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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

SIÇANLARDA KARACİĞER VE ÇİZGİLİ KAS BİLGİSAYARLI TOMOGRAFİ ATENÜASYONLARI İLE POST-MORTEM İNTERVAL TAYİNİ

POST-MORTEM INTERVAL ESTIMATION BASED ON LIVER AND STRIATED MUSCLE COMPUTED TOMOGRAPHY ATTENUATIONS IN RATS

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ÖZET

AMAÇ: Post-mortem interval (PMI) tayinini hedefleyen ölüm sonrası bilgisayarlı tomografik çalışmalara ilgi artmaktadır. Literatürde çoğu çalışma, kontrolsüz veya nispeten daha az kontrollü bir durumda yürütülmüştür. Bu çalışma, sıkı kontrollü çevresel koşullar altında PMI tahmininde karaciğer ve çizgili kasların bilgisayarlı tomografi atenüasyonlarının kullanılabilirliğini araştırmaktadır.

GEREÇ VE YÖNTEM: 30 Wistar Albino sıçanın ölümden sonraki ilk 30 dakikada ve post-mortem 12, 24, 36, 48, 72, 96, 120, 144 ve 168. saatte bilgisayarlı tomografi görüntüleri alındı. 0,12 cm^2 ilgi alanında karaciğerin lateral lobları ve paraspinal kasın tekrarlayan atenüasyonları kaydedildi.

BULGULAR: Karaciğer ve paraspinal kasların atenüasyon değerleri, ölümden 12 saat sonra daha yüksek bulundu. Hem erkek (p=0,01) hem de dişi sıçanlarda (p=0,01) otopsiden 30 dakika sonra elde edilen görüntüler ile otopsiden 12 saat sonra elde edilen görüntüler arasında anlamlı fark gözlendi. PMI tayini için karaciğer ve kas dokusu için farklı atenüasyon eşik değerleri belirlendi."

SONUÇ: Önerilen model ile karaciğer ve çizgili kasların atenüasyon değerlerinin kullanımı, PMI tayini için faydalı bulunmuştur. Atenüasyonun eşik değerleri, özellikle karaciğer için, PMI sınırlandırılmasında kullanılabileceği anlaşılmıştır.

ANAHTAR KELİMELER: Post-mortem interval tayini, Post-mortem bilgisayarlı tomografi, Karaciğer atenüasyonu, Kas atenüasyonu .

ABSTRACT

OBJECTIVE: The interest on post-mortem computed tomographic studies targeting post-mortem interval (PMI) estimations is increasing. Most studies have been conducted in an uncontrolled or relatively less controlled condition. However, this study investigates the usefulness of computed tomography attenuations of the liver and striated muscles in PMI estimation under strictly controlled environmental conditions.

MATERIAL AND METHODS: Post-mortem computed tomography images of 30 Wistar Albino rats were obtained in the first 30 min after death and 12, 24, 36, 48, 72, 96, 120, 144 and 168-hours following death. Repeated attenuations of both the lateral lobes of the liver and paraspinal muscle were recorded in 0.12-cm^2 regions of interest.

RESULTS: The attenuation values of the liver and paraspinal muscles were higher 12-hour post-mortem. A significant difference was observed between the images obtained 30 minutes post-mortem and those obtained 12 hours post-mortem in both male (p=0.01) and female rats (p=0.01). Different cut-off attenuation values for the liver and muscle tissue were determined to estimate the post-mortem interval.

CONCLUSIONS: The use of attenuation values of the liver and striated muscles with the proposed model was found to be beneficial for the determination of PMI. The cut-off values of attenuation, especially for the liver, can be used in the delimitation of the post-mortem interval.

KEYWORDS: Post-mortem interval estimation, Post-mortem computed tomography, Liver attenuation, Muscle attenuation.

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INTRODUCTION

For many decades, post-mortem interval (PMI) estimation has been a frequently studied topic in forensic sciences. Scientists from various disciplines have described several PMI estimation methods. However, there is still no universally valid method to estimate PMI in a certain manner (1, 2).

Post-mortem radiological methods have been gradually developing following the 'Virtopsy project', which was announced by a team led by Thali et al. (3). Based on this encouraging inspiration, forensic scientists have shown an increasing effort toward post-mortem radiological studies to establish a post-mortem diagnosis or determine PMI. Among these radiological studies, a few dealt with PMI estimation (4 – 7), whereas most were dedicated to enlightening the radiological characteristics of post-mortem changes compared with antemortem data (8 – 17). However, some studies were conducted under relatively controlled conditions, while some were performed in a relatively open and slightly controlled environment, which potentially exposed the study material, the cadavers, to numerous variables such as insect activity, humidity, heat and cold. The following questions led to this study: 'What is the pattern of post-mortem liver or striated muscle computed tomography (CT) attenuation changes under strictly controlled environmental conditions?' and 'How useful is this for PMI estimation in comparison to previous studies conducted in open or relatively uncontrolled environment?' Therefore, our team prepared a longitudinal design that can provide a close environment in which temperature and humidity are both controlled.

As the basic study point of this paper, the attenuation value measures the ability of an incident energy beam to penetrate a material. It is the quantity of the beam, such as sound waves and X-rays, weakened by the material while passing through. In routine radiological screening applications, CT attenuation values are revealed based on a linear density scale, as 'Hounsfield units' (HU), which is calculated by the following formula:

$$HU = 1000 \times ((\mu tissue - \mu water) / \mu water)$$

(μ stands for CT linear attenuation coefficient)

This study investigates the use of CT attenuation of the liver and striated muscle in estimating PMIs and reveals the relationship between post-mortem attenuation values of the liver and striated muscles on CT images in rat models.

MATERIAL AND METHODS

The Design of the Study

Thirty healthy Wistar albino rats (15 males and 15 females) with an age of 22 weeks were euthanised using CO^2 inhalation in the beginning of the study. Wistar albino rats were chosen because of their relatively similar enzymatic systems to humans in both striated muscles and liver tissue. The rats were weighed just after death, and the mean weight was 351.9 g (standard deviation (SD) = 12.9) for female rats and 401.1 g (SD = 12.6) for male rats. All rat cadavers were fixed in the supine position to minimise the effect of post-mortem lividity on the right (RLL) and left (LLL) lateral lobes of the liver. The rat cadavers were kept in a strictly controlled environment in a Microtest® MIT 120 test cabin (ELECTROMATIC Equipment Co., Inc., Cedarhurst, NY, USA). The temperature and relative humidity were set to 23°C and 40% respectively. The actual temperature and humidity values were measured once every 30 minutes using the automatic internal measurement device in the test cabin. The mean cabin temperature and relative humidity were 23.1°C ± 0.6°C and 40.7% ± 2.4% respectively, during the whole procedure.

CT Protocol

CT was used in this study because of its frequent use in post-mortem imaging as a cheaper, faster and easily accessible radiological screening method. The first CT scans of all animals were conducted 30 minutes post-mortem and follow-up scans were performed at 12-, 24-, 36-, 48-, 72-, 96-, 120-, 144- and 168-hours following death. CT scans were performed using a 16-row channel CT device with a 1-mm detector collimation. The cadavers were scanned in the axial plane, and images were reconstructed using the filter back projection method in an H10f filter and 1.5-mm slice thickness. The image matrix was 512 × 512. The attenuation values (in HU) of the RLL and LLL of the liver and paraspinal muscles (PSM) were measured in 0.12-cm^2 regions of interest

(ROIs) by two blinded radiologists (a certified radiologist with 25-year experience and a trainee with four-year experience). The 0.12-cm^2 ROIs are the largest homogenous parenchymal areas without any main vasculature and bile ducts in the liver and a homogenous tissue site of the left paraspinal muscle between the L1 and L3 sections of the rats in this study. Three ROIs were selected from the most appropriate central parts of the RLL and LLL without the main vasculature and bile duct of the cadavers; similarly, three ROIs were selected from the left paraspinal muscle. The averages of the three acquired ROIs of the same site were calculated. Therefore, three ROIs (averages of the main ones) (two from the liver and one from the paraspinal muscles) were selected for each rat.

Ethical Committee

Ethics approval was obtained from Animal Experimentations Local Ethics Board of Hacettepe University (decision number: 2016/29). The study was carried out in accordance with the principles of "Guide for the Care and Use of Laboratory Animals".

Statistical Analysis

Statistical Package for the Social Sciences (version 24; IBM Corp., Armonk, NY, USA) was used for statistical analysis. Descriptive statistics were performed for the general properties of rat cadavers. An analysis of variance (ANOVA) was used to identify the correlations between attenuation values of different time zones and receiver operative characteristics to determine cut-off values. This point was the peak of timeline for steady changes in attenuation values.

Inter-observer and intra-observer reliability and repeatability of attenuation values were assessed using unweighted and linear-weighted kappa (κ) statistics, given that the attenuation value is ordinal. The agreement rate and 95% Wilson score confidence interval (CI) were revealed. P values of <0.05 were used to indicate statistical significance. The κ values were interpreted as follows: $\kappa < 0.20$: poor agreement; $\kappa = 0.21–0.40$: fair agreement; $\kappa = 0.41–0.60$: moderate agreement; $\kappa = 0.61–0.80$: good agreement and $\kappa = 0.81–1.00$: very good agreement.

RESULTS

Liver Attenuation Values

All attenuation values were measured from the RLL and LLL using areas free from putrefactive gases and vascular formations until the 72nd hour images. However, there was not enough area to get 0.12-cm2 ROIs without putrefactive signs in the 96th hour liver images. 'Swiss cheese' signs were seen on all liver images obtained 72 hours post-mortem with minor intact areas. Statistical analysis was performed on the mean values of the RLL and LLL since no significant differences were found between the RLL and LLL of each rat ($p = 0.69$). All liver attenuation values were significantly higher than that obtained 30 minutes post-mortem in rat cadavers. The liver attenuation values increased with an increasing PMI **(Figure 1)**.

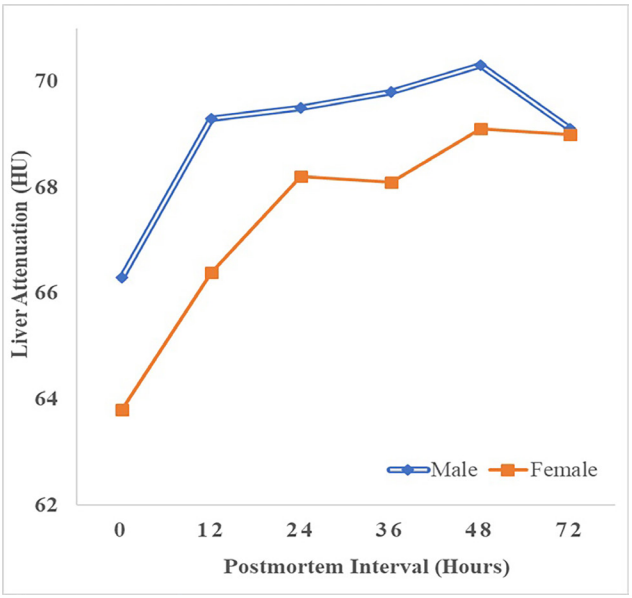

Figure1: Change of mean liver attenuations with time in male and female cadavers (HU: Hounsfield Unit, F: female, M: male)

The most significant difference was observed between the images obtained 30 minutes post-mortem and those obtained 12 hours post-mortem in both male ($p = 0.01$) and female ($p = 0.01$) rats respectively. Female cadavers showed significantly higher liver attenuation values, except in the images obtained 24-, 48- and 72-hours post-mortem. Receiver Operating Characteristics (ROC) analysis was performed to determine cut-off values to estimate PMI. Cut-off values of all male and female cadavers for 12th hour PMI are shown in **Table 1**.

Table1: Mean liver attenuation cut-off values of all individuals, male cadavers and female cadavers for 12-hour postmortem interval.

	Cut-off value	Sensitivity	Specificity	ROC AUC*
All cadavers	67.25 HU	80%	83.3%	87%
Female cadavers	65 HU	96%	73.3%	90.4%
Male cadavers	68.25 HU	81.3%	80%	86.8%

* ROC AUC: Area under the curve of receive operative characteristics

This point was the peak of timeline for steady changes in attenuation values, after which comparison of attenuation values become unreliable. The PSM attenuation values increased after death, as well. However, these values differed between different time points without showing any linear pattern **(Figure 2)**.

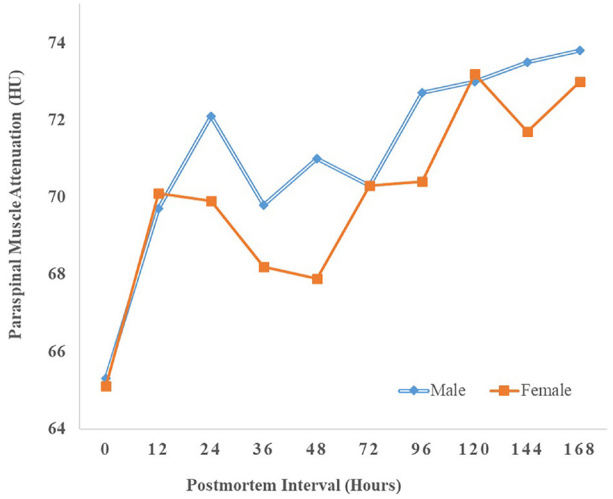

Figure 2: Change of PSM attenuations with time in male and female cadavers (HU: Hounsfield Unit, F: female, M: male)

Among mean liver attenuation values, PSM attenuation values showed the most significant increase in the first 12 hours post-mortem in both male (p = 0.01) and female (p = 0.01) rats. Liver CT images obtained 0–72 hours post-mortem are shown in Figure 3 **(Figure 3)**.

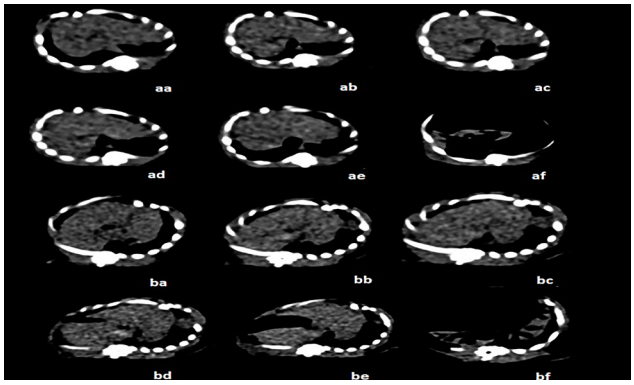

Figure 2: Postmortem Liver CT Scan Images for 0-72 hours (aa-af: Female rats, aa: 0 h, ab:12 h, ac: 24 h, ad: 36 h, ae:48 h, af: 72 h) (ba-bf: Male rats, ba: 0 h, bb:12 h, bc: 24 h, bd: 36 h, be:48 h, bf: 72 h)

PSM Attenuation Values

The PSM attenuation cut-off values of all cadavers for 12-hour PMI are shown in **Table 2**.

Table 2: PSM attenuation cut-off values of all individuals, male cadavers and female cadavers for 12-hour postmortem interval

	Cut-off value	Sensitivity	Specificity	ROC AUC*
All cadavers	68.5 HU	75.6%	86.7%	89.7%
Female cadavers	68.5 HU	81.5%	93.3%	84.6%
Male cadavers	67.5 HU	84.4%	73.3%	94.7%

* ROC AUC: Area under the curve of receive operative characteristics

After this point, changes in attenuation values of PSM were relatively unstable.

Inter- and Intra-Observer Agreement

Weighted kappa statistics indicated a high level of agreement for the inter- and intra-observer agreement, reliability and repeatability of the ROI choice and CT attenuation measurements **(Table 3)**.

Table 3: Inter- and intra-observer agreement

	Inter-observer Agreement			Intra-observer Agreement		
	RLL	LLL	PSM	RLL	LLL	PSM
n	90	90	90	90	90	90
Kappa (±se)	0.801±0.045	0.795±0.051	0.833±0.046	0.831±0.019	0.823±0.019	0.838±0.019
W. Kappa (±se)	0.861±0.035	0.856±0.037	0.903±0.030	0.892±0.012	0.893±0.023	0.892±0.013
Agreement Rate (%)	94.3	94.0	94.1	98.0	97.8	98.5
(95% CI)	80.9-93.4	77.4-91.7	80.7-94.0	85.1-90.2	84.5-90.0	85.9-91.2
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

n: number of measurements, se: Standard error, W: Weighted, CI: Confidence Interval of Wilson Score

DISCUSSION

In this study, there was an increase in attenuation values of the liver and striated muscles for both male and female rats with increasing PMI, except in CT images obtained at 72 hours post-mortem. The increase in the attenuation values has been attributed to post-mortem autolytic changes of tissues and protein degradation, which occur spontaneously in early post-mortem phases (18, 19). There was a slight decrease in attenuation values of the liver of our subjects at 72 hours post-mortem, which may be related to intra-parenchymal gas formation and increased bacterial succession. Our experimental model was designed to protect the RLL and LLL from post-mortem lividity as much as possible by placing and fixing the rats in the supine position during the procedure. On the other hand, this model caused the PSM to be affected by post-mortem lividity, a common post-mortem change, as a consequence of blood displa-

cement by gravity. Therefore, the PSM attenuation values decreased between 12- and 36-hours post-mortem1, which might be attributed to the post-mortem change secondary to lividity. PMI estimation based on radiological changes is a relatively new topic in the field. However, there are many studies in the last few years investigating the value of post-mortem CT changes in PMI estimation (4, 6, 8). The thyroid (4), heart (7, 9, 10), cardiothoracic index (8), muscle (11), liver (12), spleen (13), bone (14), brain (15), central nervous system (15, 16), lung (17), aorta (20) and surrenal glands (21) were studied to understand the changes during post-mortem processes in the literature. A summary of review of the related literature is presented in Table 4 **(Table 4)**.

Table 4: A summary of literature review regarding PMI and certain postmortem

Tissue	Finding	Target	Reference
Thyroid	Attenuation decrease on postmortem CT	Postmortem Change	(7)
Brain	Increase in both White and gray matter attenuations with loss of differentiation between gray and white matter	Postmortem Change	(15)
Heart and great vessels	Partial increase in attenuation of dependent areas and differentiated fluid levels in vessels due to postmortem hypostasis	Postmortem Change	(4, 9, 10, 21)
Spleen	Attenuation increase on postmortem CT	Postmortem Change	(13)
Lung	Hyper attenuated dependent areas due to postmortem hypostasis	Postmortem Change	(17)
Striated Muscle	Attenuation increase on postmortem CT	Postmortem Change	(11)
Liver	No significant change by increased postmortem interval	PMI	(25)
Vitreous	Attenuation increase in correlation with postmortem interval	PMI	(22)
Cerebrospinal Fluid	Attenuation increase in correlation with postmortem interval	PMI	(22)

To the best of our knowledge, this is the first study dealing with the relationship of the liver and striated muscle attenuation values and PMI estimation in a strictly controlled environment using a rat model. In a longitudinal post-mortem CT study from Japan (20), the widest and narrowest diameters of the aorta were scaled, and the ratio of these two diameters were investigated as a method of determining PMI, which is found to be useful.

This finding is parallel to the structural changes caused by post-mortem decomposition processes, as shown in this study. The radiodensities of the cerebrospinal fluid and vitreous humour were studied by Koopmanschap et al. (22). It has been stated that the attenuation values of the cerebrospinal fluid and vitreous humour was associated with PMI (16, 22). Since these biological fluids are preserved in a relatively closed environment, they are exposed to environmental conditions in a later stage compared with the liver. Therefore, i/n contrast to tissues relatively resistant to autolysis and putrefaction as suggested by the findings of this study, CT attenuation of the liver in the first 12 hours post-mortem seems to be more appropriate to be used. Okuma et al. (11) investigated the use of striated muscle attenuations in determining PMI in 33 human cadavers in a non-controlled environment within 20 hours of maximum PMI. Similar to the findings of this study, they showed a significant difference between antemortem and post-mortem striated muscle attenuations; however, in contrast to our findings, there was no strong correlation between striated muscle attenuation values and PMI in their study (11). This difference might be attributed to a wide spectrum of variables in human cadavers, such as the subjects' antemortem diseases, level of lividity, muscle mass and body mass index differences. This study revealed a correlation between PSM attenuation values and PMI, which might be attributed to the established stable environmental conditions and the use of a standard experimental rat model. In a preliminary study investigating a multi-factor methodology to estimate PMI with post-mortem CT features (23), they described morphological changes on post-mortem rabbit liver images. According to their study, they recorded the stages of post-mortem liver changes as stable, peripheral gas accumulation, liver shrinkage and separation due to intra-parenchymal gas formations. However, all these stages ended in less than 96 hours in this study, whereas it took 196 hours in Wang et al.'s study (23), which might be attributed to the differences in subjects and liver masses. Iwamoto et al. revealed that post-mortem changes of intestinal gas and portal venous gas volumes can be used for PMI estimation. Furthermore, they observed that the rate of post-mortem portal venous gas increased steeply from the 72nd hour

of post-mortem period. However, in contrast to our study, they did not examine the effects of environmental factors (24). In addition to our findings, the literature shows that the organs' minor structural decomposition due to enzymatic autolysis or bacterial decay results in changes in the CT attenuation values, which might be used in the estimation of early PMI. However, gross decomposition with excess gas formation takes longer, which makes its assessment more valuable in PMI estimation in 72 hours after death. Fischer et al. (25) also investigated post-mortem liver attenuation changes longitudinally in five human subjects in an uncontrolled environment. In contrast to this study, Fischer et al. (25) revealed no significant changes in liver tissue attenuation values in relation to PMI. This difference may be caused by the small number of cases and relatively uncontrolled conditions (e.g., age, sex and environmental conditions, such as humidity and temperature) in their study. In this study, the overall gender-related differences in CT attenuation levels of the liver and striated muscle were attributed to the antemortem characteristics of these tissues, such as weight differences, lipid metabolism and glycolysis/gluconeogenesis differences caused by the various activities of liver enzymes and female sex-hormones, and higher mitochondrial mass and oxidative/phosphorylative capacities of the female rats' striated muscle; however, as a limitation of this study, potential molecular mechanisms behind gender-related differences could not be identified (26 – 28). As another limitation of this study, a control group exposed to uncontrolled normal environmental conditions was not added since there were related data in the literature (11, 12, 25). Since excessive heat, cold and humidity strongly interfere with post-mortem decomposition processes, such as autolysis, bacterial or insect activity and decay of tissue, CT attenuation values might be extremely different from those achieved in this study. This study involved rats, which is the most important limitation, to achieve a standard model; therefore, the obtained results would not represent human subjects and cannot be directly compared with those of human studies. However, gathering a larger cohort of human subjects and providing strictly controlled conditions for such examinations on humans were impossible. Our experimental model was designed to protect the RLL and LLL from post-mortem lividity as much as possible by placing and fixing the rats in the supine position during the procedure, which unfortunately caused the PSM to be affected by post-mortem lividity. Additionally, because of a lack of similar studies, some referred articles described general comparisons between antemortem and post-mortem findings, instead of time-dependent changes after death.

The presented model suggests that a combination of liver and striated muscle attenuation values can be used as an additional and alternative method to estimate PMI at early post-mortem stages, particularly up to the first 12 hours post-mortem. Furthermore, the male and female subjects might reveal potentially different values due to gender-related differences, which suggest the importance of including subjects of both genders in such studies. In this respect, there is a strong need for further studies examining several groups of male and female human subjects under different controlled environmental conditions to determine the relationship of age, body mass index and environmental conditions with post-mortem changes in CT attenuation values of the liver and muscle.

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