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Assessing Radiological Images of Human Cadavers: Is There an Effect of Different Embalming Solutions?

Abstract

Objectives: The aim of this study is to investigate the impact of different embalming solutions including formalin, Genelyn, Thiel and Imperial College London- Soft Preserving solutions on the quality of radiological images taken from cadavers embalmed with the above mentioned techniques.

Materials and Methods: Two cadavers per embalming technique were imaged pre and post-embalming using three different imaging modalities including ultrasound, plain radiography and computed tomography (CT). Imaging criteria and a qualitative grading system for each imaging modality was adapted from the European Guidelines on Quality Criteria for Computed Tomography, the European Guidelines on Quality Criteria for Diagnostic Radiographic Images, and according to the AIUM Practice Guideline for the performance of ultrasound. Qualitative analysis was performed independently by three readers on a Picture Archiving and Communication System (PACS). The readers were blinded to both the embalmment status and the embalming agent used to preclude bias.

Results: On comparison of images pre and post-embalming, brain CT images showed a significant deterioration in image quality post-embalming, while there was no significant change in chest and abdomen/pelvic images and some improvement was observed in Genelyn embalmed cadavers. No changes were observed when using ultrasound to image the spleen and aorta, while a significant improvement in image quality was observed when examining the kidney in all embalmed cadavers with a small improvement when imaging the liver. No

significant difference was observed on plain radiography post-embalming, while a minor deterioration was observed mainly in the chest area.

Conclusion: Different embalming techniques had varying effects on image quality, in human cadavers, with the range of imaging modalities investigated in this study. Thus, no ideal embalming solution was identified, which would improve the quality of images on all imaging modalities Further research is required to compare the quality of radiological images at different stages of decomposition taking into consideration antemortal pathologies with a larger number of donors.

Introduction

Human cadavers are used in different disciplines for teaching, research and training. The dissection of the human cadaver has been considered a superior tool to teach anatomy by both anatomists and students [1]. In the early 1990s, medical curricula internationally started to change from a subject based approach to a multi-subject integrated system [2]. Though integrating anatomy with other clinical sciences was recommended only 20 years ago, the use of radiological imaging to supplement the teaching of human anatomy was first reported over 50 years ago [3]. Several studies have reported the advantages of using radiological images when teaching human anatomy [4, 5]. Some of these advantages include the use of radiological images to understand the three-dimensional aspect of anatomical structures and their complex positions [4]. Retention of the anatomical knowledge learnt in the first 2 years of the medical curriculum has always been a challenge. Studies have shown that the use of radiological images in cadaver dissection improves the students' ability to retain the ability to identify structures on plain radiography in the long and short-term [3]. This integration does not only help in improving anatomical education, but is crucial in enhancing the students' ability to understand diagnostic radiology later in their undergraduate and post-graduate careers [6].

From a clinical perspective, the use of cadavers to educate and train clinicians and postgraduate medical students using radiological imaging has been reported in the literature [7, 8]. Cadaver based ultrasound-guided regional anaesthesia training is widely employed [8-11]. Furthermore, the use of human cadavers in vascular sonography training and also for practicing common interventional clinical skill, such as femoral central venous access has proven invaluable [12].

Diverse radiological modalities (Computed tomography, magnetic resonance images and ultrasound) are also increasingly utilized in forensic medicine and pathology [13]. The use of plain radiography and CT supplements the information gained from conventional post-mortem. The number of articles published in the field of post-mortem and forensic radiology has increased from a dozen in 2000 to a dozen per month in 2011 [14]. The reason could be due to the fact that interpreting post mortem images is frequently different to in-vivo imaging, thus a new radiological post mortem sub-specialty has emerged. Though post-mortem imaging has increased exponentially, technical difficulties including lack of contrast enhancement, post mortem decomposition and difficulties with differencing "normal" post-mortem imaging findings from findings related to pre-mortem illnesses remains challenging [15]. There is also increasing interest in combining anatomic dissection in human cadavers, with imaging (e.g. CT) of these cadavers following donation, to enhance student experience. These reasons motivated this study, in which, we aimed to investigate the influence of different chemicals and embalming techniques on the quality of radiological images obtained from human cadavers.

Embalming is the process of introducing chemicals into a human cadaver to arrest decomposition of tissue while reducing health hazards [16]. This practice is common when using cadavers to deliver dissection-based anatomy teaching programs. Formaldehyde has traditionally been used to preserve the human body, but as it affects the quality of tissue, research has now focused on developing new embalming techniques and solutions to preserve a tissue compliance that is more comparable to the living human body [16]. Some of these techniques including Thiel [17], Genelyn [18], and Imperial College London- Soft Preserving solution, have been compared in a study of the use of cadavers in the training of gynaecological oncologists [19]. Negative effects of embalming on the quality of radiological

imaging have been reported previously [4, 6]. The use of Formaldehyde/phenol in an embalming solution was considered to be the primary reason for the decrease in the quality of images as they induce soft tissue oedema [20]. Recent studies have reported the advantages of embalming a human cadaver using a soft tissue preserving technique, such as Thiel, as it results in improved quality of radiological images [9].

To the best of our knowledge, this is the first study comparing the impact of different embalming techniques (formalin, Thiel, Genelyn, and London) on image quality in human cadavers on several imaging techniques routinely used in clinical practice including ultrasound, radiography and computed tomography The first study to investigate different imaging techniques on embalmed cadavers was in 2013 where only one embalming technique was assessed [21]. A further study in 2014 compared different embalming techniques using ultrasonography [22]. The aim of this study is to compare the quality of radiological images in human cadavers before and after embalming with different embalming techniques.

Materials and Methods

The study was carried out under the auspices of the 'Licence to Practise Anatomy' granted by the Irish Medical Council to the Chair of Anatomy under the Anatomy Act 1832. For the purpose of the study 8 human cadavers were embalmed using four different embalming techniques. Donors premorbidly signed written consent for the use of their bodies by the department of Anatomy and Neuroscience for education and research. Appendix A includes details on the different embalming solutions.

Cadavers were imaged pre and post-embalming using three different imaging modalities including ultrasound, radiography and CT. Imaging criteria and a qualitative grading system

for each imaging modality was adapted from the European Guidelines on Quality Criteria for Computed Tomography [23], the European Guidelines on Quality Criteria for Diagnostic Radiographic Images [24], and according to the AIUM Practice Guideline for the performance of ultrasound [25]. Qualitative analysis was performed independently by three readers (MT, FM, and OJOC) on a Picture Archiving and Communication System (PACS) (Impax 6.3.1; Agfa Healthcare, Mortsel, Belgium). The readers were blinded to both the embalmment status and the embalming agent used to preclude bias.

Computed tomography

Whole body multi-detector scanning including the brain, thorax, abdomen and pelvis was performed pre and post-embalming by two specialist CT radiographers using a 128-slice Discovery HD 750 (GE Healthcare GE Medical systems, Milwaukee WI, USA). The cadavers were scanned supine, cranio-caudally, with their arms by their sides. The following CT parameters were used in conjunction with automatic exposure control: tube voltage: 120kVp; gantry rotation time: 0.8 seconds; collimation: 40 x 0.62mm; and pitch factor: 0.98. Images were reconstructed from an acquisition thickness of 0.625mm to a final slice thickness of 1.25 mm. All images were reconstructed from the raw-data acquisitions using the standard departmental protocol employing hybrid iterative reconstruction (60% filtered back projection and 40% ASiR, adaptive statistical iterative reconstruction (GE Healthcare, GE Medical Systems, Milwaukee, USA).

Three readers qualitatively rated different structures in 3 anatomical regions according to a four-point scale: 1 = not visible, 2 = poorly visible, 3 = adequately reproduced, 4 = very well reproduced in comparison to images from patients. The results were summated per body part

to give a total raw score. A calculated score to facilitate comparisons was derived by dividing the total raw score by the number of criteria assessed.

In the brain, radiologists rated the visibility of the skull, grey-white matter differentiation and the ventricular system. In the chest, the visibility of the thoracic wall, lung parenchyma, heart, aorta, superior vena cava and vertebrae were rated. In the abdomino-pelvic area, the aorta, inferior vena cava, liver, kidneys, spleen, pancreas, intraperitoneal fat, vertebrae and pelvis were rated.

Ultrasound

Ultrasound of the solid abdominal viscera including the kidneys, liver, aorta, and spleen was performed using an Ultrasonix L14-5/ 38GPS by a single radiologist (MT). Representative images were recorded and reviewed by the other two radiologists and a final score was allocated in consensus. The visibility of the following solid viscera and vascular structures was rated: kidney: long axis (both poles visible), transverse length, renal cortex and renal pelvis; liver: left lobe, right lobe, common bile duct, and portal vein; spleen: long axis (both poles visible), transverse length; aorta: anterior and posterior abdominal wall visibility in the transverse plane and anterior and posterior visibility in the longitudinal plane.

Percutaneous biopsy of the liver was performed with an 18G automated core biopsy device by a single radiologist (MT) (Co-axial Temno biopsy device, Carefusion Corporation, San Diego CA, USA). Needle visibility was assessed on a three-point scale: 0 = not visible, 1 = poor visibility, 2 = good visibility.

Plain Radiography

Plain Radiography was performed by an experienced radiographer using an Axiom Aristos digital radiography unit (Siemens Healthcare Diagnostics, Siemens, Malvern PA, USA). Series included an antero-posterior chest (AP), AP & lateral lumbar spine, and AP pelvis. Image quality was subjectively rated by the two readers using the same four-point scale as CT.

In the chest, the visibility of the pulmonary vascular markings, trachea and proximal bronchi, cardiac borders, diaphragm, costophrenic angles, retrocardiac lung, and the upper and lower endplates of the thoracic spine were assessed. In the lumbar spine, radiologists rated the visibility of the upper and lower endplate surfaces, the cortex and trabecular pattern, the aortic course, and aortic calcifications. In the pelvis, the upper and lower endplate surfaces of the lumbar spine, iliac wings, obturator foramina, and the femoral neck cortices were rated.

Statistical Analysis

Statistical analysis was performed using SPSS statistical package version 22 (IBM Corp., Armonk, NY). Weighted Kappa was calculated to assess the level of agreement among radiologists where one-point difference was weighted as 0.6667 and two points as 0.3333. The CT visibility score was computed from the mean of the three average ratings for the body areas: brain, chest and abdomen/pelvis, while the mean of two average ratings of the same body areas was used for the plain radiographs. A 2x4 'Split Plot' ANOVA was performed to check whether there was a statistically significant effect due to embalming techniques on the difference between the pre and post-embalming scores, and if not, the same analysis was used to test the overall difference between pre and post-embalming scores.

Results

Radiologist Agreement

The level of agreement between radiologists varied depending on the imaging modalities. When scoring CT images from post-embalmed cadavers, Radiologist 1 and 2 had a very similar level of agreement with weighted Kappa score of 0.9476. Radiologist 1 and 3 had a moderate level of agreement with weighted Kappa score of 0.4575, and Radiologist 2 and 3 had a similar level of agreement with score of 0.4432. When scoring radiographs from post-embalmed cadavers, Radiologist 1 and 2 had a very similar level of agreement with weighted Kappa score of 0.9836.

Computed tomography

Raw data (scores) from CT images from the three radiologists are attached in appendix B. All radiologists considered the bony structures in the brain (skull), chest (vertebrae) and abdomen/pelvis (vertebrae, pelvis) as very well reproduced before and after embalming. Therefore, the scoring data from these images were taken out of the statistical analysis as there was an absolute no variation in scores.

Table 1 shows the mean values and standard deviations of CT visibility scores for all eight cadavers embalmed by the four different techniques of pre-embalming scores, post-embalming scores and the differences. Images from Thiel, Genelyn and ICL-SP embalmed cadavers show a negative difference and therefore the quality of images has deteriorated after embalming. Meanwhile, there was an almost zero difference in image quality pre and post-embalmment with formalin.

A 2x4 'Split Plot' ANOVA statistical analysis was performed and the results showed that there was no statistically significant effect between pre and post embalmment scores attributable to embalming technique ($F_{3,4} = 1.305$; p=0.388) but that there was weak evidence of an overall deterioration between pre-embalmed and post embalmed cadavers ($F_{1,4}=5.361$; p= 0.082).

Embalming Technique		Pre-Embalming	Post-Embalming	Difference
Thiel	Mean	2.70	2.55	-.145
	SD	.474	.594	.12
Formalin	Mean	2.96	2.97	0.02
	SD	0.04	0.03	0.06
Genelyn	Mean	3.25	3.21	-.05
	SD	.18	0.08	.11
ICL-SP	Mean	3.03	2.90	-.15
	SD	.12	0.03	.09

Table1- CT Images: Mean (SD) CT visibility scores of images taken from cadavers before and after embalming using four techniques Thiel, Formalin, Genelyn and ICL-SP (2 cadavers per technique).

The CT visibility scores included three areas: Brian, chest and abdomen/pelvis. Figure 1 shows the CT visibility scores and the three regional scores before and after embalming for the four different embalming techniques. Graph A shows that there was some decrease after embalming in 3 of the 4 embalming techniques. Statistical analysis (2X4 'Split Plot ANOVA) showed that none of the three regional scores showed a different effect between pre and post-embalming due to embalming technique. Graph B indicates that there was a statistically significant overall deterioration ($F_{1,4}= 19.868$; P=0.011) in the quality of Brain CT images

before and after embalming. Genelyn and ICL-SP embalmed cadavers had the same scores

before and after embalming on graph B. Better quality of images could be observed in the

chest and abdomen/pelvis of cadavers embalmed by Genlyn solution. Meanwhile chest

images of formalin embalmed cadavers did improve post-embalming while the rest of the

images either stayed the same or showed a very small variation.
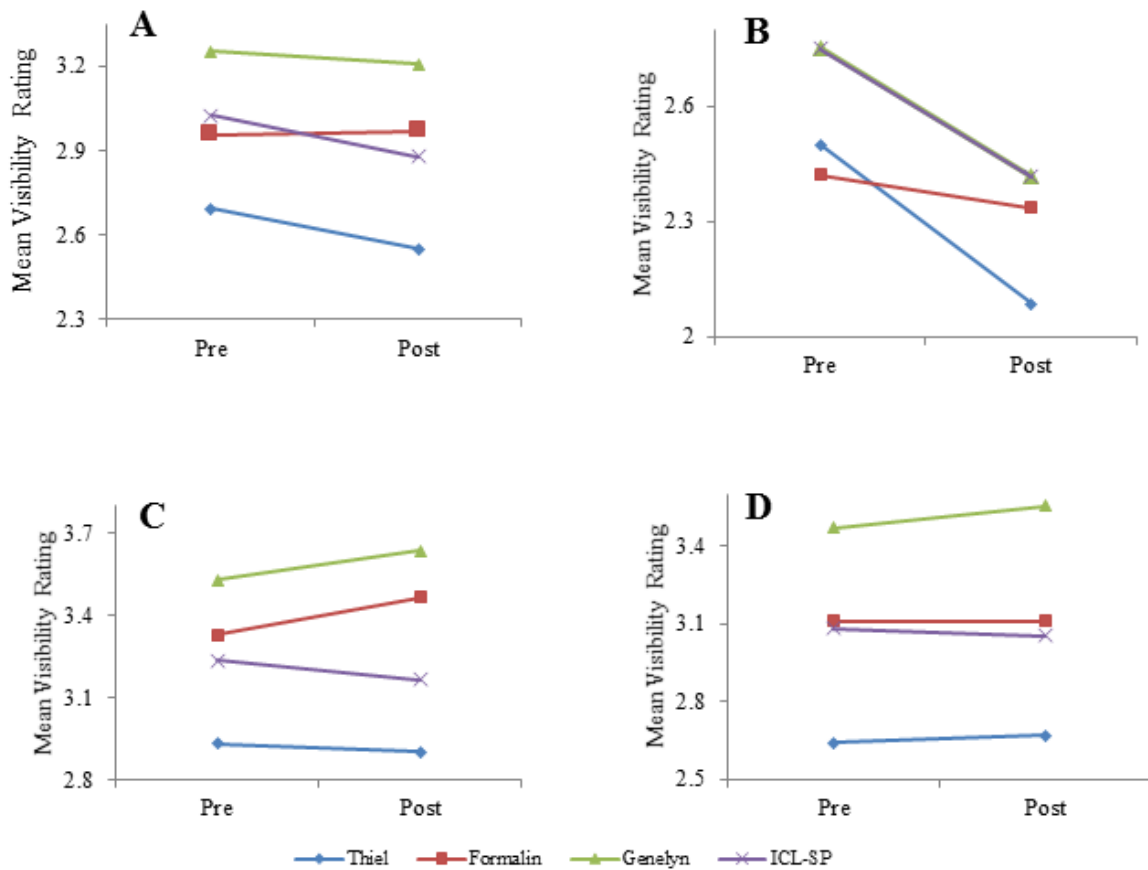

Figure 1- Overall and regional visibility scores of CT images before and after embalming using four different techniques (A=overall; B=Brain; C= Chest; D= Abdomen/Pelvis).

Ultrasound

Raw data (scores) of ultrasound images are attached in appendix B. While some difference

was noted in the US kidney images, a non-significant difference was noted in the liver and

none was observed in the images taken of the spleen and the aorta as they were not visible

before or after embalming. Table 2 shows the mean and standard deviation of image quality scores of the kidneys before embalming, after embalming and the difference. ICL-SP embalmed cadavers showed the highest improvement in the quality of images followed by Thiel and Genelyn and then formalin. Both Thiel embalmed cadavers showed an improvement in needle visibility during needle biopsy post-embalming compared to pre-embalming.

Embalming Technique		Pre-Embalming	Post-Embalming	Difference
Thiel	Mean	1.5	2.25	.75
	SD	.71	1.03	.35
Formalin	Mean	1.0	1.25	.25
	SD	0	.35	.35
Genelyn	Mean	1.37	2.13	.75
	SD	.53	.18	.71
ICL-SP	Mean	1.75	2.63	.88
	SD	.35	.88	.53

Table 2- US Images: Mean (SD) US visibility score of the kidneys of images taken before and after embalming using four different techniques (2 cadavers per technique).

The 2x4 'Split Plot' ANOVA was performed and the results showed that there was no statistically significant effect on the change between pre and post-embalming image quality scores due to embalming technique ($F_{3,4} = 0.401$; p=0.760) but that there was overall improvement between pre-embalmed and post embalmed cadavers ($F_{1,4}=9.0$; P= 0.040).

Figure 2 shows the change before and after embalming, with Thiel, ICL-SP and Genelyn showing the biggest increases.
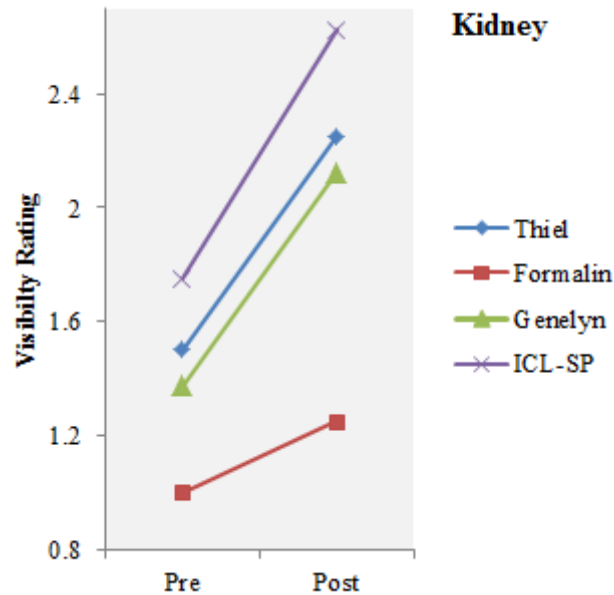

Figure 2- Visibility scores of ultrasound images from kidneys before and after embalming using four techniques.

The common bile duct and the portal vein were not visible before or after embalming across all cadavers, while some improvement was observed in identifying the left and the right lobe of the liver irrespective of the embalming solution.

Plain Radiographs

Raw data (scores) of radiographs from the two reviewers are attached in appendix B. While some difference was noted in the chest radiograph images, almost no difference was noted in the lumbar spine area and none was observed in the images taken of pelvis. Table 3 shows the mean scores and standard deviation of image quality of the Chest before embalming, after embalming and the difference. All cadavers showed deterioration in the quality of images,

but the changes were not statistically significant, except for formalin embalmed cadavers which stayed mainly the same.

Embalming Technique		Pre-Embalming	Post-Embalming	Difference
Thiel	Mean	2.38	2.04	-.33
	SD	.77	.65	.12
Formalin	Mean	2.83	2.92	.083
	SD	.24	.35	.12
Genelyn	Mean	3.29	2.67	-.63
	SD	.29	0	.30
ICL-SP	Mean	2.88	2.25	-.63
	SD	.18	.82	1.00

Table 3- Radiographs: Mean visibility score of chest X-rays taken before and after embalming using four different techniques (2 cadavers per technique).

The 2x4 'Split Plot' Anova showed that there was no effect on the change between pre and post-embalming image quality scores related to embalming technique ($F_{3,4} = 0.803$; P=0.554). Nor was there an overall difference between pre-embalmed and post embalmed cadavers ($F_{1,4} = 4.025$; P= 0.115).

Discussion

The decomposition of the human tissue post-mortem typically leads to a deterioration in the quality of radiological images [21]. While embalming is performed to reduce the health risks associated with working with a human cadaver and enables its use for a longer period of time, this study shows that the chemicals used in the embalming solution influences the quality of the radiological images taken on human cadavers. The embalming process has mainly prevented the deterioration of the quality of radiological images and has improved the quality of others. The quality of some images deteriorated post-embalming and several explanations could be provided for this neither positive nor negative influence of the chemicals on the tissue may have prevented the decomposition of the tissue; failure of the embalming process whereby chemicals did not perfuse to some parts of the body; or potentially a negative influence of the chemicals on the tissue leading to deterioration in the quality of some images.

A small number of studies have assessed the quality of radiological images taken from human cadavers after embalming. These studies compared the quality of images after embalming without comparison to the quality of images before embalming. Our study demonstrates that comparing the quality of images from cadavers post-embalming without considering their conditions before embalming is inaccurate. Figure 1 clearly shows how the Thiel embalmed cadavers had poor image quality before embalming compared to Genelyn embalmed cadavers and failure to compare images pre and post-embalming could wrongly attribute reduced image quality to the embalming agent (i.e. Thiel). Alternatively, this could have led to the conclusion that Genelyn solution improves the quality of CT images compared to Thiel solution, which is not the case.

The overall summative score of CT images shows that there was deterioration in the quality of images post-embalming. When looking into specific areas of the body, it was clear the only statistically significant deterioration was in the brain images while other parts of the body mainly improved or remained unchanged. Formalin embalmed cadavers were the only cadavers that showed a slower deterioration in image quality and this could be due to the high concentration of formaldehyde that caused the fixation of brain tissue compared to the other solutions. In this case, the deterioration is due to the neutral effect of Thiel, Genelyn and ICL-SP solution on the brain tissue. Meanwhile, figure 3 shows a clear difference between brain CT images of cadaver 1 and 2 that were both embalmed using Thiel technique. Images from cadaver 1 post-embalming (B), shows collapsed brain tissue which has been reported in literature as the influence of the Thiel embalming solution. On the other hand, this phenomenon was not observed in cadaver 2 post-embalming (D). Moreover, the main deterioration in the quality of brain tissue images from Thiel embalmed cadavers was observed in cadaver 2. This could be related to the embalming process as cadaver 2 did not show signs of being embalmed until after 6 months, whereas cadaver 1 showed signs of embalmment within 3 months which is the expected period of time after being immersed in the Thiel tank.
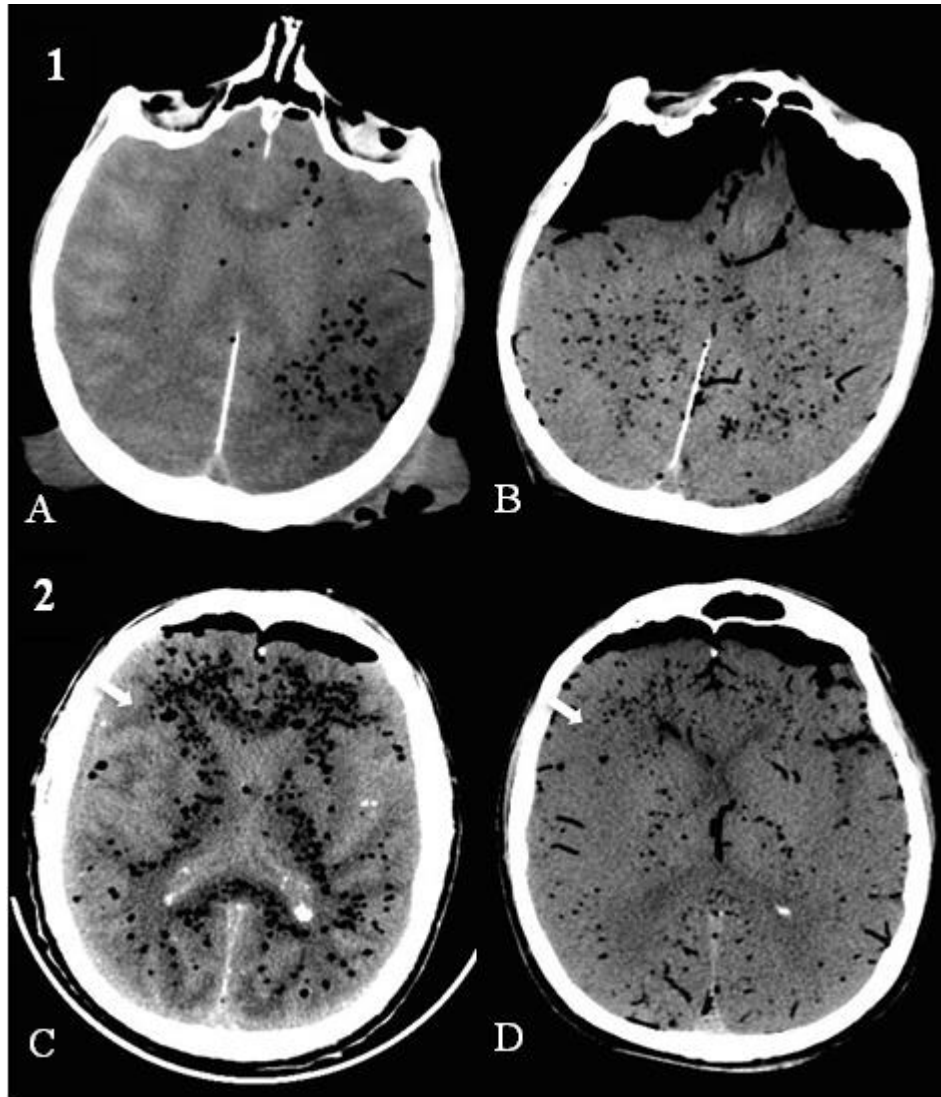
Figure 3- Brain CT images of Thiel embalmed cadavers 1 &2 with A&C before embalming and B&D after embalming.

With regard to ultrasound, while there was no change in the visibility of the aorta and the spleen, the main change was noted in the visibility of the kidneys and the lobes of the liver. The overall improvement in image quality post-embalming could be explained by the infusion of fluids into the tissue which replaces gas produced during decomposition. Fluids represent conductivity media for ultrasound, whereas gas reflects ultrasound. Thus the presence of fluid enhances image quality image quality, whereas the presence of air negatively impacts image quality. The findings of this study support the findings of other

studies [8, 10]. This could be observed in figure 4 where the left lobe of the liver (white arrow) was not visible before being embalmed using Genelyn solution and was considered as adequately imaged after embalming.
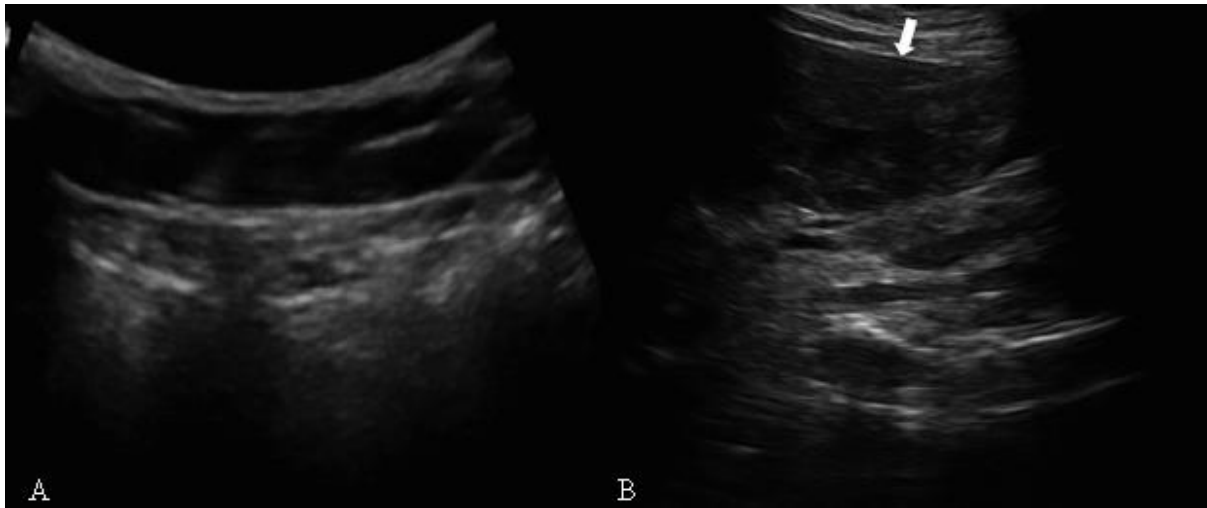

Figure 4- Ultrasound images of the left lobe of the liver taken from Genelyn embalmed cadavers with A before and B after embalming.

While all embalmed cadavers showed an improvement in image quality on ultrasound, formalin cadavers demonstrated the smallest improvement. This could be due to the lack of flexibility in formalin embalmed cadavers, which restricts the ability to manoeuvre the cadaver while performing the scans. This could explain the negative impact of formalin on the quality of ultrasound images. These findings align with other studies which concluded that satisfactory image quality on ultrasound was possible in cadavers embalmed with commonly used embalming techniques (formalin-based) and can be useful for training in ultrasound and ultrasound guided interventional radiology techniques[11].

A previous study reported very poor image quality on ultrasound examination of a formalin-embalmed cadaver. However, as no images were taken before embalming, the authors could

not conclude that the poor quality of the ultrasound images could be attributed to the embalming technique [21]. Several other studies have also looked at the quality of ultrasound images after embalming but again the studies suffer from lack of comparison with imaging taken prior to embalming [21, 22].

While the majority of plain radiography images showed no change before and after embalming, a non-significant deterioration could be observed on plain radiography of the chest region. Images from formalin embalmed cadavers showed no deterioration in the quality of images and this could be due to the high percentage of formaldehyde in the solution compared to the other solutions.

It is important to acknowledge that there are limitations to the current study many of which are common to many cadaver-based studies. With an average of 20 donations per year to the anatomy department at our institution, it was difficult to recruit a larger number of donated bodies for the study. Another limitation was a lack of knowledge regarding the donors' medical history as the Department did not have access to the donors' medical background regarding illnesses which could affect the embalming process. Thus the small sample size provides limited estimates of cadaver-to-cadaver variation and hampers the power of the study to show statistically significant differences unless such differences are very large. Hence caution needs to be exerted when interpreting statistical results and further multicentre studies, to ensure recruitment of larger numbers of cadavers, should be considered to robustly evaluate some of the findings of this study.

In conclusion, although embalmed human cadavers are frequently used in medical education, to our knowledge, there are no previously published studies comparing imaging (Ultrasound, plain radiography and CT) of cadavers before and after embalming with different embalming solutions. This study indicates that there is no ideal embalming solution which improves

image quality of the full range of imaging modalities including plain radiography, ultrasound and CT of human cadavers. Our findings show that embalming a human cadaver inhibits the deterioration in image quality due to the natural decomposition of tissue, except for CT images of the brain. Moreover, we found that embalming a human cadaver leads to better quality of ultrasound images as it leads to a decrease in the presence of gas within the tissues of the cadaveric body. Formalin, appears to offer the least benefit in terms of improving image quality on ultrasound images, perhaps explained by reduced soft tissue flexibility in formalin-embalmed cadavers.

Further research in a larger cohort of cadavers is required to investigate the quality of radiological images at different stages of decomposition taking into consideration other factors such as patient age and antemortal pathologies.

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