

An Approach to Know Effective Techniques in Processing and Cleaning of Bones

Dhanesh Kumar Sharma¹, Nikita Chaudhary²

Abstract

Introduction: As a part of anatomy teaching curriculum and three-dimensional demonstrations in Osteology, preparation of bones plays an important role. Also, to bring more information regarding sites of muscular attachments and course of neurovascular structures in a region, study of bones consistently maintains its own essentiality. Osteology is an important aspect of forensic anatomy too. This study is to know the effective techniques in bone cleaning among the various methods like maceration with water, boiling, enzymes, chemicals, bugs, leaving open above ground and burial under soil. **Methods:** Dissected cadavers buried for a period of about one year were dugout for the skeleton. Bones were also collected from the dissected cadavers directly without burial, with the help of technicians by using common instruments in the dissection hall like scalpels, scissors, and forceps to leave the bones with minimal soft tissue attachments. Then these bones were put in different solutions like H₂O₂, NaOH, Limewater and Plain water with sun exposure in daytime for a week. Finally the bones were then washed in running water and air dried under the fan. **Results:** The best method found suitable for the clean and white bone preparation in this study was using the chemical Limewater. The smell of the bones prepared and the process is not as strong/difficult and distasteful as perceived in the other methods like burial, leaving open above ground, boiling, using enzymes and bugs etc. **Discussion:** Most of the methods of bone preparation are tedious and time consuming. Also, they need training and expertise of persons doing these procedures. In present study we found that using Limewater (a clear liquid consisting of calcium hydroxide) with sunlight exposure in daytime for a week time, was the best method in the terms of less time required, the odourless and white colour and bones prepared and the simplicity of the process. So we are now able to find out the most suitable organic method by which bones can be prepared with least damage and for a good presentable appearance.

Keywords: Maceration; Chemical preparation; Burial; Osteology; Cleaning.

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Introduction

Human Anatomy is the foundation and basic subject of medical science and osteology or study of bones is the essential part of anatomy teaching curriculum and is incomparable in the ability to provide

3-dimensional presentation in osteology. There are a number of methods or techniques for bone preparation like maceration of dissected cadaver through submersion in water for a time in cold or hot water; boiling; burying in the ground; using biological washing powder, enzymes like pepsin, chemicals like H₂O₂, NaOH, Limewater, dermestid beetles; leaving bones open above ground to fasten the removal of the attached soft tissues from the bones.

Bones are very essential part of anatomy teaching curriculum.¹ Preparation of bones involves soft tissue removal or bone cleaning, bone bleaching, bone articulation and labeling.² Preparation of bones from cadavers by maceration has been shown to elicit strong and distasteful odour, is time consuming

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and takes longer time in large animals.³ Dermestids the flesh-eating beetles have been used for bone cleaning in small animals and is said to be the best method. Cold or warm-water maceration has been used as standard maceration techniques. Boiling and subsequent mechanical cleaning of skeletal material is also used.⁴ Maceration with organic chemicals can be performed with enzymes such as papain/pepsin or with washing powders containing enzymes.⁵ All solutions eventually remove or dissolve the soft tissues from the external surface of the bone. Solutions with different pH have significant and interesting effects on bones to the varying degrees.⁶

Cleaning treatments using bleach, H_2O_2 , ethylenediaminetetraacetic acid/papain, room temperature water and detergent/sodium carbonate followed by degreasing had low DNA concentrations and failed to generate nuclear polymerase chain reaction (PCR) products. Study shows that traditionally "conservative" maceration techniques are not necessarily the best methods to yield DNA from skeletal tissue.⁷ Maceration techniques remove soft tissue by the destruction of biomolecules, but the applied techniques may also affect the morphology and the molecular integrity of the hard tissue itself.⁵

Materials and Methods

In this study for bone cleaning and preparation we adopted method using Water, H_2O_2 (50%), 5% solution of NaOH and Limewater in human bones both small and large. Dissected cadavers buried for a period of about 1 year were dugout for the skeleton. We also used the bones of dissected cadavers in the dissection hall. The muscles were carefully dissected and teased from the bones using common instruments in the dissection hall (scalpels, scissors, forceps) to leave the bones with minimal soft tissue

attachments. They were then put into different plastic buckets/containers containing water, H_2O_2 , NaOH and Limewater enough to submerge the bones. Necessary precaution for handling these chemical solutions were taken. Then the bones in the buckets/containers were placed under the sunlight in daytime for 4/7 days. The bones were then washed in running water and air dried under the fan. Parameters such as colour change and odour of the bones were noted. Percentage of bone recovery was noted and thereafter photographs of the bones were taken.

Results

The best method suitable for bone preparation in this study we found is use of chemical Limewater. The method we used is not time consuming, bones appear most white as compared with other chemicals like H_2O_2 and NaOH. **Table 1** is showing comparison of bone cleaning with these chemicals. The smell of the bones and the process is not as strong/difficult and distasteful as perceived in the other methods like burial or leaving bones open above ground. The main disadvantage with some chemicals is the cracks made on the bones, which is also not found with this chemical limewater. It is also observed that when sunlight exposure is avoided then bone whiteness is less and time duration of bone preparation increases for getting similar result as with sunlight exposure. This study has highlighted the advantages of chemical method over maceration and burial methods in the preparation of bone. Amongst the chemicals, the use of limewater gave the best result. Another most obvious feature is that result in the form of cleaning and whiteness was found equal with small as well as large human bones. Result about whiteness of bones after 7 days was most satisfactory than that of after 4 days (**Fig.1**).

Table 1: Comparison of results from media used in Methods of Bone Preparation.

Parameters	Water	H_2O_2 (50%)	NaOH (5%)	Limewater
Days taken	7 days	7 days	7 days	7 days
Colour change	No change	White-brown	Creamy	White
Smell	Moderate	Moderate	Moderate	Slight
Damaging effect	Not detected	Not detected	Slight cracks	Not detected

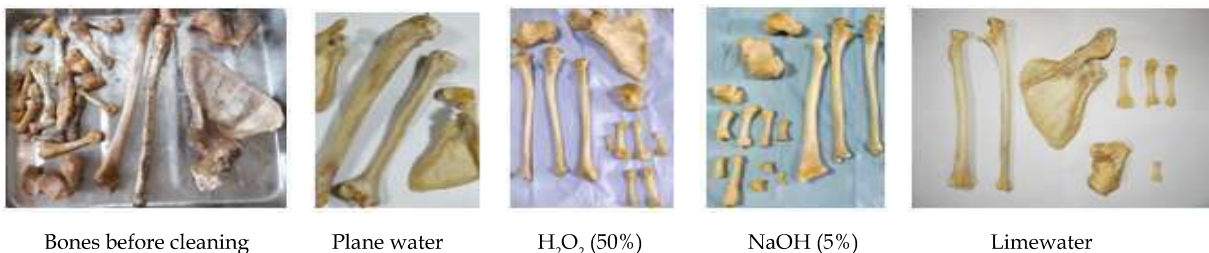


Fig. 1:

Discussion

Most of the methods of bone preparation are tedious and time consuming. Also, they need training and expertise of persons doing these procedures. In present study, chemical preparation with Limewater (a clear liquid consisting of calcium hydroxide and water) with sunlight exposure in daytime for 7 days was found to be the best method in terms of time required to complete the procedure, number of bones recovered; and colour and odour of the bones prepared. However, the chemical method has the disadvantage of dissolving and cracking the bones if the concentration used is high and prompt attention is not given to the preparation.⁸

Effectiveness of detergents for the purpose of soft-tissue removal from animal-derived specimens is comparable to enzymatic maceration but with fewer health and safety issues; and greater advantages regarding transportation and availability of materials when an investigator is in a fieldwork scenario.⁹ Rennick *et al.* (2005) employed three cleaning techniques, boiling bone in water, in bleach, and in powdered detergent/sodium carbonate, to test for their effect on nuclear and mtDNA recovery from a variety of human and non-human bones. The human bones also showed much lower yields from bleach cleaning, while the detergent/carbonate method allowed the largest segments of DNA to be amplified, indicating it may have a less degradative effect on bone DNA than either of the other cleaning processes.¹⁰

Enzyme maceration has been shown to be remarkably fast compared to the traditional warm-water procedure, which requires up to several days. In addition, the enzyme maceration eliminates the odor problem associated with the traditional procedure. This method allows preparation of skeletal material in an essentially odorless way within a matter of hours, making the method useful in particular for forensic science, private conservation workshops and educational purposes. The mtDNA was intact and all PCR products could be identified to the right species without contamination, demonstrating that both the warm water maceration and the fast enzyme preparation method had not compromised the DNA.¹¹ 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.¹¹ Using biological washing powder/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. 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.¹²

Maceration is time consuming especially with larger animals and elicits strong odours. Insects are useful as a technique to clean bones because they perform an excellent debridement of smallest cavities but several thousand are required to produce rapid cleaning and if left with bones for a long period of time can eat and destroy them. Soft tissue removal by solutions of organic and inorganic chemicals was found to be the most effective since it macerates bone in a remarkably fast and odourless way. And it also has a less degradative effect on bone DNA than the other cleaning processes. But prompt attention should be given to the preparation when using the chemical method so that the concentration used is not high in order to avoid dissolving and cracking the bones.

So, the chemical preparation of bones specimens with Limewater in terms of comparatively easy procedure, less time required in procedure (*a week*), whitest colour of the bones and minimal odour during procedure etc. can be of enormous value for many institutes to get their self-prepared bone specimens. As it is essential to constantly reevaluate the methods of skeletal preparation to ensure a product that is acceptable visually as well to touch, we have found this method of bone preparation to be very useful and easy as compared to other methods of bone preparation.

Conclusion

Chemical preparation of bones with the Limewater (a clear liquid consisting of calcium hydroxide and water) with sunlight exposure in daytime for a week was found to be the best method in terms of time required to complete the procedure, colour of the bones and odour of the preparation. We also find a similar but little inferior result with H₂O₂. The chemical method has the disadvantage of dissolving and cracking the bones if the concentration used is high and prompt attention is not given to the preparation. In bone preparation with chemical method, the smell of the bones and the process is not difficult and distasteful as perceived in the other methods like burial or leaving bones open above ground. Time duration for chemical method is also too short comparatively.

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Ethical clearance

As study was performed on bones obtained from donated bodies after routine dissection part of undergraduate study and teaching, so there is no need of any ethical clearance from Institute Ethics Committee (IEC). Donated bodies are for study and research activities in any Medical Institute.

Conflicts of interests: None.

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