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Original article

The role of new potential biomarker for medicolegal aging of wounds

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Abstract

Background: Estimating the age of a wound is crucial in the realm of forensic medicine. Determining the age and liveliness of a wound is essential for the appropriate handling of legal procedures. **Objective:** to evaluate the myostatin marker's usefulness in determining the age of medicolegal wounds. **Methods:** This cross-sectional investigation on the ante-mortem aging of surgically non-complicated wounds was conducted at the general and orthopedic surgery departments of Beni Suef University Hospital. 56 participants were divided evenly into two equal groups: Group B involved muscle injuries while Group A involved bone injuries. Each participant had blood drawn nine times following surgery: before and after zero (pre-operative), twenty-four hours, thirty-six hours, forty-eight hours, seventy-two hours, eighty-four hours, ninety-six hours, ninety-six hours, and one hundred and sixty hours (post-operative). **Results:** Over the course of the five days, there

were notable ($P < 0.001$) increases in myostatin levels, peaking 36 hours after the wound (3.73±0.42) as opposed to the bone injury group's zero time (2.07±0.76). Additionally, across the course of the five days, there were notable ($P < 0.001$) increases, with the muscle injury group's peak being reached 24 hours after the wounding (1.94±0.71) as opposed to the zero time (1.45±0.40). **Conclusion:** myostatin can be used to assess medicolegal wound age in bone and muscle injuries.

1. Introduction:

Although forensic pathologists find it difficult to determine the age of wounds, doing so can help with crime scene reconstruction and increase the possibility that a suspect will be apprehended [1, 2, 3]. Forensic pathologists have to identify wound and the duration of each wound in circumstances where many perpetrators caused the injuries, as the level of trauma reveals distinct occurrences. Nevertheless, there is presently no accurate technique or model to determine wound age despite its obvious remarkable[4, 5]. Rapid technological advancements have made it simpler to access data. Furthermore, in the past few years, a wide range of time-dependent characteristics have been investigated. Because wound age estimations involve a complex web of factors, it is necessary to look into the most effective way to utilize multiparameter databases in the setting of numerous analyses. A single parameter or even a few parameters that are may not provide enough information to assess the time that passed since an injury occurred.

Myostatin belongs to the superfamily of TGF-β. Genetic abnormalities in the myostatin gene result in a noticeable elevation in skeletal muscle mass because myostatin has a remarkable effect on the growth of skeletal muscle [6].

2. Patients and methods:

Study population and sample:

- This study was a cross sectional study conducted from October 2022 to April 2023 at Beni-Suef University Hospital in general and orthopaedic surgery departments. The ethical committee of Beni-Suef University's Faculty of Medicine gave its approval to the project. Before recruiting them for the study, all participants gave their informed consent

after being informed of the goals of the investigation. Maintaining confidentiality when managing the database was ensured. (Approval number is 02102022).

Due to the sample calculation and random participant selection, 56 patients—56 of them were male—who had surgical procedures were included in the study. They were split evenly into two groups: group (A) consisted of bone injuries and group (B) consisted of muscle injuries.

And samples were taken upon 9 intervals: Immediately pre- operative, immediately post-operative, 24h ,36h ,48h ,72h , 84h , 96h , 120h post-operative.

Patients were selected according to these inclusion and exclusion criteria:

(A) Inclusion criteria:

Non-complicated surgical wounds, Age 18-60 yrs.

(B) Exclusion criteria:

Complicated wounds from surgery, Aged under eighteen or beyond sixty, blood disorders, consumption of alcohol, tendency to bleed, loss of blood during surgery, wound inflammation, Issues that arise during the procedure.

All the patients will be subjected to the following:

1. **Detailed history taking were taken from all participants including**:

Age (years), Sex, Residence, Occupation, Parity, Smoking, History of drug intake, chronic diseases and alcohol intake

2- Examination

Temperature, BMI, Inflammation of wounds, bleeding tendency

3- Medications

Antibiotics, Antacids, Analgesics, anti-coagulants

4- Investigations

Na, K, ca, Creatinine, SGPT, SGOT, PT, PC, INR, RBCs, WBC, PLT, HGB, HCT

5- Blood Samples:

Blood samples were collected in BD Vacutainer K2EDTA applying a 21-gauge needle to tubes. Then processed at 4000 rpm for 10 min at 4°C, plasma was splited, and stored at −70°C.

- **Measurements of human myostatin in serum:**

Following the manufacturer's instructions, blood samples were utilized to test the myostatin level in the sample using a myostatin Human ELISA kit from Elbscience (catalogue number: E-EL-H5307). When it comes to myostatin detection, this assay offers great accuracy and sensitivity. This kit helps detect Human AD in given samples. There was no discernible incompatibility or conflict between Human AD and its equivalents [7].

Detection rang: 0.78-50 ng/mL, Sensitivity: 0.47 ng/mL

- Test principle

The Sandwich-ELISA method is done by this ELISA kit [8].

- Statistical analysis

The gathered information was coded, processed, and examined utilizing Windows 10's SPSS version 25.

The following tests were used:

- **Descriptive analysis** of the results
- **Cross tabulation and Chi Square test** ($\chi 2$)
- **Student t- test**
- **One way ANOVA test**
- P-values equal to or less than 0.05 were meant to be remarkable.

3. Results:

In table (1); No statistical remarkable variants between bone injury and muscle injury groups were recorded in mean age, BMI, Temperature, Serum Na, K, Ca, PH, SGOT, PT, PC nor WBCs count (p-value >0.05). While the recorded statistical remarkable variants were in mean serum creatinine, SGPT, INR, RBCs, PLT, Hb and HCT value (p-value ≤0.05).

Table (1): Comparison between groups according to basic clinical and laboratory data

	Group 1 bone injury		Group 2 muscle injury		
	Mean	Standard Deviation	Mean	Standard Deviation	P value
Age	54.50	18.31	51.11	15.81	0.461
BMI	22.96	2.74	23.00	2.58	0.960
Temp	37.13	0.13	37.12	0.13	0.918
Na (mEq/l)	139.22	3.37	139.05	4.50	0.878
K (mEq/l)	4.11	0.58	4.41	0.77	0.108
creat (mg/dl)	0.77	0.23	1.09	0.51	0.004
SGPT (U/l)	15.24	1.12	18.30	4.47	0.001
SGOT (U/l)	24.74	29.05	14.58	2.05	0.070
PT (sec)	12.43	0.84	12.43	0.76	0.997
PC %	86.79	5.98	85.07	6.33	0.302
INR	1.08	0.05	1.05	0.02	0.042
ca (mmo/l)	0.69	0.05	0.71	0.05	0.273
PH	7.45	0.04	7.43	0.04	0.187
RBCs (*10e6/µL)	4.25	0.35	3.86	0.48	0.001
WBC (*10e3/µL)	8.52	2.99	7.48	1.14	0.092
PLT (*10e3/µL)	304.68	72.76	353.07	57.01	0.008
HGB (g/dL)	12.34	0.93	11.46	0.37	< 0.001
HCT %	34.66	2.49	32.04	1.90	< 0.001

All participants in this study were males. There were no statistical remarkable variants between participants in residence and smoking as a special habit (p-value >0.05), although there were statistical variants between participants according to occupation, chronic diseases and treatment (p-value < 0.001) as shown in table (2).

Table (2): Comparison between groups regarding history of participants:

		Group 1 bone injury		Group 2 muscle injury		P value
		Count	%	Count	%	
Sex	M	28	100.0%	28	100.0%	-----
Residence	Rural	15	53.6%	16	57.1%	0.788
	Urban	13	46.4%	12	42.9%	
Occupation	Driver	0	0.0%	7	25.0%	< 0.001
	Farmer	6	21.4%	0	0.0%	
	Retired	9	32.1%	10	35.7%	
	Smith	6	21.4%	0	0.0%	
	Worker	7	25.0%	11	39.3%	
Smoking	Smoker	12	42.9%	7	25.0%	0.158
	non smoker	16	57.1%	21	75.0%	
Treatment	1alpha amrical	0	0.0%	5	17.9%	0.001
	Insulin	6	21.4%	0	0.0%	
	No	22	78.6%	23	82.1%	
chronic disease	Diabetic	6	21.4%	0	0.0%	< 0.001
	HTN	0	0.0%	7	25.0%	
	kidney disease	0	0.0%	5	17.9%	
	No	22	78.6%	16	57.1%	

There were statistical remarkable variants between participants regarding type of operation and post-operative medications (p-value < 0.001) as shown in table (3).

Table (3): Comparison between groups according to operation data:

		Group 1 bone injury		Group 2 muscle injury		P value
		Count	%	Count	%	
Type of operation	closure of colostomy	0	0.0%	10	35.7%	< 0.001
	DCL after RTA with fracture bilateral tibia and lt femer	2	7.1%	0	0.0%	
	debridment	6	21.4%	0	0.0%	
	fracture bilateral tibia	2	7.1%	0	0.0%	
	fracture femer	1	3.6%	0	0.0%	
	fracture hip	1	3.6%	0	0.0%	
	fracture right femer	1	3.6%	0	0.0%	
	I.T.F	6	21.4%	0	0.0%	
	open heart surgery	0	0.0%	18	64.3%	
	tibial osteomylitis	9	32.1%	0	0.0%	
post-operative medications	clexan/24hr alphentern/8hr declofen/12hr 500cm saline /12hr cefotax post operative	28	100.0%	7	25.0%	< 0.001
	flagyl iv/8hrs controloc 40gm/24hr averzolin 600gm/12hr	0	0.0%	10	35.7%	
	flazol danset8mg controloc40mg declofen	0	0.0%	5	17.9%	
	rocephin flazol sulbin1.5 danset8mg controloc40mg declofen	0	0.0%	6	21.4%	

For Myostatin (group A bone injury) throughout the 5 days, remarkable ($P < 0.001$) elevates with the peak at 36 hours post-wounding (3.73±0.42) compared to the zero time (2.07±0.76). Despite the remarkable ($P < 0.001$) drop in the myostatin level in the wounds 48 h-96 h old compared to the peak at 36 h wound, but significance still found compared to that in control group (zero time). Moreover, at the 5th day, the statistical remarkable was still found with mean values of (3.00±0.75) as shown in table (4) and figure (1).

Table (4): Changes in serum levels of Myostatin in bone injury group over time

Group 1 bone injury		time (hours)							
		Zero	24	36	48	72	84	96	120
myo (ng/mL)	Mean	2.07d	3.18b	3.73a	3.69a	3.37ab	2.65c	2.60c	3.00bc
	SD	0.76	0.75	0.42	0.58	0.44	0.37	0.39	0.75

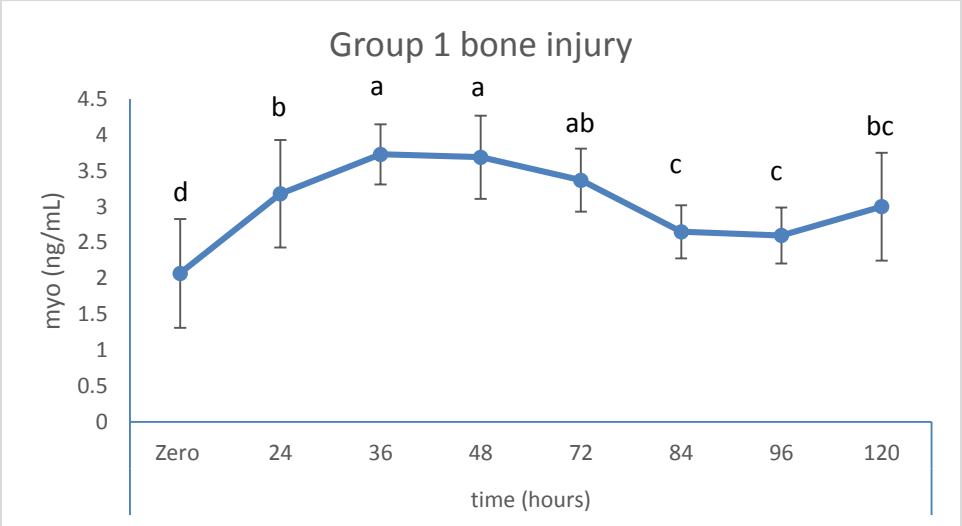

Figure (1): Changes in serum levels of Myostatin in bone injury group over time

For Myostatin (group B muscle injury) throughout the 5 days, remarkable ($P < 0.001$) elevations were noted with peak at 24 hours post-wounding (1.94±0.71) compared to the zero time (1.45±0.40). Despite the remarkable ($P < 0.001$) drop in the myostatin concentration in wounds 36–120 h old compared to the peak noted at 24 h wound; significance still found till the 5th day (1.81±0.37) compared to that in the other group (zero time as shown in table (5) and figure (2).

Table (5): Changes in serum levels of Myostatin in muscle injury group over time

Group 2 muscle injury		time (hours)							
		Zero	24	36	48	72	84	96	120
myo (ng/mL)	Mean	1.45b	1.94a	1.80ab	1.75ab	1.68ab	1.41b	1.64ab	1.81ab
	SD	0.40	0.71	0.62	0.55	0.49	0.34	0.28	0.37

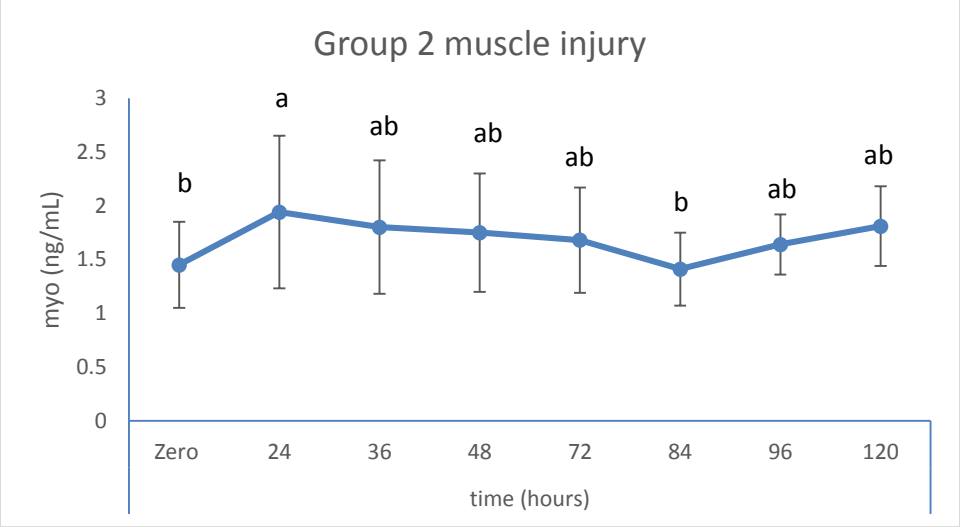

Figure (2): Changes in serum levels of Myostatin in muscle injury group over time

4. Discussion:

The medicolegal element of wound age detection is difficult. To get compelling evidence of antemortem injury, a sequence of crucial reactions following this ailment of a wound (such as bleeding, inflammatory cell invasion, and production of granulation tissue) must be taken into consederation [9]. Morphological, cytological, and molecular biology methods can be used to assess the life of a wound. According to Cecchi [10], a number of biomarkers included in physiological processes purportedly improve wound-age estimation accuracy.

Myostatin is a TGF-beta superfamily member that contrasts muscle growth and hyperplasia in order to negatively regulate skeletal muscle size. In addition to skeletal muscle cells, bone cells also express myostatin. Mice deficient in myostatin had stronger bones, more muscular mass and higher power [11].

It is generally accepted that neither documented parameters nor methods exist that produce these data. The use of a mixture of multiple characteristics may help to reduce wound-age prediction errors because it is a complex and multifaceted problem [12]. We therefore set out to evaluate the utility of myostatin as detector of medically-legal wound ageing in surgically simple lesions.

Additionally, forensic professionals may find the serum signs to be a useful method when working with live subjects. One of the biochemical techniques that produced great results with the examination of myokines and osteokines in wounds is the use of immunological assays like ELISA. Due to the fact that the mixed use of many markers in the detection of wound aging is thought to be a promising method, We have monitored the time-dependent variations in the analyzed myokine levels in wound tissue in blood for a maximum of five days following the wound [13].

No statistically remarkable variations in mean age, BMI, temperature, serum Na, K, Ca, PH, SGOT, PT, PC, or WBC count were found between bone injury and muscle injury groups in the investigation (p-value 0.05). While the mean serum creatinine, SGPT, INR, RBCs, PLT, Hb, and HCT values (p-value 0.05) showed statistically remarkable variations.

Similar characteristics were found in the study by Chetter et al.,[14]where 56.5% of participants were men, the median age was 55, and the median BMI was 28.9. Additionally, there were no remarkable variations in haematology values across groups in the study by Campbell et al., [15]. There was no thrombocytopenia detected to

explain bleeding-related adverse events. Clinical chemistry (blood), urinalysis, and endocrine function testing did not reveal any changes that were clinically remarkable.

The participants' average age was 27.56 4.20, and the two groups did not vary remarkablely in terms of age, education level, height, operation time, diastolic blood pressure, weight or temperature pre and post-operative. Although there was a substantial variation between the 2 groups in terms of BMI, heart rate

Similar to our findings, Sista et al.,[16] found no variants in the co-morbidity, pre-operative clinical characteristics, or biochemical detectors among the two patient groups.

All of the patients in our study were men. While there were statistically remarkable variants between participants according to occupation, chronic diseases and treatment (p-value 0.001), there were no statistically remarkable variants between participants regarding residence or smoking as a particular habit (p-value 0.05).

At baseline, 72.8% of participants had at least one co-morbidity and two-thirds (67%) had several co-morbidities, according to Chetter et al.[14] study. At the outset, cardiovascular disease, diabetes, and respiratory diseases were the most prevalent co-morbidities. Of the participants, 28.5% were active smokers.

In our study, there were statistically remarkable variations between patients in terms of the type of operation and post-operative medications (p-value 0.001).

69.2% of the study population reported using medication at baseline, which is similar to Chetter et al., [14]. Drugs that downregulate platelets and prevent coagulation were most frequently administered (49.9%). Chemotherapy, immune suppressants, and steroids were not commonly used by participants. Kraft et al. [17] discovered statistically remarkable variations between participants regarding operative and post-operative antibiotic medication in their study on CRP and leukocyte-count following lumbar spine surgery (p-value 0.001).

In the current investigation, myostatin levels remarkablely (P 0.001) elevated over the course of the 5 days, with the maximum occurring 36 hours after wounding (3.730.42) as opposed to the zero time (2.070.76). Even while the myostatin level in the wounds 48 hours to 96 hours old dropped remarkablely (P 0.001) compared to the peak recorded at 36 hours wound, statistical remarkable was nevertheless maintained when compared to the control group (zero time). Additionally, the statistical remarkable persisted after 5 days with mean values of 3.000.75.

Elkasrawy et al.,[18] made similar discoveries, noting that myostatin antibody staining was

prevalent in skeletal muscle fibres for the first 48 hours following surgery and in chondrocytes of the soft fracture tissue for the following 4 days. At 12 and 24 hours after surgery, myostatin expression was especially pronounced in injured myocytes that were both close to and far from the fracture site. Twelve hours after surgery, a secreted "pool" of myostatin was particularly detected in the damaged muscle fibres near the osteotomy site. Up to 48 hours after the fracture, sporadic staining might be seen in the connective tissue surrounding the fracture site. On days 4, 6, a few periosteal and endosteal cells showed intermittent very weak signals. In vivo, exogenous myostatin inhibits muscle repair and decreases chondrogenesis and fracture callus bone volume.

Furthermore, Hamrick et al.,[19] found that In a mouse model of fibula osteotomy with harm to the lateral compartment muscles (fibularis longus and brevis), blocking myostatin signaling boosted muscle regeneration and improved fracture healing. Myostatin inhibitors may therefore be helpful in promoting wound healing in orthopaedic trauma and extremities damage situations.Myostatin is remarkablely expressed in the early phases of fracture healing, according to previous research, which suggests that this protein may be important for attracting and enhancing progenitor cells in the fracture callus [20].

In particular, loading-related effects on bone cause myostatin deficiency to promote bone production [21]. myostatin remarkably highs up receptor activator of nuclear factor B ligand -mediated osteoclast proliferation in vitro [22].

The "myokines" that muscles release, which include cytokines, peptides, and growth factors, have an impact on bone resorption and creation. Myostatin, one of the most prevalent myokines, inhibits osteoblasts and activates osteoclasts to negatively regulate bone maintenance [23].

Myostatin was found to increase the production of the RANKL, a positive regulator of bone resorption, and SOST, a negative regulator of bone formation, in osteocytes in an in vitro investigation [24].

In our investigation, myostatin showed remarkable (P 0.001) increases over the course of 5 days, with the peak occurring 24 hours after wounding (1.940.71 vs. 1.450.40). Despite a remarkable (P 0.001) drop in the myostatin level in wounds 36-120 hours old compared to the peak noted at 24 hours wound, the statistical remarkable remained until the fifth day (1.810.37) when compared to that in the control group.

Mouse embryos that have no myostatin gene develop hyperplasia, which is caused by an

elevation in the muscle cell number, and hypertrophy, which is caused by an elevation in the size of each fibre, after birth [25]. Myostatin regulates the growth of skeletal muscle and reduces the mass of the muscle under physiological conditions.

Myoblast differentiation and multiplication treated with myostatin similarly decreased [26]. Elkasrawy et al.,[18] found that injured skeletal muscle fibres had intense myostatin staining between 12 and 24 hours after surgery, and plentiful injury over the first 48 hours.

Our findings could be explained by the truth that Myostatin, inhibits a lot of muscle growth by interacting to activin type II. By preventing protein kinase Akt, myostatin interacts inversly with the IGF-1 pathway in myoblasts [27].

5. Conclusion:

These findings suggest that myostatin has similar blood serum level curves, maybe as a result of its associated functions in wound healing.

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