Technique for Latex Injection and Reuse of Human Heads Preserved in Formaldehyde

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Abstract

Keywords

- cadaveric specimen
- dissection
- endoscopy
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- skull base

Background Understanding the anatomy of the skull base is paramount for every skull base surgeon, particularly in light of the expanded endoscopic endonasal approaches, and of the refined surgical technique used in both medial and lateral approaches. A comprehensive knowledge of anatomy is the cornerstone for a safe surgery, maximizing resection and minimizing complications. The best study method is the careful dissection of fresh human cadaveric heads in a well-equipped anatomy laboratory. In this study, we describe our protocol for preparing cadaveric specimens without vascular injection, which had been preserved in a formaldehyde solution after treating them with a dimethyldioctadecylammonium chloride/distearyl dimethyl ammonium chloride solution (commercial fabric softener) and injecting the vascular system with latex.

Method Six cadaveric specimens underwent our treatment and subsequent injection of the vascular system and dissection.

Results All specimens showed a good penetration of the latex and a clear improvement of the malleability of the tissues was noticed. The authors agree that this technique improved the quality of the head and facilitated studying.

Conclusion We consider this an effective treatment with latex, reaching small caliber vessels, and a greater malleability and flexibility of tissues, allowing better dissections, and greater anatomical exposure, making them suitable for skull base training, study, and research.

Introduction

A comprehensive knowledge of the skull base anatomy is required of all skull base surgeons. The development and expansion of the endoscopic endonasal approaches and the refinement of the traditional lateral skull base approaches have made this knowledge the cornerstone of a safe surgery, maximizing resection and minimizing complications.^{1–4}

Different resources can be used to understand the skull base anatomy, with an expanding number of publications on the subject and novel virtual reality and 3D software. However, most surgeons agree that the best method is the careful dissection and study of fresh human cadaveric heads in a well-equipped anatomy laboratory. Fresh specimens are not widely available and are usually expensive.^{5,6}

The specimens must be carefully prepared and preserved to avoid putrefaction and to conserve the anatomy, as similar

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to in vivo, for as long as possible. The injection of the vascular system is useful in the preparation. The method of preparation and preservation of the specimens is seldom discussed and many institutions keep this information internal, compounding the difficulty for institutions with limited resources to build an anatomy laboratory focused on skull base.⁶⁻⁹

The chemicals used for preservation of the specimens should sterilize them of any virus, bacteria, or fungi, and preserve them for a long period. They may; however, distort the anatomy, alter the flexibility and malleability of the tissue and create hazardous chemical waste.¹⁰ Formaldehyde has been, since the 19th century, the workhorse for cadaver preservation, offering a long period, low cost, easy preparation of the solution, and broad antimicrobial properties.^{6,9} On the other hand, it generates toxic fumes with an unpleasant and strong odor to work under, and it leads to tissue darkening and rigidity, distorting the anatomy. Although widely used, formaldehyde is far from ideal.^{6,7,11}

Silicon and latex have been described as suitable alternatives for the injection and study of the vascular system, which should be done as early as possible. Latex has the advantage of being ready to use, not requiring dilution or mixtures, lower viscosity, which allows a better penetration into small vessels, faster hardening, and low cost.^{5,7,9,12}

In this study, we describe our protocol of using cadaveric specimens without vascular injection, which had been preserved in a formaldehyde solution for longer than 5 years for endoscopic and microscopic surgical anatomy study, after treating them with a dimethyldioctadecylammonium chloride/distearyl dimethyl ammonium chloride solution (commercial fabric softener), and injecting the vascular system with latex.

Materials and Method

We applied our protocol to 6 human heads, previously severed at the neck from adult cadavers, which were preserved in the Anatomy Department, using a 10% solution of formaldehyde for at least 5 years. We selected only specimens without any indications of trauma, craniofacial deformities, or previous intracranial manipulation (incisions, craniotomies, or burr holes).

This study was reviewed and approved by our Institutional Review Board, under the protocol number CAAE-42014915. 9.0000.5479.

Our protocol for restoration and injection of cadaveric specimens consists of 5 sequential stages:

Stage 1: Head Selection and Dimethyldioctadecylammonium Chloride Treatment

We selected only cadaveric specimens without vascular injection, which had been preserved in a formaldehyde solution for longer than 5 years in our institution's Anatomy Department, and which did not have any indications of trauma, craniofacial deformities, or previous intracranial manipulation (incisions in the scalp, craniotomies, or burr holes).

The specimens were removed from the formaldehyde tanks and washed and were completely submerged, with the neck facing up, in a solution of dimethyldioctadecylammonium chloride/distearyl dimethyl ammonium chloride (commercial fabric softener) for 72 hours. After that they were thoroughly rinsed under running tap water and submerged in 70% ethanol for preservation. (→ Fig. 1)

After rinsing, the heads do not need to be submerged in ethanol, before moving on to the following stages. If the specimen will not be used, it should be placed in alcohol for preservation.

Fig. 1 (A) Cadaveric specimen in dimethyldioctadecylammonium chloride/distearyl dimethyl ammonium chloride (commercial fabric softener). Each head was kept in it for 72 h, completely submerged, vertically, with the neck facing up (this picture was taken before completely submerging it to show the position). (B) The heads are preserved in a 70% ethanol solution between dissection sessions.

Stage 2: Dissection and Cannulation

We identified and dissected Common Carotid Arteries (CCA), Internal Jugular Veins (IJV) and Vertebral Arteries (VA), a minimum of 1.5 cm and cannulated them using a Foley's catheter adequate to the diameter of the vessel (12 to 18 F), when available. Because of their smaller diameter, we cannulated the VA using intravenous tubing or nasogastric tubes. The Foley's catheter is superior, because its rigidity allowed us to advance it into the vessel with the flexibility for manipulation and occlusion during injection.⁷ The catheters and tubing were secured to the vessels using 0-0 cotton sutures, carefully not to occlude them. (► Fig. 2)

Stage 3: Irrigation of the Vessels

All blood clots and obstructions must be cleared for the latex to adequately enter the vascular system, including small caliber arteries. To achieve that, careful and profuse irrigation is a vital step of this technique.

We first irrigated the arterial system to avoid venous congestion and decrease the arterial flow.⁷ The order of irrigation was bilateral CCA, bilateral VA, dominant IJV (ie, greater caliber, usually on the right side) and nondominant IJV. A large 60 cm³ syringe and warm tap water were used for irrigation on both sides, until clear water emerged from the contralateral vessel. Constant and gentle pressure is applied

to the syringe's plunger. The number of repetitions needed varied with each specimen. A specific number of repetitions or volume of water should not be used as the parameter during this stage. Only, when clean water emerges from the contralateral vessel, it can be considered effectively done.

Stage 4: Latex Injection

We used prevulcanized natural latex (Siquiplás, São Paulo, SP, Brazil–1 L) with red or blue dye (Siquiplás, São Paulo, SP, Brazil–30 g) for the arterial and venous systems, respectively, using 180 mL of latex mixed with 30 g of dye. The vessels were already catheterized and we injected them in the following order: right CCA, right VA, left CCA, left VA, dominant IJV, and nondominant IJV.

If following this order, the venous system should be clamped to retain the water of the irrigation in it and avoid any red latex spilling into the venous system. The red latex was steadily injected into the right side, until latex emerged from the left CCA, which was immediately clamped, and gentle steady pressure was maintained on the plunger until another 10 cc was injected into the arterial system, so that the latex could reach smaller vessels. The right CCA is then clamped. During injection, all latex leaks at the neck were ligated for an adequate penetration of the latex into the intracranial vessels.

Fig. 2 (A) The specimen is positioned with the neck exposed facing us. We identify the CCAs, the IJVs, and the VAs, as highlighted in the image. (B) 1.5–2 cm of each vessel is dissected and cannulated, preferably using Foley's catheters, which were secured using 0-0 cotton sutures. (C and D) Latex injection into the arterial system while clamping the venous system. (E and F) Latex injection into the venous system with the arterial system clamped.

After injecting the right CCA, we proceeded to the right VA, using a similar technique, until latex emerged from the left VA. The specimen should be allowed to sit for 5 minutes. Then we open the left CCA and inject latex into it to reach all branches, particularly in the neck and face, which may not have been reached through the contralateral injection. Similarly, we then proceed to the left VA. (►Fig. 2)

The arterial system is kept closed and we inject the venous system with blue latex, starting with the dominant IJV, until it emerges from the contralateral side, which is then clamped and further 5 cc are injected applying gentle pressure. The venous system can rupture more easily than the arterial; therefore, even if less than 5 cc are injected after clamping the contralateral IJV, but the resistance increases, injection must be stopped. The specimen is allowed to sit for 5 minutes and we repeat the process on the other side. (►Fig. 2)

All the vessels must be kept closed with hemostatic clamps after injection and the heads should be allowed to sit outside for at least 20 minutes and then submerged in ethanol.

For a visual assessment of the latex penetration in the arterial system, we dissected the dorsal nasal artery (a branch of the anterior ethmoidal artery), as shown in ►Fig. 3.

Stage 5: Storage

The specimens were stored in a 70% ethanol solution, in a leak-proof container, large enough to accommodate the head vertically, with the neck facing up. The hemostatic clamps were kept in place for the first 72 hours after injection and then removed. At any time, 1 of the heads was not being dissected, it was returned to alcohol to prevent putrefaction.

All 2 heads were dissected using an endonasal rigid endoscope, simulating a transnasal approach to the skull base, noting the change in tissue rigidity compared with in vivo and with formaldehyde-preserved specimens. A transcranial approach to assess the intracranial vessels was also

performed. (►Fig. 4) The penetration of latex into the vascular system was also observed.

Results

The vertebrobasilar system was the most challenging to prepare. Dissection and cannulation of the vessels were always difficult and in 1 of the 6 specimens, it was not possible. Stage 3 (irrigation of the vessels) was also troublesome and required greater care and patience, probably due to the small caliber of the posterior circulation vessels. The CCA and IVJ, on the other hand, were easily cannulated and irrigation was not difficult, adequately clearing the vessels in all 6 heads.

During dissection, we noticed a significant improvement of tissue flexibility compared with the formaldehyde-preserved specimens without the fabric softener treatment, as agreed by all the authors taking part in the dissections. We were; however, unable to objectively quantify or measure this improvement. As expected, the darkening of tissues caused by the formaldehyde remained unchanged. A good penetration of the latex even into the smaller vessels, such as branches from the M4 (►Fig. 4) was also achieved.

During the work sections, we also noted that the heads were better to work with, free of the fumes from formaldehyde, and showed a greater similarity to a fresh specimen, although still inferior to it.

Discussion

Formaldehyde (10%) is still widely used for preservation of anatomical specimens, despite its compromise of the tissues color, malleability, and the toxic fumes, it releases. Other solutions have been suggested by different authors. Coleman et al and O'Sullivan et al used progressively lower concentrations of formaldehyde (below the traditional 10%) and were

Fig. 3 (A and B) A right and left lateral rhinotomy, exposing the dorsum nasi and the superior and inferior lateral cartilages and the dorsal nasal artery injected with latex. In B, after removing the right superior lateral cartilage, we see the nasal septum.

Fig. 4 After completing the reuse protocol in one of our heads, we did a right-sided pterional craniotomy. After skin incision, (A) the superficial temporal artery and vein were dissected, showing the latex penetration. After the craniotomy (B) the middle meningeal artery is seen filled with latex. After dural opening, (C) a thickened arachnoid (effect of the formaldehyde) partially obstructed the identification of the M4 branches with latex. After removing the arachnoid, the M4 branches are visible emerging from the Sylvian's fissure.

able to preserve specimens for a long period, while reducing tissue rigidity.^{10,13} Sanan et al showed very good preservation results abandoning formaldehyde in favor of 66% ethanol solution.⁶ Other methods and substances (polyethylene glycol, phenoxyethanol) have also been used with good results.^{13,14} In our protocol, we chose to use ethanol 70% for the preservation of the specimens, because it is a commercially available solution, relatively inexpensive and we had no putrefaction issues to date or any further damage to the complex anatomy of the skull base.

The use of dimethyldioctadecylammonium chloride/distearyl dimethyl ammonium chloride solution (commercial fabric softener) to improve the rigidity and malleability of preserved tissues was first described to rehydrate dried cadavers and to study Egyptian mummified bodies and it has been described for neurosurgical specimens.^{6,11,15,16} Using a similar technique, we imbibe our heads in commercial fabric softener for 72 hours and found them to be more malleable to work with after, allowing us to study and dissect the skull base with good results.

It has been suggested that if the vascular system was not irrigated and cleaned once the specimen is fresh, it is no

longer possible to do so, or to inject the vessels.⁵ In fact, our first attempt to use formaldehyde-preserved heads, before using dimethyldioctadecylammonium chloride/distearyl dimethyl ammonium chloride solution, was frustrated by this problem. However, after adopting this protocol the removal of blood clots and subsequent injection of latex into the vascular system was possible in all our specimens.

As it is observed with tissues, we believe that the intravascular clots were rehydrated by the fabric softener, which made them less adherent to the vessels, allowing the irrigation to adequately remove them and prepare the vasculature for injection.

Injecting the vascular system with different colors is a traditional technique, employed in its study, mainly for head and neck specimens, for both intra and extracranial dissection. Initially, this technique would use products, such as wax mixed with paint, which would be injected hot in a liquid state and would quickly solidify as it cooled. The “hot injection” was used as early as the 17th century and it was gradually replaced by “cold injections”, using a catalyst to solidify a liquid solution shortly after being mixed and injected. Anatomy books from the 19th century would

always have a section detailing the preparation and injection of the head and brain vasculature. Current literature; however, does not detail the steps of this procedure.⁷ We describe in this study a step-by-step technique of injecting the vascular system, which can be used for both fresh (excluding the fabric softener treatment) and for formaldehyde-preserved specimens.

Recent studies suggested latex as superior to silicon for injecting cadaveric heads,^{6–9} with the advantages of reduced cost, resistance and viscosity, shorter solidifying time, without the need for a catalyst. Our experience further demonstrates its simple use, low cost, and adequate filling of all the branches.

The main obstacles for a successful injection are obstructions of the vascular system, such as stenosis, atherosclerosis, and blood clots. Formaldehyde-based preservation solutions aggravate these obstructions by dehydrating the vessels' walls and blood clots. Our experience showed that using a fabric softener treatment, they can be overcome and a successful injection is possible even in formaldehyde-preserved specimens.

Hands-on cadaveric dissection courses, dissection laboratories, and specimens are not widely available and are usually expensive, particularly in developing countries. A method for improving the condition of heads stored in formaldehyde, reducing the cost of training and study would allow for a greater comprehension of the complex skull base anatomy, and improve surgical expertise and patient outcome.

Conclusion

Our technique of treating formaldehyde-preserved specimens in a dimethyldioctadecylammonium chloride/di-tearyl dimethyl ammonium chloride (commercial fabric softener) solution showed a clear improvement in tissue malleability and flexibility, although still remaining inferior to fresh specimens. Unfortunately, the tissue darkening caused by oxidation could not be improved. It also allowed us to clean and inject the vascular system with colored latex, improving the quality of dissection and anatomical exposure, making them better suited for skull base training, study, and research.

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