Postmortem Changes in Element Levels in Rat Skeletal Muscle Tissue

Sıçan İskelet Kası Dokusunda Postmortem Element Seviyelerinin Belirlenmesi

Kamil Hakan DOĞAN, MD,[a]
Gürsel GÜNAYDIN, MD,[a]
Şerafettin DEMİRCİ, MD,[a]
Sermet KOÇ, MD[b]

[a]Department of Forensic Medicine, Selçuk University Meram Faculty of Medicine, Konya
[b]Department of Forensic Medicine, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul

Geliş Tarihi/*Received:* 16.02.2009
Kabul Tarihi/*Accepted:* 28.08.2009

Yazışma Adresi/*Correspondence:*
Kamil Hakan DOĞAN, MD
Selçuk University
Meram Faculty of Medicine, Konya, TÜRKİYE/TURKEY
drhakan2000@gmail.com

ABSTRACT Objective: This experimental study was designed to determine postmortem changes in element levels in rat skeletal muscle tissue and to evaluate its relation with the postmortem interval. **Material and Methods:** Fifty-two three-month-old male Sprague-Dawley rats were sacrificed by cervical dislocation, and four rats were set aside for dissection; the remaining 48 rats were divided into two groups to determine the effect of temperature on the levels of tissue elements. One group of rats was kept at 4ºC and the other group was kept at 18 ± 2ºC. The four rats that had been set aside were dissected immediately after sacrifice; the remaining 48 rats that were kept at two different temperatures were dissected 6, 12, 24, 48, 72, and 96 hours after death, with four rats in each group. The levels of elements in skeletal muscle tissue were determined by inductively coupled plasma-atomic emission spectrometry. The values for calcium (Ca), cupper (Cu), firon (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), sulphur (S) and zinc (Zn) were evaluated by the Kruskal-Wallis test and Mann-Whitney U test with Bonferroni's correction. **Results:** Considering the results for both temperatures, Fe, Na, and K showed significant changes at 4ºC and 18 ± 2ºC. **Conclusion:** Our results suggest that these three elements in skeletal muscle tissue may be useful for determining postmortem interval. For this reason, a more detailed study on the levels of Fe, Na, and K in rat skeletal muscle tissue including a larger number of rats is planned in the near future.

Key Words: Postmortem changes; skeletal muscle LIM protein 1, rat; minerals; forensic medicine

ÖZET Amaç: Bu deneysel çalışma, sıçanların iskelet kası dokusundaki postmortem element seviyelerini tespit etmek ve postmortem interval ile ilişkisini değerlendirmek için planlanmıştır. **Gereç ve Yöntemler:** Bu amaçla, 52 adet üç aylık Sprague-Dawley cinsi erkek sıçana servikal dislokasyon yöntemi ile ötanazi uygulandıktan sonra dördü hemen diseke edildi. Diğer 48 sıçan ise, cesedin kaldığı ortam ısısının doku element seviyelerine etkisinin araştırılması amacıyla iki gruba ayrıldı. Gruplardan biri 4ºC'de, diğeri 18 ± 2ºC'de bekletildi. Daha sonra her iki ısıda bekletilen sıçanlardan dörder adedine, ölümden 6, 12, 24, 48, 72 ve 96 saat sonra disseksiyon uygulandı. Her sıçandan iskelet kası örnekleri alındı. Örnekler, mikrodalga yakma yöntemiyle analize hazır hale getirilerek, indüksiyonla birleştirilmiş plazma-atomik emisyon spektrometre cihazında element düzeyleri ölçüldü. Kalsiyum (Ca), bakır (Cu), demir (Fe), potasyum (K), magnezyum (Mg), sodyum (Na), fosfor (P), kükürt (S) ve çinko (Zn) elementlerine ait değerler, istatistiksel olarak Kruskal-Wallis testi ve Bonferroni-düzeltmeli Mann-Whitney U testi ile değerlendirildi. **Bulgular:** Her iki ısıdaki bulgular ortak olarak değerlendirildiğinde, Fe, Na ve K elementlerinin 4ºC ve 18 ± 2ºC'lerde anlamlı değişim gösterdiği saptandı. **Sonuç:** Elde edilen bulgular, iskelet kası dokusundaki bu üç elementin postmortem intervalin belirlenmesinde faydalı olabileceğini göstermektedir. Bu nedenle, yakın gelecekte daha fazla sayıda sıçan üzerinde, iskelet kası dokusundaki Fe, Na ve K seviyeleri ile ilgili daha ayrıntılı bir çalışma planlanmıştır.

Anahtar Kelimeler: Postmortem değişiklikler; sıçan iskelet kas dokusu LIM protein 1; mineraller; adli tıp

Turkiye Klinikleri J Med Sci 2010;30(4):1332-8

Postmortem changes evaluated to estimate the time interval death after, include different processes such as body cooling and hypostasis, supravital reactions, autolysis, rigor mortis, and putrefaction.[1] Forensic entomology and the findings of macroscopic eye examination are also reported to be used in postmortem interval (PMI) determination.[2,3]

Currently, there are few scientific methods based on chemical measurements that can be used to generate information regarding the PMI. Typically, such information is gained through the cooperation of trained forensic scientists who provide information based on experience and opinion. For example, estimating the PMI prior to the onset of putrefaction (36-72 h) generally involves visual inspection of the body appearance (i.e., rigor and livor mortis) and determining the core body temperature. Changes in blood and cerebrospinal fluid biochemistry are determined, however these measurements are subject to considerable error. The most useful biochemical indicator of the PMI prior to putrefaction is the K content of the vitreous humor.[4]

Most of the chemical methods used for estimation of the time after death are based on some underlying principles. One of the principles is autolysis, with breakdown of cell membranes and diffusion due to the diffusion gradient, according to Fick's law of diffusion. The higher the concentration gradient, the more suitable is the substance for the estimation of the time since death; depending on the volume of distribution.[5] Autolysis starts approximately 4 min after death. As cells of the body are deprived of oxygen, carbon dioxide in the blood increases, pH decreases, and wastes accumulate, which poison the cells. Concomitantly, cellular enzymes (e.g., lipases, proteases, and amylases) begin to dissolve the cells from the inside out, eventually causing them to rupture and release nutrient-rich fluids. After an adequate number of cells have ruptured, nutrient rich-fluids become available and the process of putrefaction can begin. Putrefaction is a complicated process, however it primarily depends on temperature and to a lesser extent on moisture, and is the result of a complex assortment of processes, ranging from enzymatic digestion and bacterial action to environmental conditions.[6] As a result of putrefaction developing after autolysis, tissue and organ unity is deformed. For this reason, liquid and element exchange occur among different tissues between intracellular and extracellular fields.

In this study, skeletal muscle tissue was selected for analysis because the skeletal muscle tissue of the extremities is topographically isolated from the large organs and blood vessels of the abdominothoracic cavity, and thus autolytic changes and putrefaction proceed slower compared to body fluids and organs. This experimental study was designed to determine postmortem changes in element levels in rat skeletal muscle tissue and to evaluate its relation with the PMI.

MATERIAL AND METHODS

Selcuk University Experimental Medicine Research and Application Center Experimental Animals Ethics Committee permission was obtained before beginning the study (Decision Number: 2004/38).

EXPERIMENTAL ANIMALS AND GROUPING

Rats were selected for this study because of their small size and ease-of-care. Fifty-two, three-month old male Sprague-Dawley rats, weighing 210 ± 10 grams, were fed by rat pellet fodder ad libitum.

Rats underwent cervical dislocation by an appropriate method.[7] Four of the rats that had undergone cervical dislocation were separated for immediate dissection. These four rats were used for determining "zero hour values" for both temperatures. The remaining 48 rats were divided into two groups to investigate the effects of environmental temperature on the levels of tissue elements. The first group of rats was kept in the refrigerator (4 ºC) and the second group was kept at room temperature (18 ± 2 ºC).

EXTRACTION OF TISSUE SAMPLES

The four rats that were set aside following cervical dislocation underwent immediate dissection. The muscles of the right frontal thigh were rid of fat tissue, and samples were taken. After 6, 12, 24, 48, 72

and 96 hours following cervical dislocation, dissection was performed on four rats in each of the two groups that were kept at different temperatures.

All the samples weighed between 0.4 and 0.6 g. The obtained samples, were kept with the methods previously used in the studies involving the levels of elements in biological samples, and were put into 10 ml polypropylene tubes, kept in 10% HNO_3 for 24 hours and washed with distilled water.[8,9] The samples were kept at -20ºC until they were analyzed.

PREPARATION OF SAMPLES AND MICROWAVE DIGESTION

In our study, the pressurized microwave oven digestion method was used.[8] Microwave digestion is a fast and effective procedure for the breakdown of biological samples and results in a thorough digestion of organic material in a reduced sample preparation time.[10,11]

The samples were put into XP-1500 Tetrafluormethaxil (TFM®; Hoechst AG, Frankfurt, Germany) tubes (CEM Corporation, Matthews, NC, USA) after they were weighed. Ten ml of 65% HNO_3 was added to each tube. The digestion took place in a pressurized microwave oven (Mars 5, CEM Corporation, Matthews, NC, USA) at 150 psi pressure and 180ºC. When the desired pressure and temperature were reached, the tubes were kept at this stage for 10 minutes and then they were cooled. After cooling, the tubes were opened and their contents were poured into 25 ml volumetric flasks washed with 10% HNO_3 and distilled water. The samples were brought to 25 ml with deionized water with 18 Ωcm^{-1} resistance (P.Nix UP 900, Human Corporation, Seoul, Korea).

Volumetric flasks were shaken for 10 minutes to homogenize the contents. Later, this liquid was placed into 10 ml polypropylene tubes which were kept in 10% HNO_3 for at least for 24 hours, were washed with distilled water and then were numbered.

The sample preparation process was compared to bovine liver NIST 1577b (National Institute of Standards & Technology, Gaithersburg, MD, USA) which was used as a standard reference material (SRM) in order to verify the accuracy of the analysis. A total of 10 samples were prepared by digestion with the microwave method for analysis, each weighing at least 250 mg.

ANALYSIS OF TISSUE ELEMENT LEVELS WITH INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY (ICP-AES)

ICP-AES was preferred due to its speed, sensitivity, inexpensive cost, and the fact that it can analyze many elements concurrently.[12,13] Before the analysis, ICP-AES was calibrated with ICP multi-element standard solution IV (Merck, Darmstadt, Germany). Ca, Cu, Fe, K, Mg, Na, P, S, and Zn levels were measured with ICP-AES (Vista AX, Varian Inc., Melbourne, Australia) in the samples which were ready for the analysis, and results were expressed as μg/g.

STATISTICAL ANALYSIS

Comparisons of the values obtained from the ICP-AES analysis were performed with the Kruskal-Wallis test for each temperature group. When a significant difference was detected with the Kruskal-Wallis test, the Mann-Whitney U test with Bonferroni's correction was used to identify the source of the difference. In addition, Mann-Whitney U test was used for comparisons of the values of two temperature groups in each postmortem interval (hours).

RESULTS

The results of the SRM (NIST 1577b bovine liver) analysis that was used to determine the reliability of the ICP-AES and the values in the SRM certificate were shown in Table 1. Repeated anaysis of the bovine liver certified reference material from NIST gave a recovery that was 100 ± 5% of the target value for all elements.

Median (minimum-maximum) values of element levels in skeletal muscle tissue of rats are given in Table 2. Among the nine analyzed elements, only Fe, Na, and K were shown to have significant changes at 4ºC and 18 ± 2ºC; Ca, Cu, Mg, P, S, and Zn did not show any significant changes under any circumstances (Table 2).

TABLE 1: Accuracy and precision of ICP-AES (Standard reference material: NIST 1577b bovine liver).

Elements	ICP-AES Wave length (nm)	Mean measure value (µg/g) (n= 10)	Certified content (µg/g)
Ca	396.847	113 ± 15	116 ± 4
Cu	327.395	158 ± 6	160 ± 8
Fe	238.204	192 ± 18	184 ± 15
K	766.491	9495 ± 172	9940 ± 20
Mg	279.553	605 ± 24	601 ± 28
Na	589.592	2340 ± 101	2420 ± 60
P	213.618	10864 ± 284	11000 ± 300
S	181.972	7510 ± 66	7850 ± 60
Zn	213.857	131 ± 12	127 ± 16

ICP-AES: Induction coupled plasma-atomic emission spectrometry.
NIST: National Institute for standards and Technology.

TABLE 2: Median (minimum-maximum) values of element levels in skeletal muscle tissue of rats kept at 4 °C and 18 ± 2 °C after death (µg/g wet weight; n=4 for all groups).

	Temperature	Postmortem interval (hours)							P*
		0	6	12	24	48	72	96	
Ca	4 ºC	77.6 (61.0-91.5)	59.7 (51.6-65.8)	58.2 (53.6-88.5)	60.1 (55.9-64.1)	95.9 (75.2-112.7)	67.0 (63.8-71.1)	62.7 (55.3-70.8)	n.s.
	18 ± 2 ºC		64.3 (59.5-91.8)	64.6 (55.0-68.8)	76.3 (63.3-93.4)	65.9 (52.8-71.2)	66.9 (63.1-73.4)	75.1 (64.1-91.6)	n.s.
Cu	4 ºC	0.42 (0.03-1.38)	0.64 (0.37-1.09)	0.14 (0.02-0.23)	0.31 (0.09-0.82)	0.99 (0.64-1.35)	1.06 (0.53-1.47)	0.35 (0.23-1.27)	n.s.
	18 ± 2 ºC		0.49 (0.04-1.50)	0.31 (0.07-0.56)	0.90 (0.50-1.01)	1.18 (0.25-1.45)	0.99 (0.72-1.30)	1.27 (0.47-1.37)	n.s.
Fe	4 ºC	15.5 (15.0-16.9)	15.2 (12.3-20.3)	14.4 (14.2-26.3)	15.2 (14.3-16.3)	26.5 (23.7-32.1)	25.8 (21.8-35.0)	32.7 (28.7-34.6)	<0.05
	18 ± 2 ºC		17.0 (14.9-26.8)	15.0 (12.7-16.0)	**27.2 (18.9-40.2)	25.8 (21.2-30.2)	26.1 (23.7-26.5)	28.0 (23.7-29.3)	<0.05
K	4 ºC	4531 (4011-4772)	4042 (3958-4175)	3704 (3642-3993)	3900 (3757-4009)	3759 (3349-3860)	3705 (3491-3933)	3570 (3146-3637)	<0.05
	18 ± 2 ºC		3926 (3770-4043)	3953 (3656-3964)	3781 (3359-3925)	3711 (3552-3832)	3577 (3483-3735)	3626 (3564-3710)	<0.05
Mg	4 ºC	315 (284-349)	302 (139-305)	289 (281-312)	299 (284-306)	294 (153-311)	293 (279-318)	289 (281-303)	n.s.
	18 ± 2 ºC		315 (299-340)	299 (292-305)	297 (295-299)	292 (280-306)	306 (291-309)	306 (294-320)	n.s.
Na	4 ºC	629 (524-692)	636 (491-720)	673 (666-695)	755 (627-767)	786 (758-837)	784 (685-847)	799 (747-888)	<0.05
	18 ± 2 ºC		721 (601-760)	709 (635-755)	770 (677-830)	784 (714-856)	822 (758-910)	914 (837-1059)	<0.05
P	4 ºC	2349 (2078-2494)	2234 (1477-2328)	2150 (2040-2207)	2161 (2127-2243)	2106 (1884-2228)	2098 (2003-2295)	2131 (1860-2168)	n.s.
	18 ± 2 ºC		2275 (2172-2388)	2155 (2102-2252)	2125 (1991-2137)	2176 (2068-2272)	2155 (2039-2190)	2089 (2039-2150)	n.s.
S	4 ºC	2709 (2439-2892)	2738 (1331-2867)	2581 (2488-2694)	2650 (2546-2723)	2502 (1702-2739)	2584 (2416-2759)	2573 (2380-2811)	n.s.
	18 ± 2 ºC		2797 (2719-2842)	2635 (2576-2674)	2566 (2531-2630)	2680 (2488-2754)	2601 (2540-2657)	2603 (2511-2706)	n.s.
Zn	4 ºC	15.4 (13.5-17.7)	13.7 (8.7-16.3)	13.2 (9.7-16.1)	12.3 (11.0-13.3)	15.0 (12.4-17.6)	12.4 (11.9-15.9)	13.8 (13.1-15.8)	n.s.
	18 ± 2 ºC		18.2 (13.1-28.2)	13.9 (9.2-21.1)	13.6 (9.0-16.1)	13.7 (11.7-15.7)	12.1 (11.9-15.5)	14.4 (9.5-17.1)	n.s.

* Kruskal-Wallis test.
**P< 0.05 compared to 40C.
n.s.= not significant.

Fe increased at 4 °C at 48, 72, and 96 hours postmortem, compared to the levels at 0, 6, 12, and 24 hours. At 18 ± 2 °C, the levels of Fe increased at 24, 48, 72, and 96 hours postmortem when compared to 0, 6, and 12 hour levels (Figure 1).

Na increased at 4ºC at 48, 72, and 96 hours postmortem compared to 0, 6, and 12 hour levels. At 18 ± 2 °C, Na increased at 48 and 72 hours postmortem when compared to 0, 6, and 12 hour levels, and at 96 hours postmortem compared to levels at 0, 6, 12, and 24 hours (Figure 2).

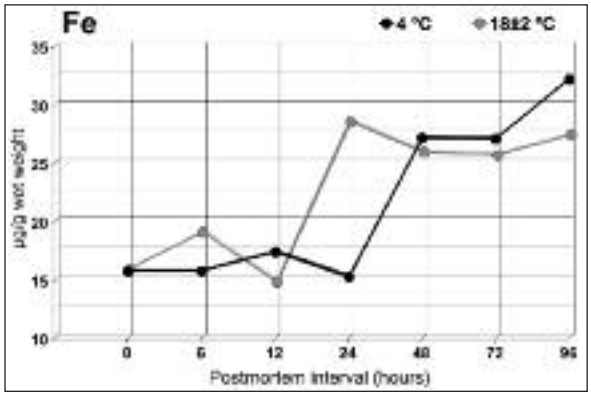

FIGURE 1: Changes in mean Fe levels of skeletal muscle tissue of rats kept at 4ºC and 18 ± 2ºC.

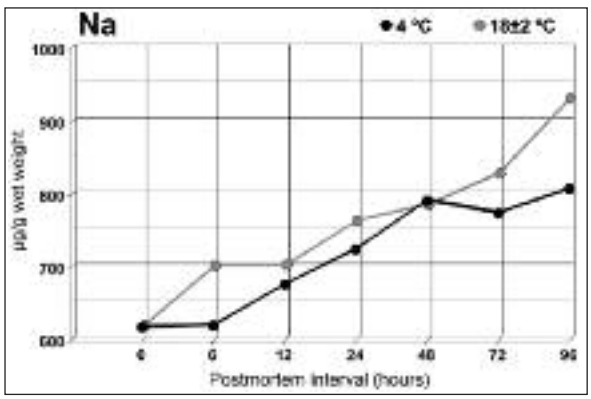

FIGURE 2: Changes in mean Na levels of skeletal muscle tissue of rats kept at 4ºC and 18 ± 2ºC.

K decreased at 4ºC at 12 and 24 hours postmortem when compared to 0 hour, at 48 and 72 hours postmortem when compared to 0 and 6 hours, and at 96 hours postmortem when compared to 0, 6, 12, and 24 hours. At 18 ± 2ºC, K decreased at 12 hours postmortem when compared to 0 hours, at 24 hours postmortem when compared to 0 and 12 hours, at 48 hours postmortem when compared to 0 hours, at 72 hours postmortem when compared to 6 hours, and at 96 hours postmortem when compared to 0 and 6 hours (Figure 3).

There were not significant differences of the element levels between two temperature groups, except Fe levels at 24 hours (Table 2).

DISCUSSION

In determining the time of death, as reflected by the advancements in technology, studies have been conducted using different analytic methods, including chemical analysis in body fluids, such as the vitreous humour, cerebrospinal fluid, blood, and synovial fluid.[1,4] With respect to studies performed on tissues and organs to determine the PMI, specific measurements have been reported as follows: in lungs, lactate and malate dehydrogenase levels; in brain, liver, heart, and kidney samples, vascular endothelial growth factor levels; in heart, tropin I levels; in muscle, creatinine concentrations; and in lungs and muscle tissue, calmodulin-binding protein activity.[14-18]

Reports suggested that the concentrations of Ca, Mg, Cu, Fe, Zn, Mn, and Al were not homogenous in the brain.[8] Cockell et al. in their analysis of rat lungs, stated that the levels of elements in the samples obtained from different parts of the lungs were compatible and added that a sample from any part of the lung could reflect the element concentration of the entire lung.[19] Thus, when it is necessary to take samples to determine the PMI, we need to know whether the tissues in people show different characteristics in different parts of the organs and we need to consider this fact. Since we took the samples from the same parts of the skeletal muscles of the rats in our analysis, we can say that there are no differences within the tissue.

In the studies in which some fluids, such as the vitreous humour, spinal fluid, blood, pericardial fluid and synovial fluid were used to determine the time after death, the changes in the levels of elements play a major role. However, our review of the literature revealed that no study existed, either

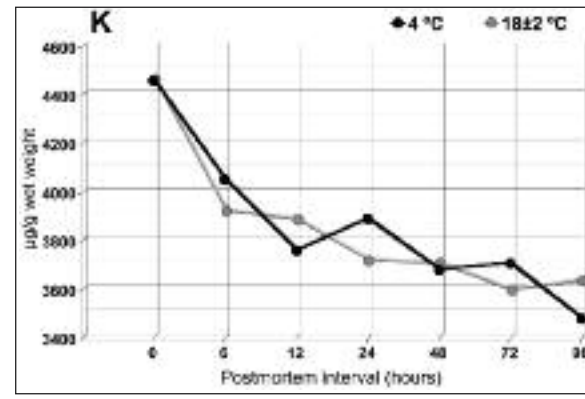

FIGURE 3: Changes in mean K levels of skeletal muscle tissue of rats kept at 4ºC and 18 ± 2ºC.

experimental or based on autopsy material, in which the levels of elements were measured to determine the PMI, making it difficult for us to evaluate our findings.

Our results revealed that Fe levels increased during the first 12-48 hours after death (Figure 1). This increase was more evident at 4ºC between 24 and 48 hours and at 18 ± 2ºC between 12 and 24 hours. Iyengar performed a study to examine the changes in levels of elements for in samples obtained from autopsy material to determine the biologic variations in the levels of tissue elements after death.[20] He stated that there were changes in the concentrations of some elements in lung samples obtained from rats and that were kept at different temperatures; however, this can be decreased by keeping the corpse in the cold, and in so doing, may not stop, but slow down the postmortem events. The fact that Fe increases earlier at 18 ± 2ºC during the postmortem process might result from the acceleration of autolysis and putrefaction at high temperatures (Table 2, Figure 1). Nevertheless, the values obtained from rats that were kept at two different temperatures revealed that Fe increased clearly after 48 hours.

Na increased during the postmortem process at both temperatures (Figure 2). In contrast, K declined during the postmortem process (Figure 3). This decrease was similar at both temperatures and was particularly evident during the first 12 hours.

There was a statistically significant difference between two temperature groups only in the Fe levels at 24 hours postmortem (Table 2). According to this finding it should be considered that, when used for PMI determination, temperature is important for Fe especially between 12 and 48 hours.

CONCLUSION

In our study, though all the samples were examined in terms of the levels of the nine elements, significant changes with the PMI only occurred for Fe, Na, and K. Na and K were affected less by the environmental temperature when compared to Fe. Our results suggest that these three elements in skeletal muscle tissue may be useful for determining PMI. For this reason, a more detailed study on the levels of Fe, Na, and K in rat skeletal muscle tissue with a larger number of rats is planned in the near future.

Acknowledgements

This study was supported by Selcuk University Scientific Research Projects Coordinating Office (Project Number: 05102009). We appreciate support by Selcuk University Experimental Medicine Research and Application Center (Project Number: 2004/38).

REFERENCES

1. Madea B, Henssge C. Timing of death. In: Payne-James J, Busuttil A, Smock W, eds. Forensic Medicine: Clinical and Pathological Aspects. 1st ed. London: Greenwich Medical Media Limited; 2002. p.91-114.
2. Tüzün A, Yüksel S. [Estimating post-mortem interval in forensic entomology investigations: Review]. Turkiye Klinikleri J Foren Med 2007;4(1):23-32.
3. Kocatürk BK, Balcı Y, Bal C. [The evaluation of the findings of macroscopic eye examination with regard to post-mortem interval (PMI) in 200 cases known death time]. Turkiye Klinikleri J Foren Med 2007;4(3):102-5.
4. Coe JI. Postmortem chemistry update: Emphasis on forensic application. Am J Forensic Med Pathol 1993;14(2):91-117.
5. Madea B. Is there recent progress in the estimation of the postmortem interval by means of thanatochemistry? Forensic Sci Int 2005;151(2-3):139-49.
6. Gill-King H. Chemical and ultrastructural aspects of decomposition. In: Haglund WD, Sorg MH, eds. Forensic Taphonomy: The Postmortem Fate of Human Remains. 1st ed. Boca Raton, FL: CRC Press; 1997. p.93-104.
7. American Veterinary Medical Association. 2000 Report of the AVMA panel on euthanasia. J Am Vet Med Assoc 2001;218(5):669-96.
8. Bush VJ, Moyer TP, Batts KP, Parisi JE. Essential and toxic element concentrations in fresh and formalin-fixed human autopsy tissues. Clin Chem 1995;41(2):284-94.
9. Rahil-Khazen R, Bolann BJ, Myking A, Ulvik RJ. Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled atomic emission spectrometry technique (ICP-AES). J Trace Elem Med Biol 2002;16(1):15-25.
10. Somogyi G, Jenei E, Szokol J, Buris L, Buris L Jr, Nagy D, et al. Distribution of inorganic elements in human autopsy tissue. Pharmazie 2000;55(3):239-42.
11. Cominos X, Athanaselis S, Dona A, Koutselinis A. Analysis of total mercury in human tissues prepared by microwave decomposition using a hydride generator system coupled to an atomic absorption spectrometer. Forensic Sci Int 2001;118(1):43-7.
12. Subramanian KS, Meranger JC. Simultaneous determination of 20 elements in some human kidney and liver autopsy samples by inductively-coupled plasma atomic emission spectrometry. Sci Total Environ 1982;24(2): 147-57.

13. Lalchev M, Ionov I, Daskalova N. Application of inductively coupled plasma atomic emission spectrometry in forensic science. J Anal Atom Spectrom 1997;12(1):21-4.
14. Gos T, Raszeja S. Postmortem activity of lactate and malate dehydrogenase in human liver in relation to time after death. Int J Legal Med 1993;106(1):25-9.
15. Thaik-Oo M, Tanaka E, Tsuchiya T, Kominato Y, Honda K, Yamazaki K, et al. Estimation of postmortem interval from hypoxic inducible levels of vascular endothelial growth factor. J Forensic Sci 2002;47(1):186-9.
16. Sabucedo AJ, Furton KG. Estimation of postmortem interval using the protein marker cardiac Troponin I. Forensic Sci Int 2003;134(1): 11-6.
17. Brion F, Marc B, Launay F, Gailledreau J, Durigon M. Postmortem interval estimation by creatinine levels in human psoas muscle. Forensic Sci Int 1991;52(1):113-20.
18. Kang S, Kassam N, Gauthier ML, O'Day DH. Post-mortem changes in calmodulin binding proteins in muscle and lung. Forensic Sci Int 2003;131(2-3):140-7.
19. Cockell KA, Fischer PW, Belonje B. Elemental composition of anatomically distinct regions of rat liver. Biol Trace Elem Res 1999;70(3):251-63.
20. Iyengar GV. Post mortem changes of the elemental composition of autopsy specimens: Variations of K, Na, Mg, Ca, Cl, Fe, Zn, Cu, Mn and Rb in rat liver. Sci Total Environ 1980;15(3):217-36.