 DOI: 10.18231/2394-2126.2017.0137

A cost effective and user friendly method for procurement of bones from formalin fixed specimens- a pilot study

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Abstract

Introduction: The Knowledge of Anatomy gained from hard part demonstration classes in 1st year MBBS needs to be stressed because of its importance in surgical, medical, radiological and medico legal practice. Traditional method of burying the bones and letting the nature do its work is the best way of procuring bones. This trusted and time tested method of procuring bones by burial is safe but time consuming and requires safe burial grounds which are available in very few institutes. With increase in number of Health Sciences Institutes, there is ever increasing need of human bones for teaching purposes. To curtail the ever increasing demand of bones, the left over parts of Formalin fixed cadavers at the end of 1st year MBBS dissection schedule available in the department of Anatomy are used to procure bones.

Materials and Method: The method is as follows: (1). Bones are freed of soft tissue by grossly cutting the attached soft tissue followed by scraping after immersion in tap water for 3 to 4 hours. (2). Bones are then immersed in solution of washing soda for 18 to 20 hours. (3). Scraped again to remove the remaining soft tissue and left in tap water for 4 to 5 hours. (4). Bones are now immersed in hydrogen peroxide solution for 24 hours. After removing from hydrogen peroxide, (5).bones are air dried for 2 to 3 days or more depending on atmospheric conditions. (6). Painted with turpentine.

Results and Discussion: 27 bones were procured in 30 to 36 working hours with very well preserved morphological features.

Conclusion: Bones procured are in natural state with all morphological features very well preserved. Expenditure incurred for making entire set of bones would be approximately 2000 to 2500 INR. Cost of artificial bone set available in market ranges from 10000 to 16500 INR and these artificial bones do not have good morphological details. The in house method is cost effective, user friendly and quick

Keywords: Procuring bones, Anatomy class, Formalin fixed specimens.

Received: 8th June, 2017 **Accepted:** 23rd October, 2017

Introduction

Hard part demonstration classes in Anatomy are an integral part of curriculum in most of the professional health science courses. Procurement of bones by burial method is time tested and most trusted one method, however it is a time consuming process. Non availability of secure and safe burial ground is one of the biggest disadvantages of this method. Similarly burial method cannot be used on formalin fixed specimen for procuring the bones. The left over formalin fixed specimens and or dissected cadavers that are readily available in the department of anatomy are usually handed over for incineration as a biological waste. These specimens and dissected cadavers can be used to procure bones. Hence development of a "Cost effective and user friendly" method for procurements of bones from formalin fixed specimens is the need of the day. Taking this in to consideration, we have developed an in house method for procurement of bones in Department of Anatomy.

Materials and Method

Formalin fixed specimens and cadavers available in the department of Anatomy after completion of routine first year MBBS dissection schedule and some old specimens preserved in the department were used to procure bones.

Clavicles are subcutaneous and have minimum soft tissue attached and are therefore used for the pilot study. They are dissected from the cadavers, immersed in tap water for 3 to 4 hours and freed from attached soft tissue by gross cutting followed by scraping (Image 1). Then the clavicles are immersed for 18 to 20 hours in solution of washing soda (20 grams (powder used for cleaning house old utensils)) in tap water (1 litre). Immersion in solution of washing soda leads to saponification of remnants of muscles, tendons, ligaments and other soft tissue and also helps in degreasing of bones. Saponification softens the tissues attached to bones and hence it can be easily scraped off from the bones. Degreasing prevents oozing of fat from bone marrow and thus prevents bones from getting dirty and sticky. After removing bones from solution of washing soda, they are washed in tap water and left in jar filled with tap water for 4 to 5 hours. After removal from tap water they are again scraped to remove the soft tissue if still attached. Bones are now immersed in 6% hydrogen peroxide (industrial grade) for 24 hours. Immersion in hydrogen peroxide bleaches the bones giving them clean look and also helps in further

degreasing (Image 2). After removing from hydrogen peroxide, bones are allowed to air dry for 2 to 3 days depending on the level of humidity and season. In dry weather air drying for one day is sufficient. After the bones are dry remaining tissue is again scraped off. The bones are then painted with turpentine and allowed to dry. Painting with turpentine (the one used for paint thinner) increases the shelf life of bones by keeping the pests away and also preventing them from further decomposition. With this method 20 clavicles were procured in 7 working days. After painting with turpentine bones are ready to use. Similar procedure was followed for procurement of 7 scapulas. Scapulas being deeply paced with lot of soft tissue attached, hence required 10 working days.

Image 1: Removal of soft tissue and cleaning the clavicles

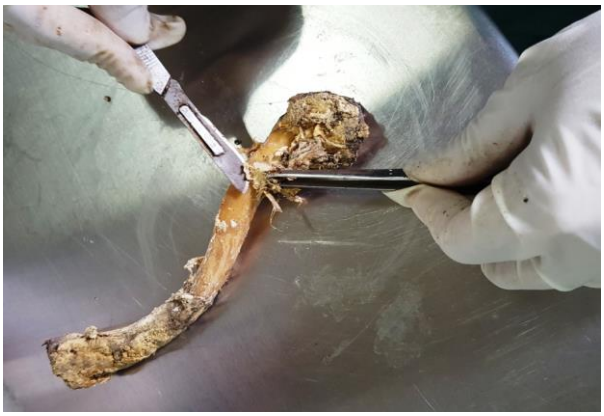


Image 2: Clavicles in Hydrogen Peroxide

Scapulas and Clavicles were taken from formalin fixed specimens and cadavers that were discarded after routine undergraduate dissection and would be handed over for disposal by incineration.

Similarly an attempt was made to procure carpals, metacarpals and phalanges for preparing articulated hand skeletons; however removal of soft tissue from the metacarpals and phalanges was difficult as these delicate bones broke off. The most likely cause was that the specimens used for this were preserved in formalin since very long time, hence making the bones brittle and fragile.

Results

The method proved to be cost effective, less time consuming and user friendly. This method does not require specialised technical support or costly and delicate automated instruments. Bones could be procured from formalin fixed specimens within the expected time period of 8 to 10 days. Fleshy part of the muscle is the easiest part to be scraped off. Tendons and aponeurosis attached to bone near the epiphyseal end needs to be removed carefully because there are chances of separation of diaphysis and epiphysis. Scraping of soft tissues should be gentle to prevent removal of periosteum.

Specimens preserved in formalin for a very long period may not yield good results because the bones become fragile if left in formalin for long duration. This is evident from (Image 3) where clavicles appear denser as compared to scapulae because scapulae were procured from preserved specimens of previous year and clavicles were taken from cadavers of current year. Specimens of hand could not yield good quality bones because the bones were brittle as they belonged to very old preserved specimens, hence authors advocate use of fresh formalin fixed cadaver or specimens for better results.

Image 3: Scapulae (less dense) and Clavicle (denser)

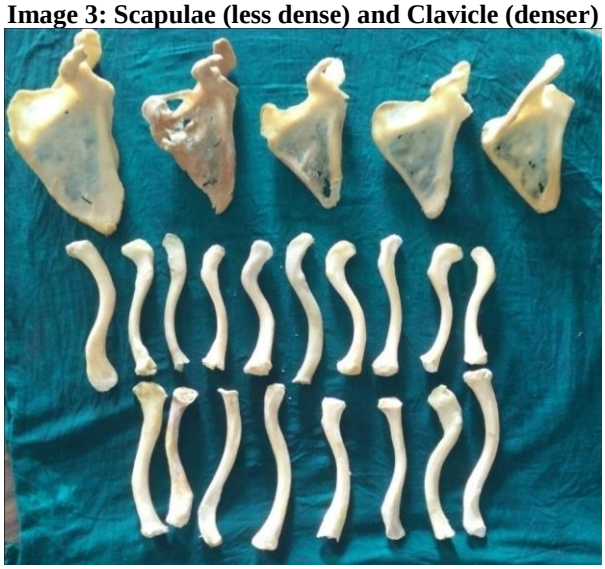


Image 4a: Natural bones

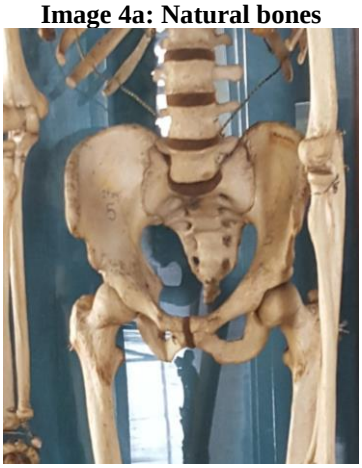
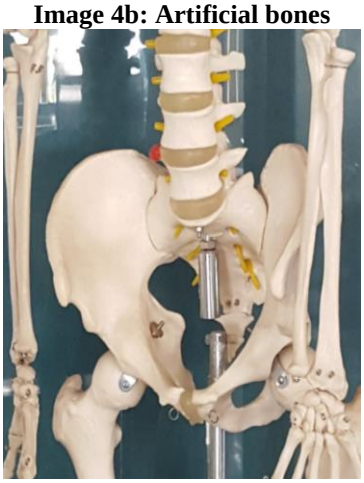


Image 4b: Artificial bones



Discussion

It is a well-known fact to an Anatomist that the most commonly used method for procuring bones is either burial or boiling. Average time period required for procurement of bones by burial method is minimum one year. If cadaver is buried without removing viscera time period may be further increased. This is further delayed and may not be successful at all if the bones are from cadavers embalmed with formalin. Process of boiling needs the availability of utensils, gas, special and secure space and most important a trained technician to monitor the end point. Sometimes bones need to be boiled after excavating from burial ground to remove the attached tissue that may be left over. Pulvertaft[(1)] has revived various museum techniques for preservation of different specimens. Various methods for preparation of skeletons have been described by Schmitt.[(2)]

Modi, Puri and Patnaik[(3)] mention that boiling with alkali based detergent further speeds up the process and have used hot air oven to dry the bones. Our method has an advantage of being economical as boiling of bones is taken care of by scraping the bones after removal from washing soda. We have not used hot air oven to dry the bones. We have dried the bones in air at normal room temperature.

Aggarwal et al.[(4)] have combined burial and use of different concentrations of hydrogen peroxide to procure bones. Buried bones were excavated after one year and if large amount of soft tissue was still found attached, bones were reburied for removal of soft tissue. Bones with minimal soft tissue were cleaned to remove the dirt and mud and then allowed to dry. Dried bones with minimum amount of soft tissue were immersed in different concentrations of 50% w/w hydrogen peroxide solution. One volume of hydrogen peroxide diluted with 2 volumes of water gave best results after immersion for 18 hrs. Bones appeared brittle and degraded with higher concentration of hydrogen peroxide. Lower concentration of hydrogen peroxide took more time to clean the bones. We have used 6% hydrogen peroxide industrial grade which is cheaper compared to routinely available laboratory grade; only to bleach the bones so that they look cleaner and are better accepted by students. Cleaning of bones by immersion in water with the help of insect larvae is yet another most commonly used and very old time tested method. However this is a long process requiring almost six to eight months or more to clean the entire skeleton. The odour emitted from left over macerated remains while changing the putrid water for cleaning the bones is highly obnoxious. Complete removal of larvae from cleaned bones is necessary because if they persist in bones, larvae will continue to use bones as their food because soft tissue is no longer available. Our experience says that, this method however does not seem to work well with formalin fixed specimens because larvae do not thrive well on such specimens. Use of larvae to clean the skeletal parts has been described previously by Hall and Russell.[(5)] They have used Dermestid beetles larvae for cleaning bones. Borell[(6)] had used similar method to prepare skulls. Gritisand Brunner[(7)] have modified the above method for preparation of skeletons in formalin fixed specimens. Pramod, Vaswani and Bindhu[(8)] used 1% KOH for maceration. Specimens were dipped in 1% KOH till bones were visible through the tissues, but this process requires close monitoring as the unattended specimen may get damaged. Onwuama et al[(9)] has compared maceration , burial and chemical method using 3 and 5% NAOH for obtaining bones of rat and has concluded that chemical method is most effective and bones could be procured in 7 hours but they needed cleaning by hydrogen peroxide. Considering the difference in size of rat and human bones our method definitely appears more promising. Hoffmeisterand Lee[(10)] have used ammonium hydroxide to clean mammalian skulls, whereas Jakway, Raskin and Thyle[(11)] made use of sodium perborate for preparation of skeletons.

Ossian[(12)] has used enzyme-based laundry presoakers for preparation of skeletons. Swift[(13)] is of

the opinion that simple detergent can be used to clean bones. Simonsen et al[14] is of the opinion that, degradation of soft tissue by use of enzymes is very fast and the yielded bones are also very clean. However all the above mentioned methods need specific chemicals and are costly due to use of various enzymes and chemicals. In house method developed by department of Anatomy is very cost effective and fast compared to other methods discussed above. It does not require segregated area or the specialized technical staff. Entire procedure like cleaning, washing, drying and immersion in washing soda or hydrogen peroxide for procurement of bones was performed in the dissection hall. Only disadvantage of this method could be exposure of research person to fine dust emitted while scraping the bones. This can be avoided with the use of appropriate mask. Formalin is a well known for its disinfectant property as it is an age old chemical used for fumigation of operation theatres so authors are of the opinion that formalin fixed specimens should be virtually free of all known pathogens.

Boyle[15] Sullivan and Romney[16] Grygon[17] have mentioned different techniques for preservation and procurement of non-human skeletons and their parts for display at museum. McDonald[18] has described "Do's and Don'ts" to get the best results for preparation and preservation of skeletons (compare Image 4a & b). Our requirement was making bones available for teaching purposes, even though all the above mentioned procedures can be reproduced with suitable modification for human skeletons, but they would not be as cost effective and quick as the one developed and discussed here. Authors further plan to procure dry specimens of pelvis and skulls and other long bones by similar in house method with suitable modification if needed in near future.

Conclusion

To overcome the acute shortage of some skeletal parts for routine undergraduate teaching, in house method developed in the department of Anatomy has proved to be cost effective and quick. The bones procured are from dissected cadavers and hence have well preserved morphological features. Artificial bone sets available in the market cannot produce morphological features with same precision. (Image 4). Perfect and precise knowledge and visual impression of morphological features on bones needs to be stressed here because of its forensic significance for determining the age and sex of the deceased and is the key factor for differentiating male and female pelvis and skull, human and non-human bones. Making use of discarded cadaver parts to procure bones at the end of dissection schedule gives great satisfaction of making use of every bit of donated body coming to the Department of Anatomy.

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