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Taher, M.M.B., Skerry, T.M., Cohen, M.C. et al. (2024) Correlation of CT measurements of total body gas volume and hounsfield units with post-mortem interval. *Indian Journal of Forensic and Community Medicine*, 11 (3). pp. 111-118. ISSN: 2394-6768

<https://doi.org/10.18231/j.ijfcm.2024.025>

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Original Research Article

Correlation of CT measurements of total body gas volume and Hounsfield units with post-mortem interval

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ARTICLE INFO

Article history:

Received 09-08-2024

Accepted 12-09-2024

Available online 23-10-2024

Keywords:

Post-mortem interval (PMI)

Hounsfield units (HU)

Computed tomography (PMCT)

Total body gas volume (TBGV)

ABSTRACT

Background: Accurate estimation of the post-mortem interval (PMI) may be a matter of crucial importance in forensic investigations.**Aim and Objective:** A preliminary investigation to assess whether longitudinal changes in organ Hounsfield units (HU) and total body gas volume (TBGV), as measured from serial PMCT scans, correlate with postmortem interval (PMI).**Materials and Methods:** Eight euthanised lambs each had five whole body CT scans performed over seven days and measurements were taken from the brain, heart, lungs, liver, kidneys and spleen. HU tissue density was measured directly from the PMCT images, while TBGV was calculated using ImageJ software. A random effect model was fitted with the subject fitted as a random intercept. Ethical and Animal Welfare approval was obtained.**Results:** The average increase in TBGV was 422 ml/Kg with a change of 77 ml/Kg per day. For each additional post-mortem day, the HU of the brain, heart, lung, liver and spleen decreased by per day since death, while HU for the kidneys increased by day since death.**Conclusion:** Expect for the kidney where it increased, there was clear and progressive decrease in tissue densities and for all organs there was an increase in TBGV over time. However, the overlap in values between cases indicates that more work is required before either HU or TBGV can be developed as non-invasive methods to reliably determine time of death in humans.This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.For reprints contact: reprint@ipinnovative.com

1. Introduction

In forensic investigations and the term post-mortem interval (PMI) the time elapsed since death is critical to refer to the interval between the time of death and examination of the body.¹ Examination of ions and chemical changes or

enzymes involved in the death process that could not usually be detected by morphological methods is known as post-mortem biochemistry involved in the death procedure that might not usually be explained by morphological methods is known as post-mortem biochemistry.²

The forensic process can provide PMI primarily based on histologic and macroscopic changes. Decomposition is a natural process that occurs in the body's tissues

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and leads to decay. The tissue degradation usually occurs by microorganism activity, bacteria, including fungi and protozoa, which are produced from normal biota in the body of humans, especially in the gastrointestinal system. This process proceeds beyond the dry remains stage, as well as the bones continue to undergo putrefaction, though at a much slower rate.³ The decomposition products formed by decay are in the form of fluid, gas or salt. Different types of gas can be produced including carbon dioxide, sulphide, methane, sulphur dioxide, ammonia and hydrogen.⁴

Post-mortem imaging such as the CT approach for autopsy is highly prospective given their minimal invasiveness and increasing popularity. Furthermore, it is vital to crafting a worldwide standard procedure for post-mortem imaging for perinatal, prenatal, and pediatric cases to better understand the cause of death, and putrefaction factors to provide an alternative to traditional autopsy.⁵ The images are easily stored, and available for review if required. These methods can provide additional support to the autopsy such as information on the expected causes of death.⁶

Hounsfield units (HU) are used to measure the density of tissue from CT scans and represent the attenuation of the X-ray photons by tissues. The values of HU rely on the anatomical structures of the tissue and thickness which the beam passes through from.⁷ HU can provide quick and simple measurement and allows for differentiation of tissues such as the nature of the intraperitoneal fluid, cystic lesions, and/or hepatic steatosis.⁸ The CT scan images can provide information about blood fluids which are characterised and differentiated based on HU magnitudes and ranges.⁹ The HU scale at all tube energies used is standardised such that the HU value for water is 0 and for gas is -1000. The HU of blood radiodensity ranged between 40 and 60, but the exact value of HU will mainly depend on the cellular content. The serous fluids ranged between 15 to 30 HU but the exact value of HU depended on the content of protein in the cells.^{9,10} Also, they pointed out that there is a strong correlation between the HU values of blood in CT on immediate PMCT after CPR within the infusion range of 1000 ml and the before-death hemoglobin levels, suggesting the possibility of before-death anemia diagnosis at least in an early period after death.¹¹

The total body gas volume from PMCT can be determined by using existing software. The relationship between the total volume of gas and the body's decomposition may provide a useful non-invasive method for estimating the time of death. This study aimed to determine the degree of correlation between 1) the volume of gas resulting from the process of decomposition and 2) organ Hounsfield units (HU) with PMI.

2. Materials and Methods

This observational prospective study of eight lambs, the age of these at time of death one month. those lambs were slaughtered by intravenous pentobarbitone overdose. The time of death for each lambe was recorded before the started the CT scan at Sheffield Children's Hospital, The time of death for each lambe was recorded before the CT scan at Sheffield Children's Hospital, within about three hours after death, to start the scanning protocol. The lambs were divided into three groups; the first group with two lambs, and three lambs in the second and third groups between 11/04/2017 and 02/05/2017. This was to reduce congestion in the Radiology Department and facilitate the PMCT scanning process. Immediately after euthanasia, each cadaver was double-bagged and kept in a covered plastic box (to avoid exposure to flies and rodents) and kept at room temperature of approximately 19°C.

2.1. Image acquisition

Whole-body PMCT scans were performed on each lamb five times during the week after death and the position supine on the left side of all lambs in the boxes was the same on all scans. The lambs were transported daily to Sheffield Children's Hospital for CT scanning (the time between death and initial PMCT was about 3 hours) then returned to the storage room. All CT examinations were performed at Sheffield Children's Hospital, by a trained radiographer, using The CT scan used a Lightspeed VCT 64-slice helical CT scanner and used standard scanning parameters as 100 Kv and 60 mA. The axial slice thickness 0.625 mm was obtained and images were reconstructed on soft tissue and bone algorithms to produce 5 mm and 1.25 mm thick slices. The time taken for the whole PMCT image acquisition was less than 5 minutes per study. The scans were reformatted, and the average HU was calculated from six sites for each organ and each time point. The picture archiving and communication system (PACS) at Sheffield Children's Hospital was used to measure all Hounsfield units.

2.2. Hounsfield unit measurements

One observer researcher reconstructed and reformatted the PMCT images. For each lamb, the average HU was recorded by an individual observer: a circular ROI with an area ranging around 2.0 cm² was placed, as follows; brain (right and left frontal lobes, temporal lobes and occipital lobes, heart (right and left ventricles), lungs (right and left upper lobe, medial lobes and lower lobes), liver (right and left lobes), spleen, kidneys (cortices of upper and lower poles). The average of the HU of these measurements was calculated for each organ (Figure 1). As far as possible, the researcher obtained repeated longitudinal measurements from the same site for each organ.

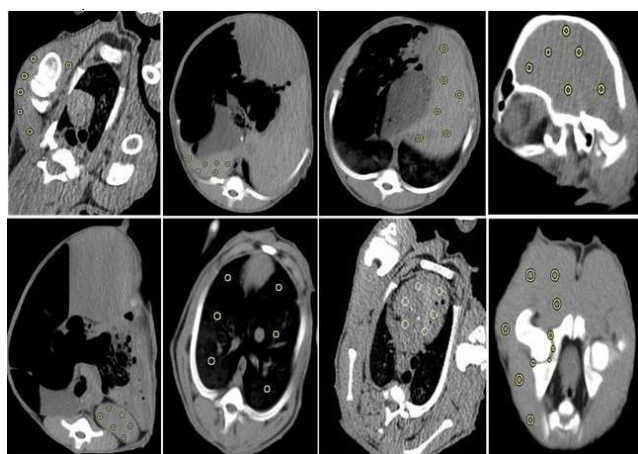


Figure 1: Shows the region of interest (ROI) and measuring of HU in different organs from six regions

2.3. Total body gas measurements

ImageJ software has grown from a simple tool to analyze two-dimensional images, into a widely utilized and used platform for modern biological and medical image analysis.¹² This software was used to measure the total body gas volume (TBGV) of each lamb at approximately 3, 16, 50, 75 and 160 hours after death. ImageJ software is a machine vision application capable of measuring size and volume. The measure of the volume of gas was by calculating total body gas volume by using ImageJ software. (All images that did not include a body area were excluded from the analysis (Figure 2 a). Artifacts were removed by cropping images in each slice in a rectangular shape (Figure 2 b). Images were then converted to 8-bit format and contrast was adjusted to distinguish gas from soft tissue (Figure 2 c) maximally. The software only allows segmentation into a maximum of two compartments. Therefore a two-step measurement process was adopted. First, for slice 1, body tissue and total gas (internal to the body and external to the body i.e environmental) were assigned different colors red and white respectively (Figure 2 d). The total volume of gas in Slice 1 (A_1) was measured. In the second step, the external gas volume in Slice 1 (B_1) was segmented from the image and measured (Figure 2 d; external gas = red). Therefore, the internal body gas volume for Slice 1 was A_1 minus $B_1 = V_1$. TBGV was calculated as the sum of body gas for all slices in the scan).

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 24 for PC (IBM Corp, Armonk, NY). Multilevel Random intercept models was used to analyses the data for the measurements of Hounsfield units and TBGV. Statistical significance was set at 0.05.

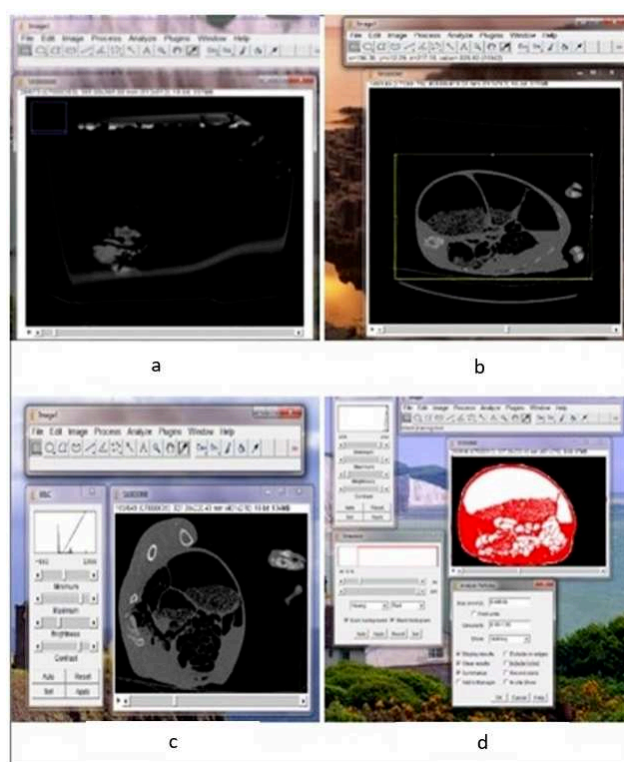


Figure 2: Steps followed to calculate total body gas volume using ImageJ software. (All images that did not include a body area were excluded from the analysis (a): Artifacts were removed by cropping images in each slice in rectangular shape (b): Images were then converted to 8-bit format and contrast was adjusted to maximally distinguish gas from soft tissue (c): The software only allows segmentation into a maximum of two compartments. Therefore, two-step measurement process was adopted. First, for slice 1, body tissue and total gas (internal to the body and external to the body i.e environmental) were assigned different colours red and white respectively (d): The total volume of gas in Slice 1 (A_1) was measured. In the second step, the external gas volume in Slice 1 (B_1) was segmented from the image and measured (Figure d; external gas = red). Internal body gas volume for Slice 1 was therefore A_1 minus $B_1 = V_1$. TBGV was calculated as the sum of body gas for all slices in the scan)

3. Results

3.1. Tissue density

However, the time interval before changes became obvious varied for the liver, spleen, brain, and heart, overall there was a significant reduction in organ HU with increasing PMI. Conversely, the kidney’s tissue showed an increase in the value of HU with time. These changes are summarised in Table 1 and Figure 3.

3.2. Gas volume

PMCT scan images showed that putrefaction of the lambs (as evidenced by observation of gas formation on the

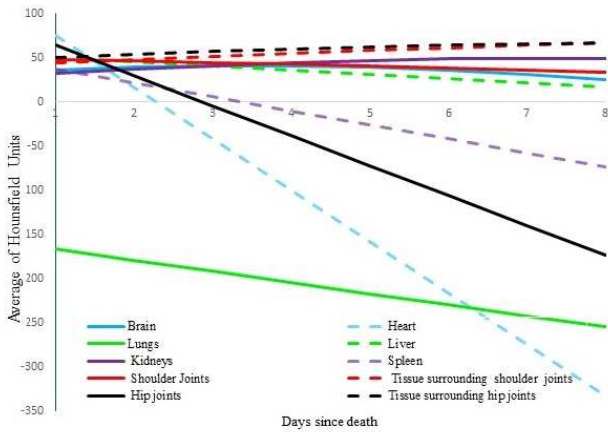


Figure 3: Change in HU of various tissues and organs in the first 160 h after death

images) began in the heart, liver, and stomach wall, and was seen at the time of the first scan (from about 3 hours after death) and visibly rising until the last scan at around 160 hours. Figure 4 illustrates this process in the heart. The average increase in TBGV was 422 ml/Kg with a change of 77 ml/Kg in the first 7 days after death. Table 2 and Figure 5 show the increase in TBGV / Weight (mL/Kg) for individual lambs and for each day following death. As can be seen, larger lambs had an increased TBGV ($p < 0.001$).

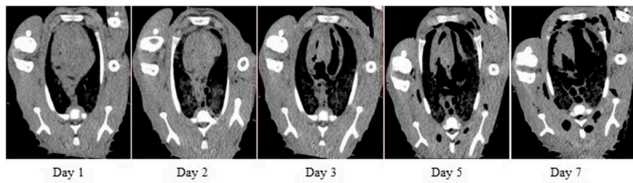


Figure 4: Axial CT images of the heart Note the progressive increase in gas within the heart that occurs with decomposition (arrows)

4. Discussion

With increasing importance on post-mortem imaging, this study sought to assess the utility of PMCT for showing measurable changes in HU and TBGV with increasing PMI estimating PMI.

To our knowledge, estimating post-mortem interval using the measurement of gas volume to estimate post-mortem interval has not been reported.

Despite several years of research, the accuracy of the time since death has not improved significantly, and there is no single specific method, that can be used reliably.¹³

Post-mortem changes occur in the early and late phases. Early post-mortem changes include rigor mortis, algor mortis, and livor mortis while late post-mortem changes

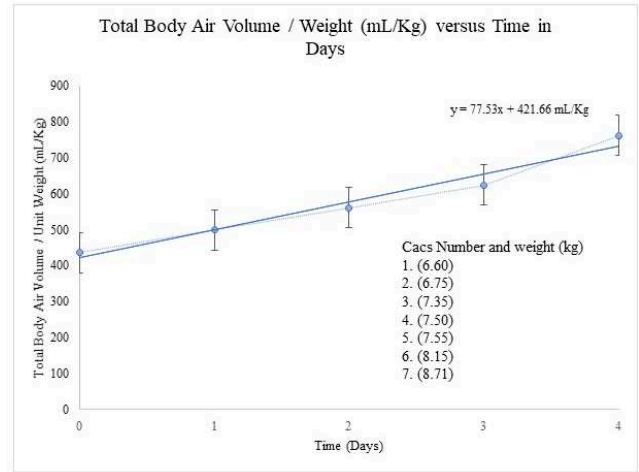


Figure 5: Change in total body gas volume for each animal over time

involve the breakdown of soft tissue leading to detectable macroscopic changes.¹⁴ After the death of every organism death will follow a natural decomposition process. In the early stages, decay may not be visible to the naked eye because the process starts at the cellular level. Then the process will move to a macroscopic level and result in many post-mortem changes.¹⁵ The current estimate of the date of death is based on a combination of examination made of the body and the scene of death. Observed conditions involving the body include algor mortis, decomposition, and livor. Also, the environment plays an important role in the processing of putrefaction and influences the levels of electrolytes and other biochemical change factors in post-mortem samples.¹⁶

These changes will occur in different degrees of constancy about their progress to their rate and an array of detailed and environmental factors. As a result of the variability can be observed the different stages of putrefaction on the same body at the same time¹⁴ and changes in the decomposition process can significantly change the estimate of the time of death.¹⁷ All of these factors make estimation of PMI difficult and the evaluation of more precise methods could be beneficial.

Regarding PMI and HU, we showed three patterns of change: initial insignificant increase in HU followed by an unimportant negative correlation in the brain tissue; immediate and constant decrease in HU in lung, liver, spleen, and heart; immediate and constant increase in HU for kidneys tissue.

Firstly, in the brain, there was a slight growth in HU (peaking at 16 hours and sustained for 3 days), following this, there was an unimportant HU reduction in all following scans. Wang et al. (2017) stated similar results for the brain tissue of rabbits, in that HU were unchanged for the first 27 hours after death. This was followed by a phase of

Table 1: Change in organ HU over time

Organ	Time before significant change in HU (Hours)	Average Hounsfield Unit (HU) for each day							Rate of decrease per day	p value	Intercept	p value
		1	2	3	4	5	6	7				
Brain	72	34	37	44	-	34	-	27	0.4	0.414	37	0.001
Heart	16	44	51	54	-	-270	-	-289	58	0.001	76	0.001
Hips–joints	16	47	47	48	-	-147	-	-141	34	<0.001	62	<0.001
Hips–soft tissue	16	47	52	56	-	61	-	65	3	<0.001	51	<0.001
Kidneys	16	31	33	41	-	45	-	48	3	<0.001	37	<0.001
Liver	16	51	47	36	-	27	-	21	5	<0.001	51	<0.001
Lung	16	-145	-	-227	-	-226	-	-245	13	0.002	-167	0.002
			156									
Shoulders–joints	16	48	47	44	-	40	-	35	2	<0.001	48	<0.001
Shoulders–soft tissues	16	46	53	55	-	61	-	66	4	0.015	43	<0.001
Spleen	16	45	44	-23	-	-34	-	-66	16	<0.001	37	<0.001

Table 2: Increase in total body air volume over time

Total body air volume - TBAV (cm3) & Time since death						
Lamb/Weight (Kg)		3 Hours	16 Hours	50 Hours	75 Hours	160 Hours
1	6.60 kg	3763 cm3 PMCT-11/04/2017	4006 cm3 PMCT-12/04/2017	4247 cm3 PMCT-13/04/2017	4769 cm3 PMCT-14/04/2017	5300 cm3 PMCT-18/04/2017
2	6.75 kg	1706 cm3 PMCT-11/04/2017	1949 cm3 PMCT-12/04/2017	2193 cm3 PMCT-13/04/2017	2436 cm3 PMCT-14/04/2017	2977 cm3 PMCT-18/04/2017
3	7.35 kg	1841 cm3 PMCT-19/04/2017	2084 cm3 PMCT - 20/4/2017	2328 cm3 PMCT-21/04/201	2868 cm3 PMCT-24/04/2017	3409 cm3 PMCT-26/04/2017
4	7.50 kg	1695 cm3 PMCT-19/4/2017	1939 cm3 PMCT - 20/4/2017	2192 cm3 PMCT-21/4/2017	2789 cm3 PMCT-24/04/2017	3330 cm3 PMCT-26/04/2017
5	7.55 kg	1806 cm3 PMCT-19/4/2017	2171 cm3 PMCT - 20/4/2017	2536 cm3 PMCT-21/4/2017	2900 cm3 PMCT-24/04/2017	3441 cm3 PMCT-26/04/2017
6	8.15 kg	1147 PMCT-25/04/2017	1390 cm3 PMCT - 26/04/2017	1634 cm3 PMCT-28/04/2017	2054 cm3 PMCT-30/04/2017	2595 cm3 PMCT-02/05/2017
7	8.71 kg	1056 cm3 PMCT-25/04/2017	1597 cm3 PMCT - 26/04/2017	2138 cm3 PMCT-28/04/2017	2382 cm3 PMCT-30/04/2017	2625 cm3 PMCT-02/05/2017
8	9.10 kg	1030 cm3 PMCT-25/04/2017	1471 cm3 PMCT - 26/04/2017	2011 cm3 PMCT-28/04/2017	2552 cm3 PMCT-30/04/2017	3112 cm3 PMCT-02/05/2017
TBAV at time of death			116 cm3	Std. Error 7	t 17	Sig. 0.000
Increasing TBAV per day after death			14 cm3	2	6	0.000

rising HU, but then with increasing putrefaction, gas volume increased, and the HU of these areas constantly reduced.¹⁸ The changes observed might be due to factors inherent to the cellular composition of brain tissue, such as neuronal karyopyknosis, Nissl degeneration, glial cell swelling, and loss of water of white matter. Liver, Heart, spleen, and lung showed a decline in HU between the first and all following scans up to and including the last scan at about 160 hours after death. Compared to the brain tissue, this might be due to the gas which may involve the putrefaction process or water loss and/or loss of enzymes in the liver, heart, and spleen as a consequence of blood stoppage in the early time after death. An increase in the body temperature due to the chemical reactions in the body while being stored at 19°C is another cause but would not explain the difference in behaviour of the brain tissue. The results of this study for the lungs and the heart are different from those of Wang et al (2017), who showed a rising in HU of these organs in the first two days after death.¹⁸ This differentiation might be due to the difference in the CT scan model or physical factors (e.g., kilovoltage or mAs) as the differentiation of HU could be observed even when using two CT scanners of the same manufacturer and type that were examined.⁸ Zech et al (2014), mentioned that the rising of the temperature and beam energy might decrease slightly of the HU values.¹⁹

In this study, we chose the lambs due to their body size being larger than rabbits closer to reflecting the size of a human infant, but it has different digestive systems from that of rabbits and humans. In this age, the ruminant stomach of the lamb has not yet fully adapted to the digestion of a vegetable diet but diverts milk directly into the abomasum, which is the chamber in the ruminant stomach most like a human monogastric stomach. Despite, it is reasonable to be cautious about the changes seen in the abdominal viscera, we believe that the other different changes we have recorded are likely to be closer to those in humans than to those in rabbits.

HU of the kidney showed a rise between the first and all following CT scans up to and including the last CT scan at about 160 hours. This maybe related to the presence the cocet of amino acids in kidney tissue caused by tissue breakdown and to the presence of oedema or due to titin and deoxyribonucleic acid or nebulin in muscle tissue in the kidneys. Kummer et al (2017) missioned that the HU value may increase due to oedma with time after death.²⁰ Hamzah et al (2014) mentioned that the decomposition process is subject to different intrinsic factors such as weight, age, before-death conditions, presence of trauma, drugs or toxins, and extrinsic factors such as environment setting, moisture level, temperature, sun exposure, layers and type of clothing, coffin and bedding and accessibility of insect.²¹ Ebuehi et al (2015) stated that the slower decay of brain, and DNA suggests that at a later PMI the brain, the liver is a preferred organ for forensic studies than the heart

and kidney.²² Yukari Tomita et al. (2004) pointed out that in the body the skeletal muscle is the most abundant and has a much greater delay in breakdown compared to the pancreas, kidney, heart, and liver tissues after death which will clearly affect the estimation of PMI.²³

In the first scan, decay was insignificant, although pockets of gas might be seen in the liver, heart, and stomach wall, which may be the gas bubble in the blood vessels mainly in the first scan.

Also, by the time of the second scan, PMCT images manifest the appearance of gas in the blood vessels. From the third scan onwards, the putrefaction of areas and the volume of gas visibly expanded throughout the body. The final scan shows that the body organs started to decay. This is due to fungi and bacteria, which cause putrefaction (followed by chemical decomposition). The postmortem changes are most significant in the areas of the body that contain the most blood since the red blood cells act as food for the bacteria. Therefore, these putrefactive changes are more noticeable in areas of livor mortis. normally, the soft tissues of the face swell first and cause eversion of the lips and eyes and then the abdominal organs become massively distended by gas.²⁴ The environmental conditions may play an important factor in the faster or slower decomposition.²⁵

The average daily increase in TBGV was 422 ml/Kg with a change of 77 ml/Kg. There is some overlap between TBGV to the single lambs; this was due to an important difference in TBGV of three out of the eight lambs. Whereas the values are similar to the remaining five lambs. This might be due to differences in body size of these lambs (two of the three outlier lambs were of larger weight than the other six). Previous authors have stated that cadavers' size affects the rate of tissue degradation, with larger cadavers decomposing faster.²⁶ Another explanation could be the time interval between the last feeding and death as it is recognized that chemical reactions to food in the stomach might increase the amount of bowel gas.²⁷

The last feed in the studied lambs was not recorded. Another limitation of this study is that although all lambs were stored at room temperature, this was uncontrolled, so there might have been changes in ambient temperature due in part to different weather conditions for the 3 groups (scans were performed in the time between 11th April to 2nd May 2017). Matuszewski et al. (2020) stated that experiments using human cadaver analogs such as pig carcasses will be easier to repeat the experiment and more practical for controlling confounding factors than studies based solely on humans and, as a result, are likely to remain our primary epistemic origin of forensic knowledge for the immediate in future.²⁸ Evaluating the influence of altered environmental parameters could provide still considerable insights into the use of non-invasive PMCT to accurately determine PMI. In this study, one observer measured the HU from six sites and TBGV which is a moreover limitation.

Lastly, in the future, it could be prudent to weigh the animals before each scan rather than just before the initial scan.

5. Conclusion

The results of this study suggested that measuring post-mortem tissue density is possible to be used as a non-invasive method to estimate the time of death. However, there was a difference in the behavior of tissues from different body organs. These variations are likely associated with the characteristics of different body organs and the different types and content of the enzymes content. Even though TBGV showed a significant correlation with PMI, there was also a significant overlap between individual bodies of animals. Anatomical variations (e.g. size variations) and varying temperature and humidity almost definitely affect the rate of change and could be considered in future larger studies. Combining the HU of various body organs and TBGV as an individual index might prove more accurate than measuring the single parameters and should be considered in any validation future studies. In further research, PMCT might be developed to determine PMI in humans.

6. Abbreviations

PMCT, post-mortem magnetic resonance imaging (PMMRI), Hounsfield units (HU), post-mortem interval (PMI), total body gas volume (TBGV).

7. Ethical Approval

The study was approved by the Animal Welfare and Ethical Review Body from Nottingham and the University of Sheffield Ethics Committee.

8. Source of Funding

None.

9. Conflict of Interest

None.

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Cite this article: Taher MMB, Skerry TM, Cohen MC, Russell J, Offiah AC. Correlation of CT measurements of total body gas volume and hounsfield units with post-mortem interval. *Indian J Forensic Community Med* 2024;11(3):111-118.