REVIEW

Techniques for Cadaveric Preservation of the Biliary Tract for Anatomical Studies: A Mini Review

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Abstract

The preservation of human cadavers for anatomical studies and preparation of prosections for teaching dates back to the third century B.C. The ability to examine the intact structural environment of the human body is crucial for understanding normal anatomy and variations. Techniques have evolved with growing knowledge, and anatomists and dedicated clinicians have developed methods to preserve entire bodies as well as internal structures with delicate and precise architecture. These techniques range from formalin preservation of the whole body to corrosion cast preparation of selected structures.

With this review, we discuss the methods that are primarily used for preservation of the biliary tract for anatomical studies and teaching.

Keywords: Bile duct preservation; Formalin fixation; Corrosion cast; Cadaver freezing; Cadaver color coding

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Introduction

Evidence of human dissections by Greek physician Herophilus of Chalcedon and his contemporary, Erasistratus, goes back to the third century B.C., but unfortunately, it was abandoned after their time (1). Scientific literature of human anatomy dates back as far as 1543, and by the 19th century, learning gross anatomy through dissection became an important part of anatomy education and medical curricula (2). The use of the human body for anatomy teaching and learning in medical education mainly focuses on full-body dissections of preserved cadavers and prosections made by preserved body parts.

Cadaveric preserving techniques have evolved over hundreds of years for teaching and learning human anatomy (3). Learning with cadavers aids significantly in linking theoretical anatomy knowledge with the real human body. The selection of the cadaver preservation technique depends on the organs, their variability in the tissue types, consistency, as well as financial feasibility. The substances and chemicals used vary from alcohol to heavy metals (4). Various preserving techniques of embalming have been used to maintain tissue and organ color and shape (3, 5).

The biliary tract consists of the gallbladder and bile ducts inside and outside the liver. This is mainly a hollow system containing bile (6). Therefore, preserving biliary tissue is a process that requires delicate handling of the ducts, depending on the indication of the preservation, whether it is for routine cadaveric dissection by medical undergraduates or for producing prosections. While there are numerous studies that are based on gross specimens of the hepato-biliary tract, only a handful mention the method of preservation of the study samples. Even a smaller number discussed these techniques in detail so that a budding researcher could build on them. We aimed to gather information on cadaveric preservation of the biliary tract, primarily with the intention of using them for anatomical teaching, studies, and to prepare prosections.

Methods

This study reviews the methods used for cadaveric preservation of the biliary tract for gross anatomical studies and to prepare prosections. The authors searched articles in two medical databases (PubMed and Google Scholar), and conducted a focused literature search using Elicit AI-powered research assistant, using the following key terms: ((‘biliary tract’ OR ‘bile duct’ OR ‘hepatic ducts’) AND (‘human cadaver’ OR ‘human autopsy’) AND (‘preservation methods’ OR ‘processing methods’ OR ‘preparing methods’)). The database and Elicit-AI search yielded 153 records. First, two reviewers independently screened the titles and abstracts of articles selected from the databases mentioned above using the keywords for eligibility. After this initial screening, 24 studies were included in full-article analysis. Full texts were assessed, and discrepancies were resolved with discussions or with the opinion of the third reviewer. Leaving 13 studies eligible for data extraction. Six additional studies were identified through manual searching, bringing the total to 19.

Inclusion and exclusion criteria

Articles published in English, with complete information on preservation methods and human studies, were included in this review. Articles with information only on preservation of human tissues for histology or pathology slides, and preservation of animal specimens were excluded.

Discussion

The anatomy of the biliary tract has been studied using different methods. While the most traditional method was using gross specimens, with the development of new technologies, this has shifted towards imaging, such as cholangiography, endoscopic retrograde cholangiopancreatography, and magnetic resonance cholangiopancreatography. However, the use of gross specimens either as fresh autopsies or as fixed specimens for research and teaching in this region is still widespread.

Techniques of preserving the biliary tract

Injection of a chemical to veins to prevent putrefaction

In the past, many chemicals were used to prevent the process of putrefaction (alcohol, tannic acid, mercuric chloride, arsenic, bismuth, glycerin, and formaldehyde). However, recently, chemicals with heavy metals have been used sparingly to avoid unwanted side effects (4).

Fixing with formalin

Formalin fixation of the full-body or the excised and separated hepatobiliary system with Kaiserling's solution is a widely used method. Here, diluted 40% formalin (up to 10%) is injected through the femoral artery, with perfusion facilitated by gravity. Postmortem clotting can be a limiting factor for poor perfusion (7, 8). The fixed-cadaver/the hepatobiliary apparatus is then preserved in 5% formalin solution, or at 4 degrees Celsius (9, 10). However, in some studies, the fixed specimens were preserved in 60% alcohol solution until dissection (7).

Freezing

Freezing is another technique in which the body/specimen is preserved in a cold chamber or a freezer at 4 degrees Celsius (11, 12). A comparative study done using freezing and embalming techniques in preserving the biliary tract has highlighted that both techniques distort the biomechanical properties, leading to fiber network deterioration (10).

Injection of Polymers into Vessels of Fixed Specimens

With time, polymers came into the picture, which have the benefit of durability in addition to preventing putrefaction and less biological risk of exposure compared to previously used chemicals (4). Silicone injection into vessels in formalin-fixed cadavers using Rhoton's Lab technique, then distinguishing them from naturally bile-stained bile ducts, and dissecting with a surgical microscope, was a method used by several authors (13, 14). They have differentiated and removed the vascular branches at the end to totally expose the bile ducts (13). Colored, synthetic latex is another polymer that can be used (14).

Injection and fixation

This involves the injection of a polymer into the vessels of a fresh cadaver/specimen and then fixing it. Water-soluble absolute latex resin can be used for this purpose, and the resin is injected into an artery of the liver specimen until the subcapsular vessels are visualized. After injection, the specimen is fixed with 10% formalin. This way, the naturally bile-stained biliary tract can be easily distinguished from the arterial system and can be dissected (9). Injection of polymers into the bile duct and then fixing with 10% formalin is also carried out (15).

Injection and corrosion

This involves the injection of a hardening substance into a hollow system, followed by degeneration of the surrounding soft tissue, and finally obtaining a 3-dimensional polymeric specimen which depicts the internal structure of the initial intended organ. This method allows a detailed study of the hollow structures (4, 16). Therefore, it is more suitable for studying the biliary tract than the traditional techniques.

This technique dates back to the early 16th century, when Leonardo da Vinci used beeswax injected into the ventricular system of the bovine brain, followed by careful scraping of brain matter to study its configuration. Historically, the substances used as fillers included hot beeswax, molten metal mixtures, sheep tallow, and pine resin (16). More recently, several injectable substances, such as acrylic, epoxy resin, polyester resin, silicone, liquid latex, and polyurethane which can be used for this technique (4, 13, 17, 18). Fresh specimens give the best outcome with these techniques. Though in the past, the process involved washing the specimen with Ringer Lactate, pre-mortem anticoagulation or fixation with formaldehyde, these steps are currently considered unnecessary (4).

For this procedure, first, the specimen should be prepared by careful dissection of the main ducts without damaging them and washing thoroughly with water to remove debris. The polymer injection phase requires skill and patience and has three steps: dissection and identification of the ducts, isolation and cannulation of the ducts, and preparation of the polymer and injection. To allow the polymerization process to happen in the ideal shape of the organ, it can be submerged in a sodium chloride/water solution in the anatomical position. Substances used for corrosion could be biological, enzymatic, or chemical (16). Use of chemicals is common, the most popular one being sodium hydroxide (NaOH)/caustic soda. Using NaOH in a concentration below 25% (weight/volume) was found to be more effective (4). Concentrated hydrochloric acid (HCl) is another chemical that can be used for the corrosion of the liver tissue (18, 19).

Correct technique in all steps of corrosion casting will be important in the quality of the final result (16). This method is popular and has become the rule due to easy handling, safety, better hardness of the final product, and maintaining the size of the structure at its original (4). Therefore, corrosion casting has become a method of preparing anatomy models with high academic value.

Color coding

Whether it is a gross specimen or a cast, a color code to accentuate the vascular and biliary structures would be very helpful in identifying the anatomy. A color code has been established for polymer casting, which uses green for bile ducts, while other colors have been proposed for artery, vein, airway, and urinary tract (red, blue, white, and yellow, respectively) (4, 15). In Doppler ultrasound studies, the arteries are depicted in red, and the veins are depicted in blue (20). As bile is greenish in color and urine is pale yellow, this proposed color code seems reliable. However, some studies have used the yellow color for bile ducts (15, 19). Usually, it is the resins that are colored. Farias et al. used green English vegetable dye 37 as the injectable to color the biliary tract (11). A study in Nepal used methylene blue dye to color the bile duct (21).

Challenges

The blind-ending nature of the biliary tract poses the challenge of selecting access and drainage points, as the enclosed space must be fully drained while preventing the leakage of cast medium. To achieve this objective, a catheter of comparable size should be used to introduce the casting material, and the other outlets should be ligated with sutures to prevent leakage. (4, 15). If a catheter of the right diameter cannot be found, then a smaller one should be used/or if a bigger one is the only available option, a wedge cut in the introducing end can be utilized (4). Usually, the biliary tract is fully drained before retrogradely filling the system with casting material (16). The ducts can be initially purged

with water or hydrogen peroxide (H_2O_2 1-2%) in very wide ducts (although this is mainly used for airways) to remove clots, stones, or possible blockages, facilitating the injection of polymer, followed by a water wash if H_2O_2 is used (4). Both drainage and introduction of cast material become difficult when there is pressure coming onto the ducts from the neighboring tissues (19).

Formalin fixation poses a major challenge when specimens cannot be thoroughly perfused with formalin. This happens when there is excessive postmortem clotting (8).

Conclusion

Dissection of the human body as a part of anatomy teaching was used from the 19th century. With this educational objective and research purpose, preservation techniques of the human body have been evolving over the past centuries. The biliary tract can be preserved with 10% formalin en-block or frozen at 4 degrees Celsius for the basic requirement of dissection. For the purpose of

preparing prosections or research, the specimens can be prepared with the injection of polymers into the vessels of fixed specimens, injection of polymers into the vessels of fresh specimens and then fixing them, and injection of polymers into the vessels and bile ducts of fresh specimens and

then liquifying the surrounding tissues with a corrosive agent. Color codes have been proposed that can be used to keep the uniformity in these casts. Though there are many challenges, bile duct preservation techniques are still evolving and improving, leading to a brighter future ahead.

Conflicts of interests

The authors have no conflicts of interest to declare.

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