%) in the ninth decade.

In **2007, Ibrahim** found that the vast majority of victims were in the 2nd decade (9 cases), 3rd decade (14 cases) and 4th decade (10 cases) and the least number of cases were found in the 6th and 7th decades (3 cases each).

For estimation of time between injury and death, it ranged between 0.1 and 24 hours with a mean of 1.98 ± 4.03 hours, the median time was 1 hour.

Considering time between death and autopsy, it ranged between 1.5 and 48 hours with a mean of 24.49 ± 8.13 hours, the median time was 24 hours.

FGFs (fibroblast growth factors) are a diverse group of polypeptide growth factors involved in cell signaling, proliferation, and differentiation. KGF/FGF-7 (keratinocyte growth factor/fibroblast growth factor-7) was discovered to be secreted by mesenchymal cells and to have a mitogenic impact on keratinocytes. In a wound healing setting, KGF is created through a paracrine interaction between fibroblasts and keratinocytes. A number of growth factors and cytokines have been identified as capable of increasing KGF expression, including transforming growth factor alpha (TGF-), interleukin-1b (IL-1-b), platelet-derived growth factor-BB (PDGF-BB), and tumour necrosis factor- (TNF-) **(Wang et al., 2010).**

TGF-α does not affect epithelial morphology nor does it affect the distribution of the Triton soluble or -insoluble pool of E-cadherin **(Chung et al., 2005).** TGF-α induce an equipotent stimulation of fibroblast migration and proliferation. (TGF-α) has been shown to stimulate adult stem cell VEGF production **(Herrmann et al., 2012).**

Concerning TGF-α in the current study; it was present (positive) in 26 cases (52%) & absent (negative) in 24 cases (48%).

Furthermore, TGF-α was positive in 7 females and 19 males with the total number of 26 cases while TGF-α was negative in 4 females and 20 males with the total number of 24 cases. Chi-Square test revealed that there was no association between sex and TGF-α levels.

These results were in accordance with those of **Domenick & Saqib (2012**) They observed that their results in limb salvage do not seem to be negatively influenced by gender or age, with amputations happening less commonly in octogenarians. Women seem to have comparable results to men after tibial artery endovascular intervention but may need many operations to attain parity with men.

TGF- and TGF-b1 have roles in wound healing. TGF-, on the other hand, serves a distinct biological function than TGFb1. TGF- stimulates the proliferation of epidermal cells like epidermal growth factor (EGF), while TGF-b1 is a powerful fibrogenic growth factor required for the deposition of extracellular matrices like collagen fibres.

For the relationship between wound age and TGF-α; it was found that TGF-α was negative in 10 cases and positive in 8 cases in wound age less than 24 hours, with total number of 18 cases. In case with wound age between 24 and 48 hours, TGF-α was negative in 13 cases and it was positive in 15 cases, with total number of 28 cases. At wound age more than 48 hours TGF-α was negative in 1 case and it was positive in 3 cases, with total number of 4 cases.

Chi-square test found that there was no connection between the wound age and TGF-α

This disagreed with **Grellner et al. (2000).** They detected that the quantitative analyses of pro-inflammatory cytokines differentiated There was a significant difference between the wound samples from surgical treatments and those from postmortem investigations. They discovered that this quantitative approach had benefits, particularly in the very early post-traumatic phase of less than 5 minutes.

Concerning the relation between the type of the wound and TGF-α, it was found that TGF-α was negative in 24 cases (48%) of the total number of cases; 8 (44.4%) cases were contused wounds, 7 (70%) cases were cut wounds and 9 (40.9%) cases were stab wounds.

However, TGF-α was positive in 26 cases (52%) of the total number of cases; 10 (55.6%) cases were contused wounds, 3 (30%) cases were cut wounds and 13 (59.1%) cases were stab wounds.

Regarding the type of wounds 70% of the cut wounds were negative to TGF-α and 30% of the cut wounds were positive to TGF-α. While, 49.1% of the stab wounds were negative TGF-α and 50.1% of the contused wounds were positive to TGF-α. According to the Chi-square test, there was no association between type of wound whether cut, contused or stab and TGF-α.

This result was not in accordance with **Grellner et al. (2000).** They reported significant increase in cytokine having the lowest survival time in stab and incised wounds As a result It made logical to evaluate the cytokine response in the skin directly using wounds. by sharp force, according to their findings.

Concerning the relation between time between injury and death and TGF-α it was found that:

-In time interval up to 30 minutes, there were 18 cases (75%) with negative TGF-α and 2 cases (7.7%) with positive TGF-α.

- With time interval from 31 to 60 minutes, there were 6 cases (25%) with TGF-α negative and 7 cases (26.9%) with TGF-α positive.

- With time interval from 61 to 120 minutes, there were no cases with TGF-α negative (0%) and 10 cases (38.5%) with TGF-α positive so, all cases were positive for TGF-α in this time interval.

- With time interval more than 120 minutes, there were no cases with TGF-α negative (0%) and 7 cases (26.9%) with TGF-α positive so, they were all positive for TGF-α.

There was a significant association between time between injury and death and TGF-α value ($p<0.000^{**}$). This finding was a very precious and valuable one being used for the estimation of wound age.

Similarly, **Kondo (2007)** reported that the TGF-α the expression was increased during the first hour following damage, indicating that they may be utilized to determine wound liveliness.

Interleukin 1 (IL-1) and IL-6, as well as tumor necrosis factor (TNF), all play essential roles in the stimulation of inflammatory and fibroblastic responses. In forensic pathology, the expression of these biological molecules in

skin wounds was used to determine wound age. **(Rebolledo et al., 2000).** IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-α and IL-1, and activation of IL-1ra and IL-10 **(Febbraio and Pedersen, 2005).**

The current investigation discovered substantial associations between the victim's age and the levels of both IL6 and IL1-.

These findings were somewhat consistent with those of **Yasui et al. (2008),** who discovered that blood levels of IL-4 and MCP-1 in postmenopausal women were considerably greater than in perimenopausal women (p0.05*). Other serum cytokine concentrations did not alter substantially between premenopausal and postmenopausal women. As regards the relations between the gender of victim and cytokines; it was found that there was no significant difference between male and female concerning the cytokines IL1-β and Cytokines IL6.

This was in accordance with **Duckworth et al. (2012)** who decided that For radial head and neck fractures, male patients had a younger mean age than female patients, with no discernible gender preponderance. The mechanism of injury was influenced by gender, with female patients often suffering their fracture after a low-energy fall.

This was also in accordance with **Grellner et al. (2000)** who found their results statistically independent of age, gender, anatomical site or postmortem interval.

Furthermore, this result was in accordance with **Domenick & Saqib (2012)** who concluded that Gender or age seem to have little effect on limb salvage results, which tend to be equal in octogenarians, with amputations happening less often. Women tend to have comparable results as males after tibial artery endovascular intervention, but may require repeated interventions to achieve equivalent.

About the relation between time interval between injury and death and, interleukin 6 and interleukin 1-β in the studied cases it was found that:

-When the time interval was up to 30 minutes (time interval A), the IL6 levels ranged between 8.10 and 88.20 with a mean value of 33.89 ± 21.36. In this time interval the mean value of interleukin 1-β ranged between 2.40 and 24.30 with a mean of 13.94 ± 6.62.

-When the time interval was up to 31-60 minutes (time interval B), the IL6 levels ranged between 8.50 and 54.20 with a mean value of 32.29 ± 13.76. In this time interval, the IL1-β levels ranged between 2.90 and 27.50 with a mean value of 18.95 ± 7.64.

-When time interval was up to 61-120 minutes (time interval C), the value of interleukin 6 ranged between 8.60 and 52.70 with a mean of 24.10 ± 14.45. In this time interval, IL1-β ranged between 1.60 and 24.20 with a mean value of 14.39 ± 8.72.

-When time interval was more than 120 minutes (time interval D), the interleukin 6 levels ranged between 8.60 and 56.30 with a mean value of 29.44 ± 19.04. In this time interval, the interleukin 1-β levels varying between 2.70 and 29.50 with a value mean equals 13.00 ± 9.99.

Thus according to the non-parametric Kruskal Wallis test, no statistical significant difference was found between the time between injury and death and both IL6 and IL1-β values.

This finding partially disagrees with **Kondo et al. (1999)** who performed immuno-histochemical and Morphometric analysis of the temporal expression of interleukin-1a (IL-1a) on 40 different ages of human skin wounds In wounds, polymorphonuclear neutrophils mainly responded positively to IL-1a immunohistochemically. aged 4 h to 1 day, but neutrophils were no longer detectable as wound age increased, and macrophages and fibroblasts were positively stained. The IL-1a-positive cell ratio increased significantly in wound specimens aged 4 hours to 1 day, with a maximal ratio of 46.5 percent in a 7 hold wound. The mean value of the IL-1a-positive ratio in 10 wound specimens with wound ages ranging from 4 h to 1 day was 32.8 9.7 percent. In most instances, the ratio of IL-1a-positive cells steadily fell to less than 30% in wounds aged 1.5 to 21 days, with a mean value of 17.5 7.2 percent (n = 27). These findings imply that IL-1a-positive cell ratios more than 30% indicate a post-infliction period of one day or fewer. In the current study, when we studied the relation between the time between death and autopsy and the cytokines level and when a nonparametric correlation (Spearman's rho) was performed it was found that:

-There was a significant negative correlation between time between death and autopsy and the value of IL1-β (r= -0.390, p< 0.005**) however, similar correlation was not detected concerning IL6.

-Furthermore, there was a significant positive correlation between IL6 and IL1-β levels (r= 0.490, p< 0.000**)

Concerning type of wound in relation to Cytokines IL 6 and Cytokines IL1 -β; Kruskal Wallis test revealed that there was no significant difference between type of wound whether cut, contused or stab and IL1-β or between type of wound and IL 6.

The results of the present study pointed to the beneficial effect and usefulness of the pro-inflammatory cytokines, using histochemical studies, for either estimation of the time between injury and death (TGF-α) or the time between death and autopsy (IL1-β) as well as for wound age determination in general.

Since there was an association between time between injury and death and TGFα level thus, TGFα is a suitable immunohistochemical tool or marker for the estimation of the early part of the wound age (between injury and death) in the studied period.

Similarly, IL1-β was a suitable histochemical marker for the estimation of the late part of the wound age (between death and autopsy), in the studied period, being negatively correlated with the time between death and autopsy.

Furthermore, both TGFα and IL1-β were found to be independent predictors for wound age determination while IL 6 was a dependent one.

Similarly, Kondo et al. (2002) Through morphometrical analysis in their research, they determined that wound specimens with ages ranging from 1 to 4 days, corresponding to the inflammatory phase, had the greatest mean positive ratio for these chemokines. So, from the standpoint of forensic pathology, their analysis revealed that IL-8, MCP-1, and MIP-lc were adequate as wound age indicators.

Regression equations were constructed to obtain the value of the unknown wound age when one or more of these cytokines is known.

In this study the following regression equations were constructed:

-Time interval between injury and death = 1.597− 0.001 * IL6 − 0.009* IL1-β − 0.165 (if TGF is + ve).

- Wound Age =22.997 + 6.077 *1− 0.343 * IL1-β − 0.018 *IL6 (if TGF is + ve).

-Time between death and autopsy = 2.919− 0.011 * IL6 −0.017 *IL1-β −1.862 (if TGF is + ve).

Thus by the use of these equations, it will be easy and practical to obtain the value of the wound age providing that we have the value of TGFα and providing that this tissue uptake this marker (TGFα positive).

The results of this study were in accordance with (**Grellner et al., 2000**) as they concluded that: Given the conditions, quantitative examination of pro-inflammatory cytokines in wound extracts may aid in the assessment of vitality and wound age, particularly in the very early post-traumatic interval (vitality of typical stab wounds) and also in the later stages of the wound healing process.

Conclusion and recommendations: In this study, the estimation of the wound age, time between injury and death as well as time between death and autopsy could be calculated from the following equations:

-Time interval between injury and death = 1.597− 0.001 * IL6 − 0.009* IL1-β − 0.165 (if TGF is + ve).

-Wound Age =22.997 + 6.077 *1− 0.343 * IL1-β − 0.018 *IL6 (if TGF is + ve).

-Time between death and autopsy = 2.919− 0.011 * IL6 −0.017 *IL1-β −1.862 (if TGF is + ve).

The proposed recommendations are: Autopsy should be performed as soon as possible in cases of traumatic events to avoid loss of important findings due to autolysis of tissues. Using biochemical (inflammatory cytokines) and immunohistochemical (TGF-α) techniques as tools for determining wound aging in cadavers. Further research, using larger sample, are needed to spread light on the role of inflammatory cytokines and growth factors in determination of wound age.

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الملخص العربي

تقدير عمر الجروح: المحركات الخلوية المحفزة للالتهاب مقابل القياس الكيميائي المناعي للانسجة

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في مجال الإختبارات الطبية الشرعية هناك اهمية قصوى لتحديد إذا ما كان الجرح الموجود عند تشريح الجثة قد تم احداثه قبل أو بعد الوفاة و إذا كان قد حدث قبل الوفاة فما هو عمر الجرح حتي حدوث الوفاة؟

هذا العمل كان موجها لمقارنة تقنيات الكيمياء الحيوية للمحركات الخلوية الالتهابية و الكيمياء النسيجية المناعية (عامل النمو المتحول- ألفا) كوسائل لتحديد عمر الجروح في الجثث وعلاقتهم بالعوامل الأخرى الخاصة بالجرح .تم أخذ عينات من الجلد و نسيج ما تحت الجلد من 50 جثة بهم جروح غير نارية و معلوم الفترة التي مرت على وفاتهم - وقد كانوا 11 أنثى (22%) و 39 ذكرا (78%)و كان معدل أعمار الضحايا 30.3 سنة و معامل الانحراف 19.88 و العمر الوسطي كان 26.5 سنة و أصغر عمر 4 شهور و اكبر عمر 82 سنة.

تم أخذ عينات الجروح من كل حالة لتقدير المحرك الخلوي انترلوكين 1 بيتا و المحرك الخلوي انترلوكين 6 و أيضا لتقييم عامل نمو الأنسجة الفا بواسطة القياس الكيميائي المناعي للأنسجة.

وقد لأظهرت النتائج ما يلي: وجدت علاقة ذات مغزى ما بين عمر الضحية و قيمة الإنترليوكين 6 و ما بين عمر الضحية و قيمة الانترليوكين 1 بيتا .

لم يكن هناك فرق ذو مغزى بين الذكر و الأنثى في اختبار المحركات الخلوية الانترليوكين6 و الانترليوكين 1 بيتا.كذلك لم يوجد فرق ذو مغزي بين الزمن مابين الإصابة و الوفاة و قيمة كل من انترلوكين 6 و انترلوكين 1 بيتا.وجدت علاقة سلبية ذات مغزي بين الزمن مابين الوفاة و التشريح و فيمة انترلوكين 1 بينا و لكن لم توجد علاقة مماثلة بالنسبة لانترلوكين 6

بشأن معادلة التراجع لعمر الجروح وجد ان كلا من عامل نمو الانسجة الفا و انترلوكين 6 مؤشرات مستقلة لتحديد عمر الجروح بينما انترلوكين 1 بيتا مؤشر غير مستقل.

في هذه الدراسة امكن تقدير عمر الجروح, الزمن بين الاصابة و الوفاة و ايضا الزمن بين الوفاة و التشريح باستخدام معادلات التراجع المحددة.و يمكن ان نستنتج من هذه الدراسة ان التحليل الكمي للمحركات الخلوية المحفذة للالتهاب في مستخلص الجروح يمكن ان يشارك في تحديد حيوية و عمر الجروح.