

AN ALTERNATIVE APPROACH TO BONE CLEANING METHODS FOR ANATOMICAL PURPOSES

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ABSTRACT

Introduction: Many methods of bone cleaning have been mentioned and followed by various experts over period of years. Most of them are tedious and time consuming. Also they need training and expertise of persons doing these procedures. In this study we have tried to modify some of the older methods to make the extraction and cleaning of bones easy and less time consuming. Also we expect that the bones extracted by this method will be more presentable for study. Thus the preparation of bones specimens by this method can be of immense value and time saver for many research institutes to get their self prepared bone specimens and thus help them in becoming self sufficient.

Materials and Methods: Lots of the donated bodies under the ongoing body donation programme are being used in the department of Anatomy, AIMSAR. After dissection and autopsy in the dissection hall, the cadavers which have been buried for a period of about 1 year were dug for the remains. The bones were collected with the help of technicians and washed thoroughly with tap water. The bones were then put in varying concentration of H₂O₂ and observed for desired changes.

It was also recorded that how much time did each concentration take to clean the bone of its soft tissue. Also the amount of bleaching/whitening of bone will be noticed. Care was taken that none of the original features of bone were lost or attenuated. If at lower concentration some of the tissue was not cleaned off the bones, then higher concentration of hydrogen peroxide were tried.

Conclusion: Finally we were able to conclude that at what concentration the bones clean easily and at earliest without damage and with required amount of bleaching. They were then dipped in solution of fixative for preventing damage and giving them a presentable look.

KEY WORDS: Bones, Cleaning, Hydrogen Peroxide.

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INTRODUCTION

The study of Human Anatomy is an integral part of medical sciences. No course in medical field is complete without the subject of Human Anatomy. Among the various parts of Human anatomy, study of bones is very important to understand the organization of structures in human body. Demand of bones is increasing these days because of increased number of seats for medical stream [1]. Bone cleaning is also important for forensic science and from medicolegal point of view [2].

The ethical issues and cost involved makes the procurement of bones difficult from the market. Though some Medical Colleges face scarcity in availability of bodies for study purposes, but in our college we have a body donation programme [3] under which we have received ample number of donated bodies. These bodies are being used in fresh as well as in embalmed state for autopsy and dissection purposes.

The cadaver dissection is an important part of study of Human Anatomy. The disposal of cadaver after complete dissection is usually done by burying it in burial ground which is a part of the Anatomy department of every Medical College. This is because some anatomists think that burying and letting nature do its work is the best way of cleansing skulls and skeletons [4]. As defleshing of tissues is best done by burying, so after burial of about 1 year the bodies are taken out for the bones. The bones procured by this manner are mostly clean from their soft tissue but still in some bones a small amount of tissue is stuck to the ends of long bones or joint of bones like sutures in the skull. This study is about a method which can be adopted for easy cleaning of such cadaver bones. Different methods of bone cleaning like boiling [5], manual cleaning by scraping, maceration with water or use of detergents [6] have been tried by many. Use of various chemicals like enzymes [7], hydroxides [8] and even of bugs [9] are practiced and available in the text, but they seem difficult, time consuming and may need some special kind of place or equipment. These procedures might also need experienced manpower.

Apart from these original one specimens, arti-

cial bones are also available for study, but these are not of much use in medical colleges because any artificial bones cannot be exactly like the original bones. In the present study an attempt has been made to find a different method of bone cleaning for Anatomical purpose which is less expensive, needs less manpower and consumes less time.

MATERIALS AND METHODS

The present study was carried out in the Dissection Hall of Anatomy Department at our institute after taking the ethical clearance from institutional ethical committee. Materials used were the bones that were dug from the burial ground of department of anatomy, hydrogen peroxide and wood polish which was used as fixative for bones.

Method and detail of steps are as follows:

Skeletal remnants of cadavers (formalized as well as unformalized) were excavated after one or more years of burial from the burial ground of anatomy department.

Bones with scanty amount of soft tissue were taken for study. Those with pending large amount of soft tissue were reburied.

These remains were washed thoroughly with water to remove all the dirt and mud and dried till the left over soft tissues on bones looked completely dry (fig.1). The bones were then ready to be cleaned with a solution made of hydrogen peroxide. Necessary precautions for handling these chemicals were taken.

Fig. 1: Unclean bones after digging and washing (with soft tissue stuck at some points) ready to be cleaned.



The Hydrogen Peroxide solution is available in market at various concentrations. We used the solution with concentration of 50% W/W. It was taken in four different concentrations with water in the ratio of 1 (H₂O₂): 0.5 (water), 1:1, 1:2 and 1:3. Each container was labeled as A,B,C and D respectively.

The bones prepared from step 3 were then dipped thoroughly under these solutions and the containers were covered with loose lids (Fig. 2)

Fig. 2: Dipping of bones in solution and bubbles being formed.



Fig. 3: Appearance of bones after cleaning, washing and drying.



Within half an hour bubbles were seen in the solutions. These solutions with bones dipped were kept in dark to prevent inactivation/degradation of H₂O₂.

After about 18 hours of dipping, the bones were taken out of the solution. Changes seen in

bones are presented under observations. Only some of the bones needed brushing with a soft bristled tooth brush to look completely clean which was done wherever required.

The bones which were completely clean and looked appealing for handling, display and study were taken out and put under water bath of slow flowing water for about half an hour so that any remnants of hydrogen peroxide in bones were washed away.

After washing, the bones were dried in shade on a cotton cloth till they were completely dry.

The dry bones were then painted with a thin solution of wood polish. The wood polish is easily available in the market. The procedure of polishing worked better by dipping the bones (all types, even skull) in the solution of wood polish. The bones were dipped so that every part of it is well covered with solution. They were then taken out and thoroughly dried in shade because the moisture softens fragile bones, making it further susceptible to further damage.

Fig. 4: Final appearance of bones after polishing ready to be used.



OBSERVATIONS

Container A: (Concentration - 1(H₂O₂):0.5 (water)) On using these higher concentrations the bones appeared as if they were dissolving in solution. When taken out, the bones looked eroded and on draining the solution, powdery bone tissue was seen at the bottom of the jar. So this concentration was not suitable for bone cleaning as it dissolved the bones.

Container B (conc. - 1:1) The bones were cleaned of its stains and tissue, but looked chalky white and had powdery coating on the surface. On testing such bones for their strength,

they proved brittle and were not as strong as those treated with lesser concentration (1:2) of H₂O₂. The results were not satisfactory so this concentration was also discarded.

Container C (conc. - 1:2) Most of the bones obtained was clean and devoid of soft tissues (fig.3). Bones with remnants of soft tissues or which looked unclean/dirty were put back to solution, which took another 24 hours to get cleaned. Only few bones needed little brushing to get completely cleaned. We found this concentration of H₂O₂ best for our cleaning purpose within the specified time period of about 18 hours duration.

Container D (conc. - 1:3) The bones were clean and bleached to satisfactory level but the little amount of soft tissue stuck even after 18 hours was more than those from container C. So, the bones were again dipped for 24 hours and then taken out. This time the bones were as clean as those from container C. This container though cleaned the bones after again dipping them, but because this took more time and repetition of method we consider container C concentration the best to clean the bones to required level in minimal time. So we discarded container D concentration also.

By this method all the bones were cleaned properly. We did not observe any difference in the quality of cleaning of any of the bones. The base of the skull appeared totally clean and we even opened one of the skulls by separating individual bones of the skull. They were also completely clean from inside. So this method was able to provide satisfactory results.

Fig. 5: Appearance of bones after cleaning compared to original bones without cleaning.



The polishing solution gives them a neat polished look and also acts as a fixative so that even the smaller chips of bones are not broken apart. Finally the bones looked appealing and very presentable (Fig. 4). Difference of bones before and after cleaning by this method has been shown above. (Fig. 5)

DISCUSSION

The success of any bone cleaning technique is determined by the time taken, the resources required, and the results obtained in relation to the intended purpose for which cleaning is required [10].

As more and more bones were always the need in the department, we had been constantly trying to clean bones in the department by already mentioned methods in the text. Some of the methods tried before performing the above said cleaning method were boiling the bones in locally available detergent, manual scrubbing with brushes and maceration of soft tissue in water. Chemicals solutions of sodium hydroxide, soda bicarbonate were tried. Cleaning with dilute hydrochloric and sulphuric acid was also done.

These methods gave variable results. Boiling and scrubbing gave best results among previously tried methods but there were several shortcomings which have been discussed below.

Then we tried hydrogen peroxide solutions to clean the bones. Initially desired results were not being obtained, but after trying many times we developed the method mentioned here.

Though boiling is the time tested method but it requires big equipment and special place for the process. Lot of time required for this process and some smell is also emitted while boiling the bones, because of which the process cannot be carried out at all places. During the process of boiling one person needs to be around to keep checking the endpoint of the process [5].

Detergent is also an effective method and readily available, but it requires manual cleaning by scrubbing off the soft tissue or by boiling. This scrubbing process can be time consuming and also requires manpower.

Insect consumption method though is very effective method of bone cleaning as mentioned

in the text, but it requires the help of an entomologist for rearing of beetles. Usually demisted beetles are used. Also it takes more time and regular observation because beetles when deprived of food may even eat away the bone.

Enzymatic maceration has been declared to be the fastest and bones obtained are quite clean, but the problem is the obnoxious smell that develops during the process and high cost of enzymes used [7].

Maceration by water is simplest method with good results, but is time consuming. Need of replacement of putrid water periodically and release of bad smell during the process are some of the limitations of this method. At times the bones may need manual cleaning [6].

So we have concluded that the method for cleaning bones with H₂O₂ as mentioned here is easy and can be done in all medical colleges having dissection hall with burial grounds. Also the chemicals required in this procedure are easily available in the market. It does not require much of heavy equipment as needed for boiling the bones.

The manpower used in this method is very less as only a single person without much expertise can do the entire work. Also the time needed is quiet less. All the procedures from digging, to the bones being ready can be done in less than 1 week time during dry sunny days.

Also lots of bones can be cleaned at one time. Bones of about 10 cadavers were prepared together in our department in 1 week's time. Also the procedure can be done in a small area, so no special place for performing the procedure is needed.

Use of hydrogen peroxide as bleaching agent has been commented by many in the text but here we have used it for cleaning the soft tissue stuck to the bones as well as bleaching agent for destining the bones. In this procedure manual scrapping of bones before bleaching them is not required. So by this method both the steps of scrapping and bleaching happen together in the hydrogen peroxide solution. The strength of bones extracted from embalmed and unembalmed cadavers was maintained after this entire procedure of bone cleanup.

Over all the other advantages of this method is that the chemical once used, can be reused. Though the effect is less on reusing the solution for the second time, and the procedure takes more time than that for the first batch. By adjusting the concentration, that is by adding more H₂O₂ every time about 4-5 batches of bones can be cleaned by solution once it is made.

So we conclude that by this method even an inexperienced person with minimal supervision can clean the bones. Necessary precaution for handling hydrogen peroxide solution must be taken. This method of cleaning bones can be helpful in increasing the availability of bones in Anatomy departments for study and that also at very cheap rates, thus helping the medical colleges to become self sufficient in this requirement of original human bones.

As it is essential to constantly reevaluate the methods of skeletal preparation to ensure a product that is visually as well as acceptable to touch, we have found this method to be very useful and easy with acceptable results, as compared to other available methods.

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Conflicts of Interests: None

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