Original article

Estimation of Tissue Homogenate Cytokines and MicroRNAs might help to determine Wound Vitality and Dating

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ABSTRACT

Aim of the work: To assess the applicability of estimated levels of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, and gene expression levels of microRNAs (MiR)92a and MiR-214a in tissue homogenate (TH) of skin biopsies harvested from wound for discrimination between antemortem and post-mortem wounds and to suggest the post-injury interval (PII).

Material and methods: A 2-cm skin incision was made under anesthesia and full thickness punches were obtained from wound edge immediately (C-group) and at 30-min, 2-h, 6-h and 24-h after wounding in living animals (L-group) or animals were decapitated immediately after wounding and biopsies were obtained at the same periods after decapitation (D-group). Tissues were homogenized to be used for ELISA estimation of TNF-α and IL-6 levels and qRT-PCR expression levels of MiR-92a and MiR-214a.

Results: TNF-α, IL-6 and MiR-92a levels were significantly higher in L-group than other groups. Estimated TNF-α and IL-6 levels showed biphasic increases at 30-min and 2h, respectively and at 24h for both, while the peak levels of MiR -214a and MiR -92a were at 2h and 6h, respectively. MicroRNAs levels showed non-significant differences between all D-group specimens. Regression analysis defined high IL-6 levels as the significant variate to identify PII as either 2h or 24h and high levels of MiR-214a could suggest PII of 2h, while high levels of MiR-92a and TNF-α as the significant variate to suggest PII of 30-min and 6h, respectively. Multivariate analysis defined high IL-6 as the persistently significant predictor for victim's vitality at wounding, while ROC curve analysis defined high MiR-214a levels as the sensitive identifier for victim's viability during wounding.

Conclusion: Estimation of expression levels of MiR-92a and MiR-214a in TH might define the probable PII and differentiate antemortem from postmortem wounds, respectively. However, estimated TH levels of TNF-α and IL-6 alone are undependable for provision of knowledge about vitality and timing of wound, so combined markers might increase the accuracy of wound-dating.

Keywords: Wound dating, Wound vitality, MicroRNAs, Inflammatory cytokines

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I-Introduction

The wounding process in the living subject due to insult to skin integrity stimulates multiple cellular and extracellular cascades that are tightly regulated and occur in a coordinated fashion **(Wilkinson & Hardman, 2020)**. Injury of epithelial cells with resultant loss of intra-cellular adhesion usually triggers the epithelial cells' migratory machinery upon their neighbors **(Chen et al., 2022)**. In-vivo wound healing requires the reduction of the generation of pro-inflammatory cytokines, especially interleukin-6 (IL-6), and upregulating the anti-inflammatory cytokines as IL-10 with the release of angiogenesis-related cytokines **(Wei et al., 2022)**.

The differentiation between antemortem (AM) and postmortem (PM) wounds is still a matter of debit and controversy especially if the wound was alleged to be the cause of death **(Tickle et al., 2023)**. Further, the estimation of post-injury interval (PII), which is the time passed since wound inflection has a significant impact in the medicolegal context **(Tsellou et al., 2022)**.

Determination of vitality and wound dating is the primary goal of a forensic pathologist in trauma deaths **(Khalaf et al., 2019)**. Multiple techniques to examine the morphological, cytological, and biological changes were applied to techniques used to assess wound vitality **(Han et al., 2021)**. However, estimations of markers involved in vital and supravital reactions increase the accuracy of wound age estimation **(Pennisi et al., 2022)**.

MicroRNAs (MiR) are non-coding 18-24 nucleotides RNAs that have regulatory functions for many biochemical mechanisms in the human body and their levels in body fluids and tissues may alter through varied pathophysiological mechanisms, and thus might be employed as biomarkers for various diseases and conditions **(Rocchi et al., 2020)**. Further, MiR are involved in the process of skin wound repair, so could be used as biomarkers for the estimation of the time of skin injury **(Cheng et al., 2021)**. This animal-model study tried to evaluate the role of microRNAs and inflammatory cytokines for differentiation between AM and PM wounds and assessment of PII.

II-Material and methods:

II.1. Setting and Ethical considerations:

This study is a prospective animal model comparative study at departments of Forensic and Clinical Toxicology, and Medical Biochemistry and Molecular Biology, Faculty of Medicine, Benha University. It followed the Guidelines for the Care and Use of Laboratory Animals **(Clark et al., 1996)** and the protocol was approved by the Local Ethical Committee in the Faculty of Medicine, Benha University by RC: 4.12.2022.

II.2.The study Animals

The study included 54 healthy, normally growing adult male albino rats, weighing 200-250 g and aged 8-10 weeks that were purchased from The Animal Farm and were grown in the Animal House, Faculty of Veterinary Medicine, Benha University for two weeks before the start of the experiment to be acclimatized to the new farming conditions. Rats were provided with a standard diet and free water supply under standard living conditions at a temperature of 20°C, humidity rate of 60%, and 12-hs day/night cycle.

III.3.Methods:

A) **Surgical wound inflection:** All animals were anesthetized with

intramuscular injection of a mixture of xylazine and ketamine hydrochloride in a dose of 5 and 50 mg/kg body weight, respectively. The dorsal skin was shaved and sterilized using povidone-iodine and a 2-cm long skin incision was made using a scalpel as previously described **(Guan et al., 2000)**.

B) Specimen harvesting: Two full-thickness skin punches (0.5 x 0.5cm) were obtained one from each of the wound edges at the predetermined times after wounding.

C) Grouping: Animals were divided into three groups as shown in Figure 1:

1. The control group (C-Group; n=6): the specimens were obtained immediately after skin wounding and the wound edges were approximated and allowed to heal.
2. The living group (L-Group; n=24): The wounds were dressed without edge approximation, this group was subdivided into 4 subgroups according to time of obtaining the specimens: at 30-min, 2-h, 6-h, and 24-h after making the surgical incision under anesthesia.
3. The dead group (D-Group; n=24): these animals were anesthetized and after making the skin wounds, the animals were immediately killed by cervical dislocation and then this group was subdivided into 4 subgroups according to time of obtaining the specimens: at 30-min, 2-h, 6-h, and 24-h after death to equalize the PII in both living and dead animals.

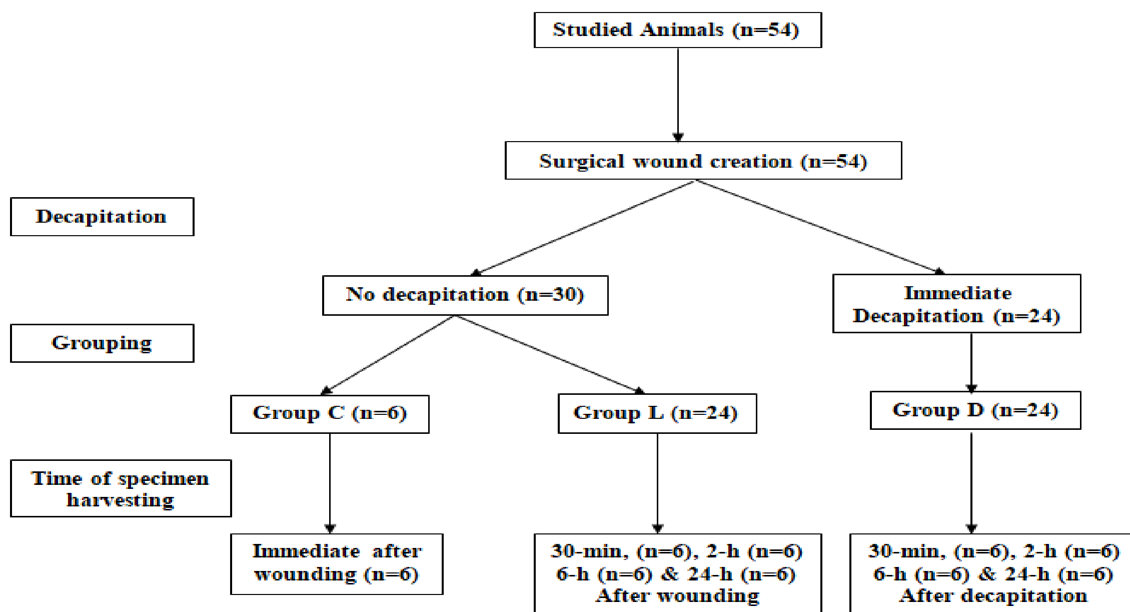

Figure (1): Study Flowchart

D) Specimen Preservation: Immediately after specimen taking, it was put in a dry sterile clean tube and kept frozen at -80ºC till being processed.

E) Tissue Processing for obtaining tissue homogenate (TH): Tissues processing was performed as previously described **(Zubaidi et al., 2010)** as follows: after thawing of each tissue specimen, it was

rinsed five times using 5 vol. of phosphate-buffered saline (PBS) containing 2 mM phenylmethylsulfonyl fluoride and 2 mM sodium azide at pH 7.2. Then, it was centrifuged for 5 min at 1000 g, the tissue pellet was homogenized in PBS to which 1 mM EDTA [5 vol. (wt/vol.)] was added. Homogenization was performed on ice in a Polytron (Kinematic PT, Dispersing and Mixing Technology; Lucerne, Switzerland) at 5-10 × speed for 4 times with 10-sec intervals. The obtained homogenate was centrifuged for 20-min at 2000 g for removal of the large tissue particles and re-centrifuged for 90-min at 13,200 g to obtain a clear supernatant. Supernatants of each tissue specimen were divided into 2 aliquots in clean dry tubes and labeled by specimen label and numbered according to the number of the animal. Both aliquots were stored at −80°C. Before each experiment, the extracts were thawed and re-centrifuged for 5-min at 13,200 g to remove debris.

F) **ELISA estimation of TH levels of IL-6 and Tumor necrosis factor-alpha (TNF-α):** estimation of TH levels of IL-6 and Tumor necrosis factor- alpha (TNF-α) by enzyme-linked immunosorbent assay kits (ELISA kits, Abcam Inc., San Francisco, USA; Cat No. 222503 and 46105) according to the manufacturer's instructions and were read using a 96-well microplate ELISA reader (Dynatech MR 7000).

G) **Estimation of expression levels of MiR-92a and MiR-214a in TH:** using the quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) as described previously **(Yang et al., 2021)** according to the manufacturer's instructions. Extraction of the total RNA in the homogenate including microRNA was performed using the MiRNeasy Mini Kit (QIAGEN, Germany). The relative quantitation of MiR-92a and MiR-214a was conducted using the 2-step Real-time PCR by Maxima SYBR Green (QuantiTect SYBR Green PCR Kit: QIAGEN). The cDNA was synthesized using miScript II RT Kit (QIAGEN, Germany). The mixture of RNA starting amounts, buffers for reverse transcription reactions for quantization of MiRNAs, and the recommended RNA input were incubated for 60 min at 37°C, for 5 min at 95°C to inactivate the miScript Reverse Transcriptase Mix then placed on ice and later diluted by 40 µl RNase-free water to the 10 µl reverse transcription reaction and mixed gently then briefly centrifuged and continued with real-time PCR using QuantiTect SYBR Green PCR Kit according to manufacturer's instructions (SYBR). The PCR reaction mix was prepared in a total volume of 25µl/tube (12.5 µl of 2x QuantiTect SYBR Green PCR Master Mix, 2.5 µl of 10x miScript Primer Assay, 2.5 ml of 10x miScript Universal Primer, Template cDNA up to 250 ng and RNase-free water). The real-time cycler was programmed using ABI 7900HT Fast Real-Time PCR System, (Applied Biosystem, Singapore). The amplification level for MiR-92a was programmed with a denaturation step at 95°C for 30 sec, followed by 40 cycles at 95°C for 10 sec and 60°C for 30 sec, and for MiR-214a was programmed as initial activation for 15-min at 95°C, denaturation at 94°C for 15-sec, annealing at 55°C, for 30-sec and extension at 70°C for 30-sec, and the process is repeated for 40 cycles.

The expression levels of microRNAs in each sample were determined after correction with the GAPDH expression level. Controls were chosen as the reference samples, and fold changes in the levels of microRNAs were determined by the $2^{-\Delta\Delta}$CT (cycle threshold) method and expressed as fold change (FC) using Step One software (Applied Biosystems, USA).

The sequences of the used primers for the detection of the expression levels of the studied microRNA

Items	Sequences
MiR-92a-F	5′-CACCTATATTGCACTTGTCC-3′
MiR-92a-R	5′-TGCGTGTCGTGGAGTC-3′
MiR-214a-F	5′-GACAGCAGGCACAGACA-3′
MiR-214a-R	5′-GTGCAGGGTCCGAGG-3′
GAPDH-F	5′-CCACCCATGGCAAATTCCATGGCA-3′
GAPDH-R	5′-TCTAGACGGCAGGTCAGGTCCAC-3′

Statistical analysis

Software used for statistical analyses was IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA). Results were analyzed using One-way ANOVA test, Tukey post- hoc test and Regression analysis (Stepwise Method) of variate was performed to determine the significant predictors. The receiver characteristic curve (ROC) was used to determine the significant predictors as judged by the significance of the difference between the variate area under the curve (AUC) versus the area under the reference area (AUC=0.5). The cutoff point for significance was $P<0.05$ (**Franklin & David, 1995**).

III-Results

Mean values of TH levels of TNF-α and IL-6 group L specimens were significantly higher than estimated levels of control group and of the corresponding subgroups in group D specimens. Estimated levels of IL-6 in specimens obtained at 2-h and 6-h were non-significantly lower than control levels while TH levels estimated in other specimens were significantly higher than control levels. Estimated levels of TNF-α showed time-course decreasing values till 6-h specimens but in 24-h specimens' level of TNF-α re-increased than levels estimated in 6-h specimens. On contrary, levels of IL-6 showed two peaks in TH of specimens obtained at 2-h and 24-h in group L and specimens of group D obtained at 30-min and 24-h (Table 1, Figure 2).

Table (1): comparison between control and studied groups regarding mean values of ELISA estimated levels of TNF-α and IL-6 and qRT-PCR estimation of expression levels of Mir-92a and Mir-214a in tissue homogenate by using one way ANOVA test andTukey post- hoc test.

Mean ±SD / Parameter	Control group	Living groups (L)				Dead groups (D)			
		Living group (30-m)	Living group (2h)	Living group (6h)	Living group (24h)	Dead group (30-m)	Dead group (2h)	Dead group (6h)	Dead group (24h)
TNF-α (pg/ml)	32±4.5	314±36.1 ^{A}	191±27 ^{A}	102.5±23.8 ^{A}	121±14.8 ^{A}	225±29.2 ^{AB}	86.5±24.8 ^{AB}	23.5±6.8 ^{AB}	72±11.5 ^{AB}
IL-6 (pg/ml)	69±8.9	114±18.2 ^{A}	193±26.2 ^{A}	113±23.4 ^{A}	311±32.4 ^{A}	103±15.3 ^{AB}	53±18 ^{B}	68.5±13.8 ^{B}	103±15.3 ^{AB}
MiR-92a	0.63±0.25	1.65±0.28 ^{A}	2±0.47 ^{A}	3.67±0.79 ^{A}	2.27±0.44 ^{A}	0.8±0.24 ^{B}	0.78±0.26 ^{B}	0.65±0.3 ^{B}	0.62±0.27 ^{B}
MiR-214a	0.079±0.026	0.12±0.028 ^{A}	0.18±0.068 ^{A}	0.145±0.076	0.13±0.1	0.09±0.05	0.08±0.03 ^{B}	0.07±0.02	0.067±0.03

A: indicates significant difference in comparison to control group; B: indicates significance versus the corresponding living group; SD: Standard deviation ANOVA: Analysis of variance TNF-α: Tumor necrosis factor-α, (IL)-6: interleukin- 6, MiR: microRNAs

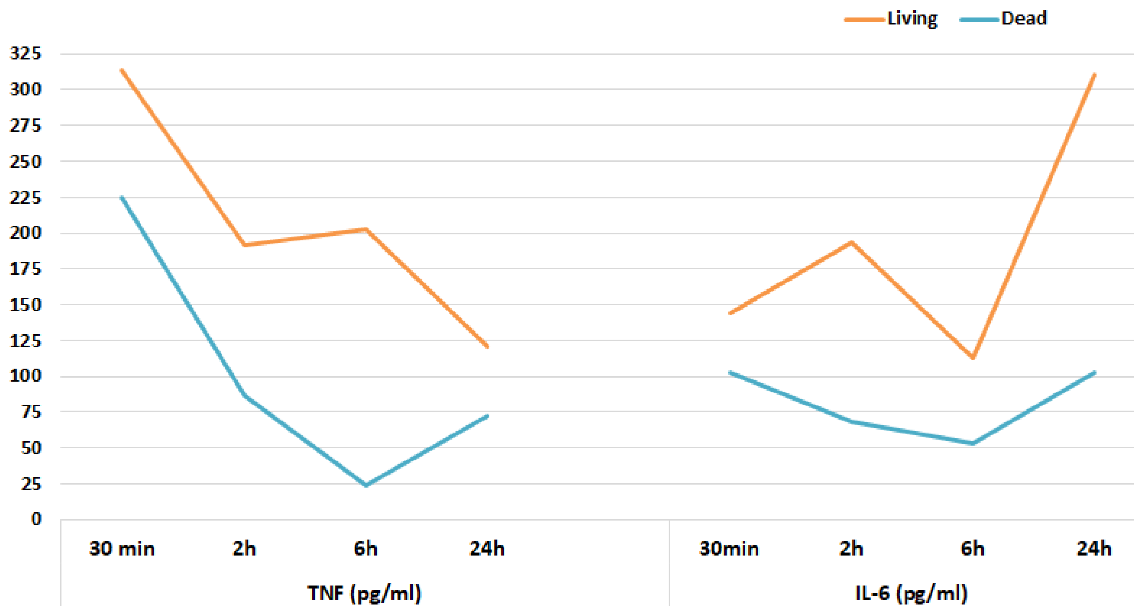

Figure 2: Time-course changes in the levels of TNF & IL-6 in TH of studied specimens

Estimated tissue expression levels of MiR -92a in TH of group L specimens were significantly higher in comparison to control and to the corresponding subgroups in group D specimens. Dead specimens with significantly higher levels in TH of specimens obtained at 6-h than other specimens of animal of group L. On contrary, expression levels of MiR -214a in TH of group L specimens that were obtained at 30 min and 2-h were significantly higher than control specimens. Specimens obtained at 2-h were higher than the corresponding subgroup in group D. However, the differences between TH levels of MiR -214a in other group L specimens showed non-significant differences. (Table 2).

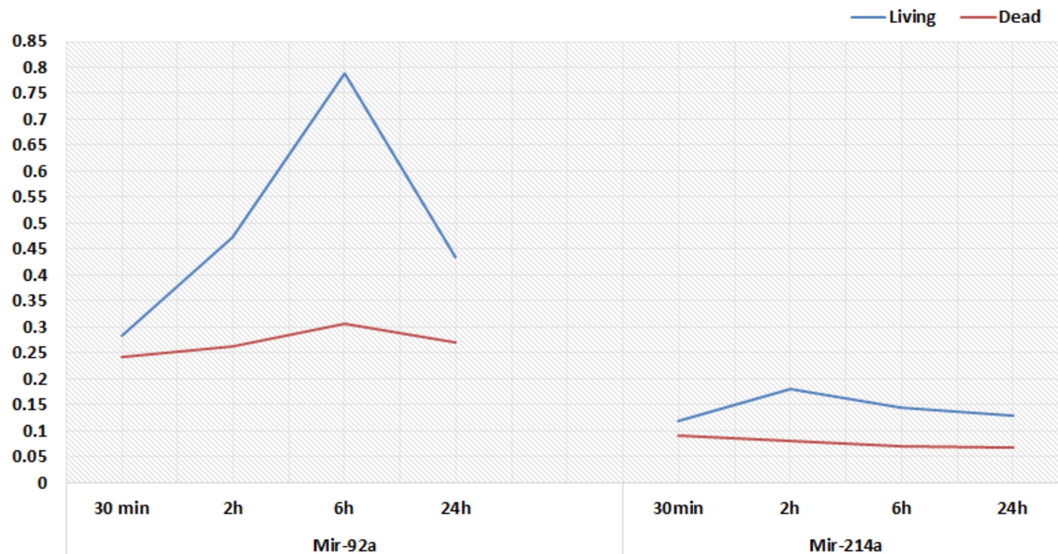

Figure 3: Time-course changes in the gene expression levels of Mir-92a & Mir-214a in TH of studied specimens

The ROC curve analysis defined high levels of TNF-α and IL-6 and high gene expression levels of MiR-92a in TH of all of the 4-time specimens as predictors for the post-injury interval, but unfortunately, their AUCs are equal (Figure4a-d). Also, Regression analysis using the Stepwise method defined high IL-6 levels in TH of specimens obtained at 2h or 24h as the only significant variate to identify wounds inflected since either 2h or 24h, but could not identify which is the most probable. High levels of gene expression of MiR-214a could only suggest a time-lag of two hours since trauma inflection as suggested by its significant AUC, while its AUCs for other times were non-significant and thus if coupled with a high TH level of IL-6 may determine which wound was inflected since 2h or 24h. Regression analysis defined high TH levels of MiR-92a in specimens obtained at 30-min as the significant variate to suggest that the wound was inflected since 30-min and high levels of TNF-α in TH may indicate that the wound might be inflected since 6h (Table 2).

Table (2): The Receiver Operating Characteristic Curve and Regression Analysis of TH levels of TNF-α, IL-6, MiR-92a and MiR-214a as a predictor of Post-injury Interval

Probable PII	Variate	Receiver Operating Characteristic Curve Analysis				Regression analysis	
		AUC	SE	P-value	95% CI	β	P-value
30-min	TNF-α	1.000	0	0.004	1.000-1.000	0.401	0.085
	IL-6	0.944	0.067	0.010	0.814-1.000	0.299	0.189
	MiR-92a	1.000	0	0.004	1.000-1.000	0.872	<0.001
	MiR-214a	0.639	0.176	0.423	0.295-0.983	0.192	0.246
Two hours	TNF-α	1.000	0	0.004	1.000-1.000	0.347	0.045
	IL-6	1.000	0	0.004	1.000-1.000	0.956	<0.001
	MiR-92a	1.000	0	0.004	1.000-1.000	0.319	0.020
	MiR-214a	0.986	0.027	0.005	0.932-1.000	0.078	0.584
Six hours	TNF-α	1.000	0	0.004	1.000-1.000	0.984	<0.001
	IL-6	1.000	0	0.004	1.000-1.000	0.171	0.056
	MiR-92a	1.000	0	0.004	1.000-1.000	0.160	0.332
	MiR-214a	0.750	0.159	0.150	0.439-1.000	0.033	0.652
Twenty-four hours	TNF-α	1.000	0	0.004	1.000-1.000	0.218	0.437
	IL-6	1.000	0	0.004	1.000-1.000	0.976	<0.001
	MiR-92a	1.000	0	0.004	1.000-1.000	0.121	0.455
	MiR-214a	0.639	0.171	0.423	0.303-0.975	0.041	0.594

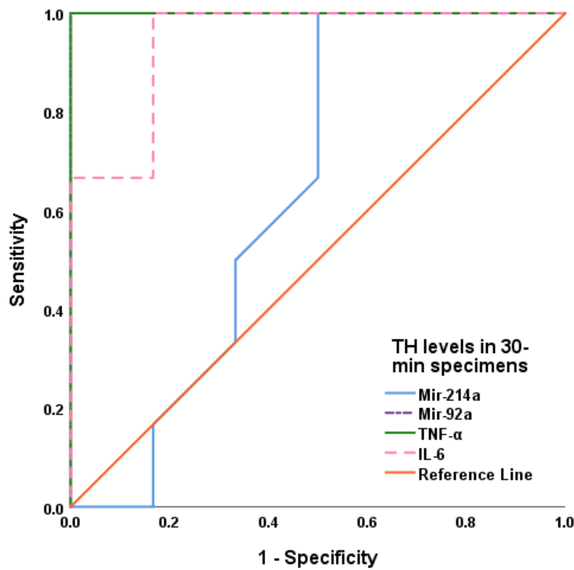

Figure (4a): ROC curve analysis of TH levels of estimated variate in 30-min specimens as predictors for PII

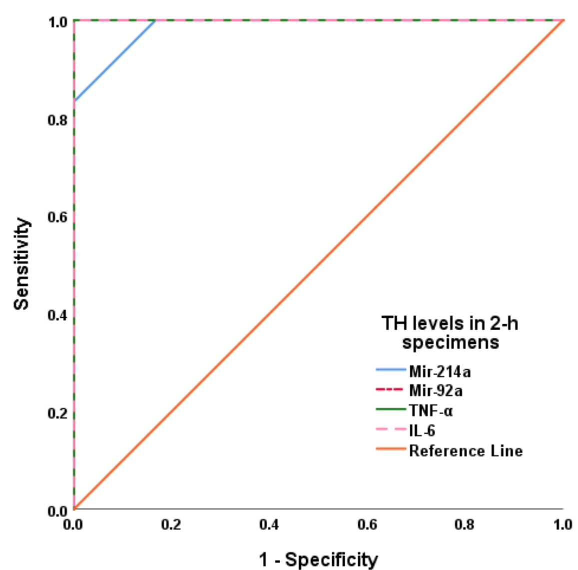

Figure (4b): ROC curve analysis of TH levels of estimated variate in 2h specimen as predictors for PII

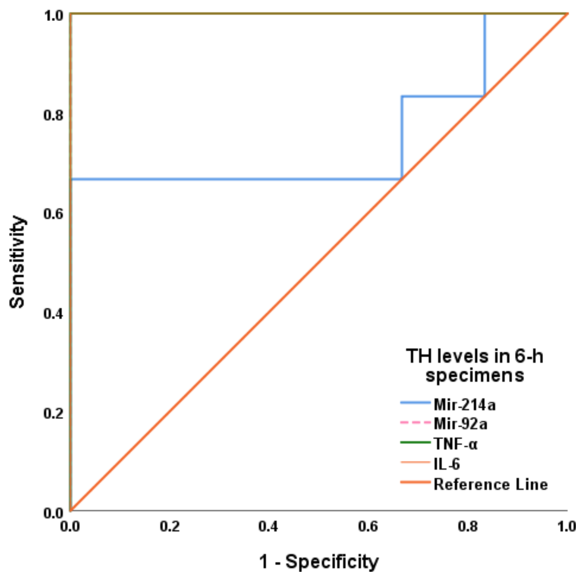

Figure (4c): ROC curve analysis of TH levels of estimated variate in 6h specimen as predictors for PII

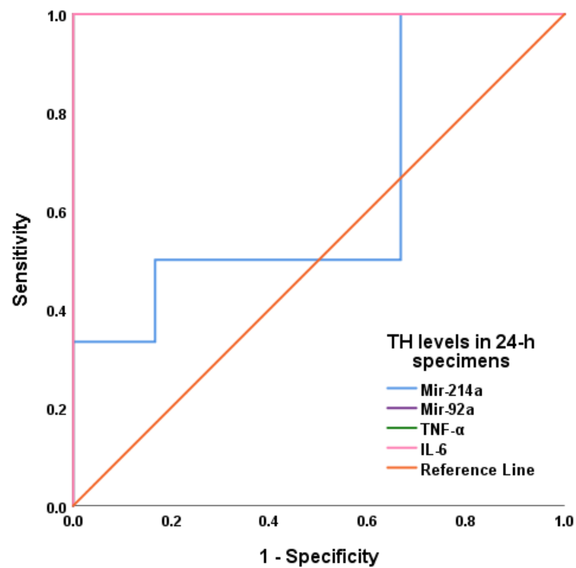

Figure (4d): ROC curve analysis of TH levels of estimated variate in 24h specimen as predictors for PII

The Multivariate Regression analysis using the Stepwise method defined high IL-6 and TNF-α levels and high levels of MiR-92a gene expression levels as significant positive predictors for the vitality of the victim at the time of trauma inflection, while levels of MiR-214a gene expression were excluded in model-1 of analysis. In model-2 of analysis, high levels of IL-6 and MiR-92a gene expression were the positive predictors. In model-3 of analysis, high IL-6 levels were the persistently significant variate for indicating viability of the victim during wound inflection. On contrary, ROC curve analysis defined high MiR-214a gene expression levels in the specimen as the probable sensitive identifier for viability of the victim during wound inflection with significant AUC (0.215±0.084, p=0.035; 95%CI for AUC= 0.050-0.381), while the other variate showed zero AUC (Figure 5).

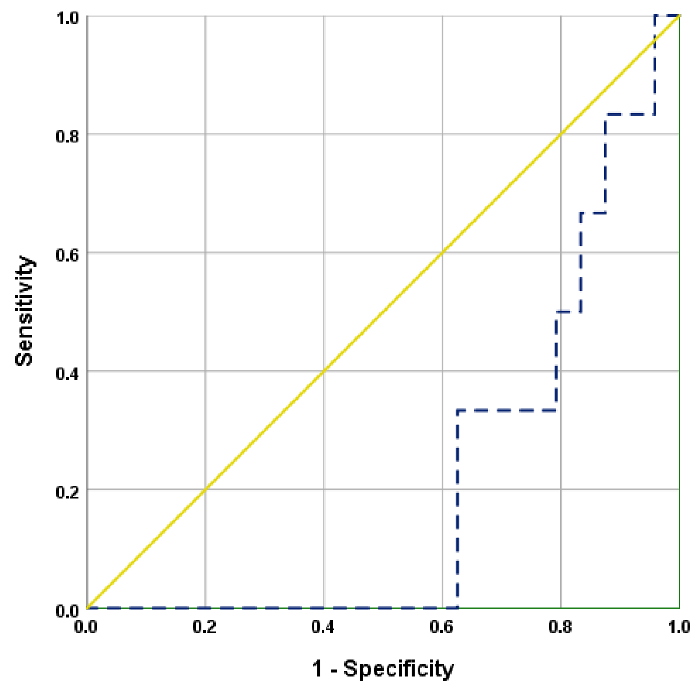

Figure (5): ROC curve analysis of TH levels of MiR-214a as a predictor for AM wound

IV- Discussion

Determination of wound dating and whether it was inflected antemortem (AM) or postmortem (PM) is still a challenge; multiple trials were performed to provide solutions for this dilemma. The current study reported biphasic secretion of IL-6 and TNF-α with the 1st phase of secretion of TNF-α occurring earlier than that of IL-6 and these findings were detected in specimens obtained from both AM and PM wounds. The early detection of IL-6 and TNF-α after wound inflection even in PM wounds assured the previously documented that keratinocytes contain high amounts of various cytokines, especially IL-6, which are stored there as inactive precursors or in the active form thus acting as continuous sources for this cytokine for a longer duration **(Haroon et al., 2000; Holzheimer & Steinmertz, 2000)** and this was also documented recently **(Giantulli et al., 2021)**. The reported different time-course releases of IL-6 and TNF-α supported the previous studies, which found TNF-α was the first cytokine to be released and peaked at 30 min after surgery, while other pro-inflammatory cytokines, such as IL-1β and IL-6, were detected thereafter **(Cibelli et al., 2010; Terrando et al., 2010)**. The reported biphasic secretion and the relation between the phase of secretion and wound vitality indicated different pathogenesis for both phases. The 1st phase of secretion of TNF-α might be attributed to the initiation of the inflammatory phase of cutaneous tissue response to injury, while that of IL-6 which peaks at 2h after injury might be attributed to its release from injured keratinocytes and as a component of the inflammatory cascade. In line with these findings, one study detected elevated cytokines levels on subcutaneous administration of recombinant TNF-α and attributed this to its activating action on Toll-like receptors 4 (TLR4) **(Fei et al., 2020)**.

Regarding the late phase, in living tissue may be attributed to starting the response to infection secondary to keeping the wound exposed without any line of local or systemic treatment, similarly, a recent study detected the increased release of

inflammatory cytokines, especially TNF-α and decrease in anti-inflammatory cytokines due to bacterial-dependent induction of the transcription factor Krüppel-like factor-4 in polymorphonuclear neutrophils which is the first line of humoral immunity released in infected injured sites **(Bhattacharyya et al., 2021)**.

The suggested pathways for secretion of inflammatory cytokines by injured keratinocytes were variant, one study found activation of TLR 5 in keratinocyte led to intra-nuclear translocation of nuclear factor-κB (NFκB), which bind to *IL-6* promoter leading to activation of IL-6 production by the keratinocyte **(Ryu et al., 2019)**. Another in-vivo study found the released intracellular adhesion molecule-1 and heat shock protein family A member 8 from keratinocyte on skin wounding, in mouse and humans, act as costimulatory molecules for the proliferation of the epidermal resident γδ T cells with subsequent interleukins' production and up-regulation of its receptors **(Johnson et al., 2022)**.

Statistical analyses defined high tissue homogenate TNF-α levels as a predictor of recent injury at 30-min, while IL-6 might identify wound inflected since either 2h or 24h and both could predict vitality during the wounding process especially IL-6. In line with these data, a previous study indicated that the expression of inducible nitric oxide synthase and IL-6 proteins in AM-burnt skin showed a time-dependent course with significantly different expression patterns from PM burn **(Abo El-Noor et al., 2017)**. Thereafter, another study detected significantly higher cytokines levels in vital than PM wounds and suggests that cytokines might be used as useful biomarkers of skin wound vitality and estimation of cytokines' levels using the immunoassays is more sensitive than immunohistochemistry to identify wounds with short survival time **(Peyron et al., 2021)**.

Estimated levels of gene expression of MiR-92a and MiR-214a in TH were higher in AM and PM wounds, but the difference was significant only with MiR-92a. Further, the expression levels of MiR-92a were the significant predictor for wound creation at 30-min, thus it can differentiate between early and late inflected wounds, but its role to determine the vitality was negligible. However, ROC curve analysis defined the expression of MiR-214a as the only significant predictor for AM inflection of the wound. Similarly, one study detected overexpression of the MiR-214a and MiR-92a in the ligature marked skin samples than the non-injured skin samples and attributed the statistical significance in expression levels of these microRNAs with anti-inflammatory activity as a body defense against the induced inflammation by hanging process **(Neri et al., 2019)**. Another study showed significantly higher expression levels of other microRNAs in skin samples 24-h PM and was continuously expressed up to 48-h PM but their levels decreased with time progress, however, this study could not detect a relationship between the time passed after death and MiR expression **(Ibrahim et al., 2019)**.

The obtained results spotlight the possibility of using the expression levels of microRNAs as discriminators for wound vitality and dating. In support of this assumption, multiple earlier studies recommended the use of microRNA as a forensic tool for the determination of PM interval for the advantage of their stability at varying temperatures and in various tissue and organ specimens **(Li et al., 2014; Pan et al., 2014; Li et al., 2016; Tu et al., 2019)**, while this stability was lacking for DNA, RNA and protein samples **(Lv**

et al., 2017; Tozzo et al., 2020). Also, a recent systemic review demonstrated a difference in the differential MiR expressions in AM and PM wounds and recommended further studies to evaluate their reliability as distinguishing markers for wound vitality (**Manetti et al., 2021**).

Interestingly, the current study found high expression level of MiR-214a could predict injury date at 2h and thus in combination with high levels of IL-6 could assure this timing. Thus, using a combination of markers might increase the diagnostic yield for wound dating. In support of this suggestion, a recent review article concluded that the use of microRNAs is promising and its application with other markers may increase the sensitivity and specificity to validate systems or models for determining wound vitality in forensic practice (**Pennisi et al., 2022**).

V- Conclusion

Biomarkers could be used as tools for forensic medicine to resolve the dilemma of wound dating and vitality. Estimated TH levels of TNF-α and IL-6 could provide knowledge about vitality and timing of wound. Estimation of expression levels of MiR-92a and MiR-214a in specimens' homogenates might define the probable post-injury interval and differentiate AM from PM wounds, respectively. Combined markers might increase the accuracy of wound dating.

VI- Recommendations

Wider scale studies are mandatory to establish the obtained results and verify the best biomarkers combinations to be used. Further, similar trials for the estimation of these biomarkers in human specimens are required to establish their clinical utility in forensic practice.

Conflicts of interest: no conflict of interest.

Funding: This research did not receive any financial assistance or specific grant from funding organization.

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الملخص العربي

قياس مستويات السيتوكينات والحمض النووي الدقيق قد يُساعد في تحديد حيوية الجرح وزمنه

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الأهداف: تهدف هذه الدراسة تقييم قابلية تطبيق نسب محددة من عامل النخر الورمي-ألفا، والانترلوكين-6، ومستوى التعبير الجيني للحمض النووي الريبيوزي الدقيق 92-أ ،214-أ في المستخلص المتجانس لانسجة العينة الجلدية المأخوذة من الجرح للتمييز بين الجروح المحدثة قبل وبعد الوفاة ولتحديد تقريبي للفترة ما بين احداث الجرح والحصول علي العينة.

الطرق و المواد المستخدمة: تم عمل شق جلدي طوله 2 سم تحت تأثير التخدير الكلي والحصول علي عينة من الجلد بعد احداث الجرح مباشرة (المجموعة أ)، وبعد 30 دقيقة، وساعتين، و6 و24 ساعة من احداث الجرح في الحيوان الحي (المجموعة ب)، أو بعد نفس المواقيت من قطع رأس الحيوان مباشرة بعد الجرح (المجموعة ج). تم اعداد المستخلص المتجانس لانسجة العينة الجلدية لاستخدامه في قياس مستويات عامل النخر الورمي-ألفا، والانترلوكين-6 باستخدام اختبار الاليزا وقياس معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق 92-أ،214-أ باستخدام تفاعل البوليميراز المتسلسل.

النتائج: وجد أن معدلات جميع العوامل التي تم قياسها أعلى بفارق ذودلالة احصائية في عينات المجموعة (ب) عن المعدلات المُقاسة في عينات المجموعتين الأُخريين، أظهرت معدلات النسب المقدرة لعامل النخر الورمي-ألفا، والانترلوكين-6 ازدياد ثنائي الطور عند 30 دقيقة، وساعتين لكلا العاملين علي التوالي، وبعد 24 ساعة لكلا العاملين. بلغت معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق أ-92، أ-214 بعد ساعتان و6 ساعات على التوالي. علي الجانب الأخر أظهرت معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق 92-أ، 214-أ ختلافا طفيفا بين جميع عينات المجموعة (ج). أشار التحليل التراجعي الى ان النسب المرتفعة من الانترلوكين-6 تعتبر المتغير الأهم لتوضيح فترة ما بعد الإصابة اثناء ساعتان، او 6 ساعات، والنسب المرتفعة معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق 92-أ من الممكن ان تشير الى أن فترة ما بعد الإصابة ساعتان، بينما يُعتبرارتفاع معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق92-أ وعامل النخر الورمي-ألفا هي المؤشر الاهم لتوضيح فترة ما بعد الإصابة اثناء 30 دقيقة، و6 ساعات على التوالي. حددت التحاليل متعددة المتغيرات أن النسب المرتفعة من الانترلوكين-6 هي المؤشر الأشد دقة وحدد المنحنى المُميز للأداء المستقبلي المستوى المرتفع من معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق214-أ كمؤشر حساس لحدوث الجرح الجرح حدث أثناء حياة الضحية.

الخلاصة: تقدير معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق92-أ، 214-أ في المستخلص المتجانس لانسجة العينة الجلدية من الممكن ان تساعد فى تحديد الفترة مابين الإصابة والحصول علي العينة، والتمييز بين الجروح المُحدثة قبل وبعد الوفاة على التوالي. كما يمكن ان تزيد المؤشرات المجمعة من دقة تحديد وقت الجرح.

التوصيات: توصي هذه الدراسة بالتوسع في اجراء دراسات لتحديد أهم الدلالات الحيوية المستخدمة في تحديد عمر الجرح في الطب الشرعي، كما توصي باجراء مزيد من الدراسات تقدير معدلات النسخ للتعبير الجيني للحمض النووي الريبوزي الدقيق92-أ، 214-أ في المستخلص المتجانس لانسجة العينة الجلدية في العينات البشرية لإثبات فائدتها السريرية في ممارسة الطب الشرعي .