A Pilot Comparative Study of Submerge vs. Non-Submerg Saturated Salt Solution Human Cadavers Embalming Method by Gross, Histological, and Microbiological Evaluation

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ABSTRACT

Objective: To investigate and develop the saturated salt embalming method and evaluate the cadavers.

Materials and Methods: Eight cadavers were embalmed with a saturated salt solution (SSS) by submerged (SG, N=2) and non-submerged (NSG, N=6), then evaluated by gross dissection, which compared to living humans, fresh and Thiel's cadavers. The histological evaluation was compared to textbook pictures. The assessments were recorded on a Likert scale from 0 (no resemblance) to 5 (most resemblance). Pre-and post-embalming swabs were collected for bacterial and fungal cultures and lung tissues for acid-fast staining and mycobacterial cultures. Comparisons between the evaluated items were performed using the Kruskal–Wallis test. The Likert scale results were reported by percentage.

Results: The submerge method (N=2) was terminated after three months of embalming because it showed insufficient quality for dissection. Six cadavers in NSG had gross tissue qualities that resembled living humans or fresh cadavers on a scale of 3 or 4. NSG had excellent joint flexibility. The histological tissues showed similarity to textbook pictures, with a scale of 4 or 5. There were bacterial and fungal cultures at the end of embalming. The pathogenic bacteria were *Clostridium perfringens* and *Pseudomonas aeruginosa*. Mycobacterium cultures were negative.

Conclusion: Injected SSS, 80% total body water volume, is a promising embalming method that yields cadavers with high tissue quality, flexible joints, and good histological structures. However, this technique cannot eliminate bacteria and normal flora. It may result from the tropical climate setting.

Keywords: Saturated salt solution; embalming; cadaver; dissection (Siriraj Med J 2022; 74: 431-439)

INTRODUCTION

Well-known cadaver preservation methods were fresh frozen, Thiel's, and formalin embalming cadavers. Fresh cadavers are non-chemically treated. Their color, consistency, and flexibility resemble living patients.[1,2] However, they have a short usage time, need to be stored in a freezer, and may host infectious organisms. Thiel's cadavers have excellent joint flexibility and soft tissue

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consistency.[1] Their weaknesses are a poorly preserved brain, muscular disintegration, complex preparation, and expensive.[3-6] Conventional embalmed cadavers have hard tissue consistency and inflexible joints. Also, the high formaldehyde content in conventional embalming solution is toxic because of its carcinogenicity.[7,8]

Coleman and Kogan introduced a saturated salt solution embalming cadaver (SSS). The cadaver had excellent dissection properties and well-preserved histological structures.[9] Hayashi et al. published a study of SSS made from Coleman and Kogan's solution, comparing this solution with 20% formaldehyde and Thiel's solution. Cotton swabs were taken at the pharynx and rectum for pre-and 14-day post-embalming bacterial and fungal cultures. Body cavities fluid were collected during dissection for cultures. The post-embalming results showed the solution had a bactericidal effect.[10] The SSS had flexible joints and high tissue quality and were equally suitable for surgical simulations as Thiel's cadavers.[10] However, their study assessed only two cadavers per embalming type. Coleman's publication did not mention the number of cadavers. Hayashi et al.'s embalming technique differed from Coleman's. They injected 6 liters (L) of SSS with an undetermined amount afterward, while Coleman and Kogan used 21 L for a small cadaver. Hayashi et al. had stored a cadaver in a plastic body bag and kept it at room temperature, but they did not report the storage time duration. In contrast, Coleman and Kogan placed cadavers in thick polyethylene sheeting and stored them in an embalming solution at 18 °C for at least three months and up to 1 year or longer.[9,10]

The present study aimed to investigate, develop, and clarify the saturated salt embalming method. Furthermore, we radically evaluated SSS in all possible aspects: gross anatomy, applicability, histology, microbiology, and period of use.

MATERIALS AND METHODS

This study took place at the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. The university institutional review board granted documentary proof of exemption, SIRB Protocol No. 500/2561 (Exemption). The study design is a descriptive study.

Eight legally donated cadavers with negative serologic tests for HIV, hepatitis B, hepatitis C, and venereal disease research laboratory (VDRL) were recruited. The cadavers were randomized into two groups: the submerged group (SG) and the non-submerged group (NSG), with equal gender distribution. The groups indicated the method of embalming. In case of embalming method failure, such as putrefy cadaver, unsuitable for dissection, observed along the process, the soon-to-be embalmed cadaver would be processed with the better method. Hence, the total number of recruited cadavers was eight (Table 1).

Oral and rectal swabs under sterile technique for bacterial, fungal culture and identification had been performed before embalming. The embalming solution contained 40% formaldehyde 0.5 L, isopropyl alcohol 4 L, phenol 0.2 L, glycerin 0.5 L, tab water 19.8 L, and 20 kilograms (kg) of sodium chloride resulting in a 25 L solution.[10] The solution was injected into a cadaver via the femoral artery with a pressure pump at 10 pounds/

TABLE 1. Demographic data and injected solution volumes.

Cadaver type	Code	Sex	Age (years)	Height (cm)	Weight (kg)	Cause of death	Death time to embalming		Embalming solution	
							Hours	Minutes	Planned amount (mL)	Injectable amount (mL)
Submerged cadavers`	SS6101	Female	83	149	70	Pulmonary Embolism	11	22	16	16
	SS6102	Male	68	150	60	Respiratory failure	24	0	17	17
Non-submerged cadavers	SS6103	Male	69	167	70	Cardiopulmonary failure	67	16	30	30
	SS6104	Female	74	150	70	Aging	92	10	26	26
	SS6105	Male	63	165	55	Hypertension	34	30	26	20
	SS6106	Male	86	150	45	Pulmonary infection	35	30	17	17
	SS6107	Female	64	170	70	Heart failure	70	30	26	24
	SS6108	Female	58	158	55	Asphyxia	57	10	23	23

inch.[3] The injected amount was based on the total body water, which was calculated by Watson's formula using the average height for Thai males (171 cm) and females (161 cm).[11,12] The mean ages came from the Siriraj cadavers' database, collected between 2012-2015 A.D. (70.5 years old from 805 males and 71.5 years old from 615 females). The NSG were injected with solution equal to 80% of their total body water, while the SG were injected with fluid equal to 50%. The actual injected amounts are presented in Table 1. Then the NSG were stored in a polyethylene bag, one bag per cadaver, at room temperature, the same method as Hayashi et al.[10] The SG were placed in a tank containing the embalming solution with the same composition as the injected. The storing process stopped after six months. Then, post-embalming oral and rectal swabs were taken for bacterial and fungal cultures.

Each cadaver underwent gross anatomy evaluation by randomly invited five physicians or anatomists in our department, who would be asked about their prior experiences regards experiences in the operating room as an assistant or a surgeon, experiences with each cadaver type, and cadaver number they have encountered for dissecting, instructing, and teaching. The pre-dissection evaluation assessed general appearance, joint flexibility, and consistency compared to living humans, fresh cadavers, and embalmed cadavers. The post-dissection evaluation items were the resemblance of tissues/body regions/organs compared to living humans or fresh cadavers; eye/nose/skin/hand irritation during dissection; odor; and suitability (e.g., for medical student dissection or surgical training). The evaluations results used a Likert scale, where 0 = no resemblance/no symptom/unpleasant odor/unsuitable for use, up to 5 = most resemblance/severe irritation/acceptable odor/most suitable for use.

Additional swabs for culture assessment were performed during dissection at the ileum (2 inches proximal to the ileocecal valve) and sigmoid (2 inches proximal to the rectum). Lung tissues were sent for acid-fast bacilli (AFB) staining and mycobacterial culture. Histological specimens were also collected in this process, including skin, skeletal muscle, smooth muscle, cardiac muscle, artery, nerve, ligament, heart, liver, intestines, and kidney. After performing hematoxylin and eosin (H&E) staining, the histological structures were evaluated on a Likert scale of zero (no resemblance) to five (most resemblance) by a pathologist comparing the specimens with standard textbook descriptions and photos.[13]

Statistical analysis

Data analysis was done using PASW Statistics software, version 18 (SPSS Inc.). Comparisons between evaluated items used the Kruskal–Wallis Test. P values <0.05 were defined as statistically significant. The Likert scale results were reported as frequency percentages.

RESULTS

Eight cadavers were recruited for the study: two cadavers in the SG and six in the NSG. Because after embalming for three months, the SG showed an insufficient quality for dissection due to stiffening, shrinkage, and distorted hands and feet (supplementary material). Consequently, the submerged method was then terminated.

Most of the evaluations (87%) were done by evaluators who have experience as surgeon assistants or surgeons (Total N = 30, five evaluators per cadaver). They had encountered more than 40 embalmed cadavers before this study. Eighty-three percent of them had dissected 1-10 fresh cadavers, and 97% had experienced 1-10 Thiel's cadavers. They mostly rated the NSG's consistency resemblance to living humans with a Likert scale of 3 (43.3%), fresh cadavers with a scale of 3 (70%), and Thiel's cadavers with a scale of 4 (56.7%). Kruskal–Wallis H test showed a statistically significant difference of $\chi2(2) = 8.081$, $p = 0.018$, comparing these three consistency evaluations. There was no statistically significant difference, $\chi2(2) = 3.531$, $p = 0.171$, comparing the NSG general appearance to a living human, fresh cadavers, and Thiel's cadavers. Table 2 shows the gross anatomy resemblance post-dissection evaluation results compared to living humans or fresh cadavers of various organs and body regions.

Fig 1 shows the thoracic region, abdominal region, and joint flexibility of the NSG. This method excellently preserved subcutaneous fat and muscles. The skin darkness was seen only in the superficial layer. After peeling this off, the dermis color was like a Thiel's cadavers skin color. The small and large NSG joints could be moved like in living humans. The selected Likert scales of the internal organs were mainly 3 and 4. However, cadavers SS6105 and SS6107 had putrefied thoracoabdominal organs. SS6105's ascending colon was perforated. Tumors were seeding in the intestinal wall, and there were numerous blood clots in the thorax. While SS6107's abdominal organs were thin and fluffy. There were tumors on both sides of the SS6107's lungs.

Forty-three percent of the evaluations showed no eye or nose irritation during dissection. Thirty-three percent were rated on a scale of 3, and the others scale of 2. Forty-seven percent had no hand irritation, thirty-three percent reported on scale 3, and the others on scale 2. The odor during dissection was highly acceptable (70% selected scale 4). The odor was an anchovy-like odor. However, it became more intense as time passed.

TABLE 2. The gross anatomy resemblance post-dissection evaluation compared to living humans or fresh cadavers of various organs and body regions.

Tissue resemblance to living humans or fresh cadavers	Selected likert scale (%)					
	0	1	2	3	4	5
Skin	0	10	63.3	23.3	3.3	0
Subcutaneous tissue	0	0	0	46.7	46.7	6.7
Vessel	0	0	0	30	60	10
Nerve	0	0	0	66.7	26.7	6.7
Fascia	0	0	0	13.3	76.7	10
Muscle	0	0	3.3	33.3	56.7	6.7
Cartilage	0	0	0	46.7	46.7	6.7
Ligament	0	0	0	60	36.7	3.3
Lymphatic tissue	0	0	0	56.7	40	3.3
Heart	0	0	3.3	30	63.3	3.3
Lung	0	3.3	0	60	33.3	3.3
Stomach	0	3.3	0	30	60	6.7
Small intestine	0	3.3	0	0	90	6.7
Large intestine	0	3.3	0	3.3	86.7	6.7
Kidney	0	3.3	0	70	20	6.7
Spinal cord	0	3.3	0	16.7	23.3	56.7
Brain	13.3	3.3	6.7	70	6.7	0
Body wall region	0	0	16.7	53.3	23.3	6.7
Head neck region	0	0	20	36.7	40	3.3
Pharynx and larynx region	0	0	6.7	36.7	53.3	3.3
CNS and organ of special senses region	0	0	13.3	43.3	43.3	0
Upper and lower limb region	0	0	0	66.7	23.3	10
Thorax region	0	0	3.3	53.3	36.7	6.7
Abdominal region	0	0	0	36.7	56.7	6.7
Perineum and pelvic region	0	0	16.7	36.7	40	6.7

Likert scale 0 = No resemblance, 5 = Most resemblance

The suitability for medical student anatomy class was a scale of 3 (53.3%) and 4 (46.7%), whereas suitability for surgical training was scale 4 (60%) and 3 (30%). The NSG brains were very soft and liquefied in most areas.

Table 3 shows the results from the pathologist who compared histological structure quality with textbook pictures. The tissues had some autolysis and pale nuclear staining but had identifiable structures. The captured histological slides are shown in the supplementary materials.

Before embalming, there were organisms in both the mouth and rectum, mostly microbiota. After embalming, the organisms were still present (Table 4). But all the intestinal and rectal swabs were negative for *Salmonella, Shigella, Vibrio, Aeromonas,* and *Plesiomonas shigelloides.* There was no mycobacterium in the AFB stains and no mycobacterial growth in the lung specimens. After dissection was finished, we observed the possible period that these cadavers could be used by leaving them in the laboratory at room temperature. After 4-6 weeks, we found fungal colonies growing on all the cadavers.

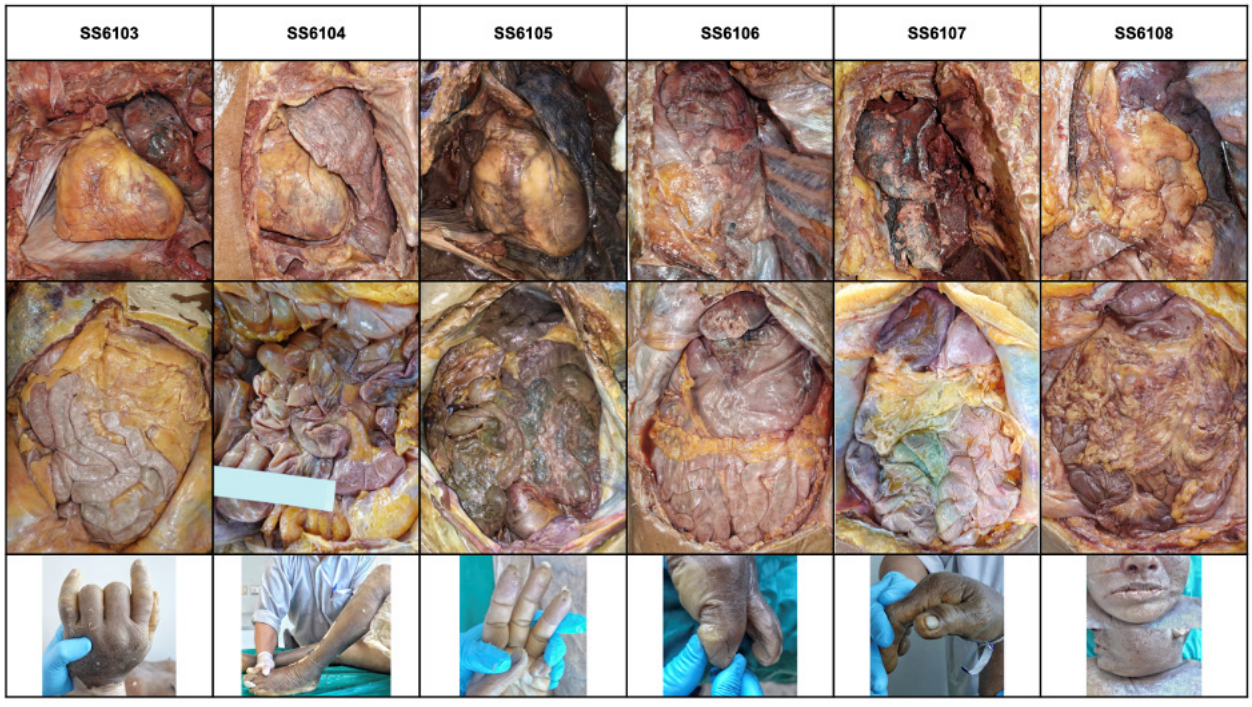

Fig 1. The thoracic region, abdominal region, and joint flexibility of the NSG.

TABLE 3. The histological resemblance compared to the reference picture in the standard textbook.

Tissue	Selected likert scale (%)					
	0	1	2	3	4	5
Skin (epidermis, dermis, subcutis)		0	60	0	40	0
Skin (basal layer)	40	0	0	0	60	0
Skin (melanocyte)	40	0	0	0	60	0
Skeletal muscle geography	0	0	0	40	60	0
Skeletal muscle	0	0	0	40	60	0
Smooth muscle	0	20	0	0	80	0
Cardiac muscle	0	40	20	40	0	0
Cardiac muscle: intercalated disc	80	20	0	0	0	0
Muscular artery	0	0	0	0	60	40
Small-sized artery	0	0	0	0	20	80
Medium-sized vein	0	0	0	0	40	60
Small-sized vein	0	0	0	0	0	100
Peripheral nerve	20	0	0	0	0	80
Ligament	0	0	0	0	40	60
Heart geography	0	0	0	0	60	40
Liver geography	0	20	0	0	0	80
Portal area	20	0	40	40	0	0
Duodenum	0	0	0	100	0	0
Jejunum	0	20	40	40	0	0
Ileum	0	40	40	20	0	0
Colon	0	20	40	40	0	0
Kidney geography	0	20	0	0	80	0
Renal Cortex	0	20	20	40	20	0
Renal Medulla	0	60	40	0	0	0

Likert scale 0 = No resemblance, 5 = Most resemblance

TABLE 4. Pre-embalming and post-embalming culture results

Organ	Culture type	SS6103		SS6104		SS6105		SS6106		SS6107		SS6108	
		Pre-embalming	Post-embalming	Pre-embalming	Post-embalming	Pre-embalming	Post-embalming	Pre-embalming	Post-embalming	Pre-embalming	Post-embalming	Pre-embalming	Post-embalming
Oral	Bacteria	Numerous mixed bacteria	Few mixed bacteria	Moderate microbiota	Rare microbiota	Numerous microbiota	Few microbiota	Numerous microbiota	Few microbiota	Numerous microbiota, Moderate Yeasts	Numerous microbiota	Numerous mixed bacteria, Numerous *Pseudomonas aeruginosa*, Numerous *Prevotella* spp.	Few microbiota
	Fungus	*Candida albicans*	Bacterial overgrowth	*Candida albicans*	Yeast seen in direct examination but no growth on culture	No growth	Bacterial overgrowth	*Candida albicans*	Bacterial overgrowth	Candida albicans	Bacterial overgrowth	*Candida tropicalis, Candida krusei*	Bacterial overgrowth
Rectum	Bacteria	Moderate microbiota	No growth	Numerous microbiota	No growth	Microbiota	*Clostridium perfringens, Clostridium* spp.	Moderate microbiota	No growth	Numerous microbiota	No growth	Numerous microbiota	*Clostridium perfringens, Clostridium* spp.
	Fungus	Bacterial overgrowth	No growth	Bacterial overgrowth	No growth	Bacterial overgrowth	Bacterial overgrowth	*Candida albicans*	No growth	Bacterial overgrowth	No fungal detected due to bacterial overgrowth	*Candida albicans, Candida species* (non-albicans, non-tropicalis, non-krusei)	No growth
Ileum	Bacteria	N/A	Microbiota	N/A	*Costridium perfringens*	N/A	No growth	N/A	No growth	N/A	No growth	N/A	No growth
	Fungus	N/A	No growth	N/A	Yeast seen in direct examination but no growth on culture	N/A	No growth	N/A	Bacterial overgrowth	N/A	Yeast seen in direct examination but no growth on culture.	N/A	Bacterial overgrowth
Sigmoid	Bacteria	N/A	No growth	N/A	*Clostridium perfringens*	N/A	*Clostridium perfringens*	N/A	*Clostridium perfringens*	N/A	No growth	N/A	No growth
	Fungus	N/A	No growth	N/A	Yeast seen in direct examination but no growth on culture	N/A	Bacterial overgrowth	N/A	Bacterial overgrowth	N/A	Bacterial overgrowth	N/A	Hyphae seen in direct examination but no growth on culture.
Lung	AFB	N/A	No AFB observed	N/A	No AFB observed	N/A	No AFB observed	N/A	No AFB observed	N/A	No AFB observed	N/A	No AFB observed
	Mycobacterium	N/A	No growth	N/A	No growth	N/A	No growth	N/A	No growth	N/A	No growth	N/A	No growth

DISCUSSION

Embalming technique and the gross evaluations

There are few publications concerning the human embalming technique with the saturated salt solution.[9,10,14] There is no standardization. Apart from Coleman and Kogan's and Hayashi et al.'s studies, Burns et al. injected 15-20 L of embalming fluid on the embalming day with an additional 1-5 L on the next day.[9,10,14] We hypothesized the volume should be at least 80% of the total body fluid in the NSG. Because in a 50 kg male cadaver, the injected fluid amount is 24.8 L by our calculation method, which is close to Coleman's study.[9] Our NSG storage technique was different from that of Coleman and Kogan. Their method involved storing a wrapped embalmed cadaver in the solution. Despite these differences, the gross evaluation of the NSG showed consistency, resembling Thiel's cadavers on a scale of 4 (56.7%) and fresh cadavers on a scale of 3 (70%). The gross tissue qualities resembled that of living humans or fresh cadavers on scales of 3 and 4 (Table 2). The NSG had excellent joint flexibility (Fig 1). These results are similar to previous studies performed in both human cadavers and rats.[6,10,14-16]

The NSG's brains were soft and mostly liquefied. This finding is similar to Thiel's solution embalming method, which has been shown to poor preserve the brain.[3,17] There is no prior report of brain morphology. However, a study of different salt concentration solutions to preserve human brain and liver slices found that saturated salt fluid can preserve brain slices without signs of decomposition.[18] This discordancy suggests there is room for further study.

We also tested the submerged method by directly placing cadavers in the fluid and decreasing the injected fluid amount. However, this was unsuccessful, which could have resulted from the cadavers' body fluids diffusing into the higher osmotic embalming fluid.

Histological evaluations

Coleman and Kogan reported the saturated salt method preserved adipose tissue, striated muscles, liver, and spinal cord microanatomy.[9] Beger et al. compared seven preservative methods on rat cadavers. They found that Thiel's and the saturated salt method had the same qualities as freshly preserved assessed by appearance, joint flexibility, and consistency. But Thiel's solution skin and internal organs were too pliable. The saturated salt method's skeletal muscles and tendons had similar properties to the fresh. But Thiel's solution muscles had less nuclear prominence and muscle fiber integrity.[16] We used another way to assess histological quality by comparing H&E-stained tissues to textbook pictures. Most of them were rated on Likert scales 4 and 5 (Table 3). It is presumed that the saturated salt with the non-submerged technique has preservative properties for the histological structures.

Microbiological evaluations

At 25 °C, 1 L of water can dissolve 360 grams of sodium chloride. The saturated salt solution molarity was 6.2 molar (M).[19] A study of pathogenic bacterial inoculated natural sheep casing showed that no non-spore-forming bacteria could be identified in the casing from brine at 6.2 M. The pathogenic bacteria in that study were *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens,* and *E. coli* O157:H7. The results showed that Gram-positive and spore-forming bacteria were more resistant to high osmotic pressure than the others, resulting in a need for at least 30-day preservation to eliminate the pathogens.[20]

Our study findings contradicted Hayashi et al.'s results. We found bacteria and fungus at the end of the embalming. The post-embalming pathogenic bacteria were *Clostridium perfringens* and *Pseudomonas aeruginosa*. Burns et al.'s study found fungal growth on SSS after using them for 15 days. There was no microbiological evidence of putrefaction in Burn et al.'s report. But we found bacteria in every NSG's intestine since the first dissection. We speculated that the climate in our country (Thailand) plays a role in microorganism growth since no prior SSS studies have been done in a tropical country.[6,9,10,14,21]

The putrefied cadavers

We found that the death records and post-mortem signs in cadavers SS6105 and SS6107 were mismatched. Both cadavers' internal organs had disrupted surfaces, whereby microbiomes could leak into the body cavities and ingest tissue right after death.[22] This also may have caused the lower injected fluid amount than planned in these two cadavers (Table 1). The cadavers might have entered the bloated stated before we embalmed.[22]

Application

A questionnaire-based study asking medical students, residents, and specialists from five Israel medical schools about how anatomy should be taught showed 87.8% of participants (Total N = 678) thought anatomy is relevant to general practitioners even in the age of the imaging and 68.8% agreed that dissection should be taught based on dissection, while only 13% agreed with image-based.[23] A preclinical education study found active learning enhanced learning outcomes and soft skills such as responsibility,

honesty, and kindness.[24] Cadaveric dissection is one way of active learning, which needs teamwork, responsibility, and intention to complete the assignment. Moreover, longitudinal integration such as cadaveric biopsy during dissection is helpful in pathology learning correlation, and practice suturing during dissection makes medical students at ease in the clinical year.[25,26] Recent research shows cadaver-based simulation also increases resident confidence and augments operative autonomy.[26] From previously studies and this research, saturated salt cadaver shows good quality for all these applications, but the embalming technique is still in need of perfection.[9,10,14,15,18]

Limitation

We have a limited cadaver number in this study since it's a pilot study. Moreover, the gross anatomy evaluators were randomly invited to avoid bias. In future research, more cadaver numbers and randomly selected staff forming an evaluator team to evaluate every cadaver may yield a different result.

CONCLUSION

The embalming technique by injecting the saturated salt solution at up to 80% total body water volume is a promising embalming method that yields cadavers with high gross tissue quality, flexible joints, and good histological structures. However, this technique cannot eliminate bacteria and normal flora. It may result from the tropical climate setting. Further study of this technique adjustment, such as changing the solution composition and storing cadavers in low-temperature conditions, is advised.

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