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Comparison of Laundry Detergent and Antiformin Solution Methods to Retrieve Clean Bones from Embalmed Cadavers

Comparación de los Métodos de Solución de Antiformina y Detergente de Lavandería para Recuperar Huesos Limpios de Cadáveres Embalsamados

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YADAV, P.; SHRESTHA, S.; BARAL, P. & KHANAL, L. Comparison of laundry detergent and antiformin solution methods to retrieve clean bones from embalmed cadavers. *Int. J. Morphol., 40(4)*:1566-1573, 2022.

SUMMARY: Traditional methods of bone retrieval from embalmed cadaver are not able to meet the demand of medical colleges as they are time consuming & tedious, thus there is need of evaluating an alternative approach that includes use of laundry detergent. The purpose of the study was to compare & establish the most effective method between laundry detergent and 10 % antiformin solution methods to procure clean bones. Thirty-two bones of the right side that were included in the inclusion criteria obtained from the four embalmed cadaver were cleaned by laundry detergent and of the left side by 10 % antiformin solution methods. Retrieved clean bones were evaluated for their cleanness using the scale from 0 to 5. The mean cleanness scores of the bones cleaned by laundry detergent method were not significantly different at 95 % confidence interval than the mean cleanness scores of the bones cleaned by 10 % antiformin solution method. The study found that though there is no significant difference in the mean cleanness score of the bones cleaned by two employed methods nevertheless, bones were found to be cleaner by using 10 % antiformin solution method and bones obtained by using laundry detergent method had smooth surface as well as more suitable for flat bones.

KEY WORDS: Bone cleaning; Embalmed cadaver; Laundry detergent; Antiformin solution.

INTRODUCTION

Anatomy is one of the core subject in modern medical education which is taught during the inception of medical carrier of an individual and the acquired knowledge is applied throughout his/her carrier (Paranjape *et al*., 2017). Two hundred and six bones that form the human skeleton are demonstrated & taught to the undergraduate students with the purpose of making them understand how the body framework is formed & provide them the knowledge of the location, structure, parts as well as muscles attached to each individual bone.

In 1978, Institute of Medicine (IOM) started the MBBS course in Nepal for the first time (Karki & Dixit, 2004), since then till date there are twenty medical colleges where the students are being enrolled for MBBS course which are either an autonomous institute i.e. B.P. Koirala Institute of Health Sciences (BPKIHS) & Patan Academy of Health Sciences (PAHS) or are affiliated to Kathmandu University & Tribhuvan University (Medchrome, 2009).

Despite of a twenty-fold growth of medical college in the last forty-two years, the culture of body donation for medical education has not been well established in Nepal. Apart from academic purpose retrieval of clean bones, are also required by the forensic experts to deal with the medico legal cases (Aggarwal *et al*., 2016). Traditional methods of bone retrieval by manual cleaning, cooking, water maceration, insect consumption and enzymatic maceration are not able to meet the demand of medical colleges as they are time consuming & tedious (Modi *et al*., 2014). Thus there is a need of evaluating alternative approach to procure clean bones from embalmed cadaver that is convenient to process and is feasible.

MATERIAL & METHOD

In this comparative cross sectional study approved by the Institutional Research Committee (IRC), BPKIHS

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(Ref. No. Acd. 484/076/077), upper & lower limbs were disarticulated and further defleshed using scalpel without causing any damage to the bones from four embalmed cadavers.

A total of 32 bones of right side, included in the study (scapula, humerus, radius, ulna, femur, tibia, fibula & patella) were cleaned using scouring pad & blunt knife (where necessary) by laundry detergent (LD) and 32 bones of the left side by 10 % antiformin solution (AFS) methods. Evaluation for their cleanness was done using the scale from 0 to 5 where 0=no effect; 1= small amount of tissue removed; 2= appreciable amount of tissue removed but still can't see the bone; 3= can see the bone but still see tissue over it; 4=almost clean, can see the bone but still feel tissue over it & 5= completely clean (Motz *et al*., 2008).

For every liter of distilled water, the laundry detergent solution contained 35 g of detergent (Ariel Matic top load washing machine powder; Procter & Gamble, India). The defleshed bones of right side were immersed in the solution that was pre heated to the temperature of 60 ºC, contained in the airtight heat resistant plastic container (12-gallon Life Latch drum, USA). The container was placed back in the bacteriological incubator where the temperature was maintained at 60 ºC. After 24 h the bones were removed from the solution, cleaned & evaluated (Mairs *et al*., 2004).

Bones of left side were placed in 10 % antiformin solution prepared by combining solution of 150 g sodium carbonate (Thermo Fisher scientific, Mumbai, India) in 250 mL of distilled water & 10 g calcium hypochlorite (HiMedia Laboratories, Mumbai, India) in 750 mL of distilled water; shake at 30-min interval for 3 h. The solution was filtered using cotton cloth & equal volume of 15 % sodium Hydroxide (Loba Chemie PVT.LTD, Mumbai, India) in distilled water was added (Motz *et al*., 2008). After 24 h the bones were removed, cleaned & evaluated.

In both methods, bones that were not completely clean after 24 h were placed back in their respective solution for another 24 h after which they were again removed from the solution, cleaned & reevaluated. The process was repeated till the completely clean bones were retrieved or till the seventh day of the procedure.

The cleaned bones obtained by both methods were immersed in solution of 6 % hydrogen peroxide (Merk Life Sciences PVT LTD, Mumbai, India) for 15 minutes (Sullivan & Romney, 1999) then rinsed under running tap for 5 min and immersed in pails containing water for next 24 h. Next day the bones were removed & dried under the sunlight for 5 h each day for four consecutive days (Aggarwal *et al*., 2016). The bones procured by both methods were reevaluated on twelfth day for the last time.

All cleaned and dried bones were immersed in turpentine for half an hour, then were allowed to dry for next 24 h (Paranjape *et al*., 2017). Finally, a coat of wood polish (touchwood ®, Asian paints, India) was applied to all the bones (Aggarwal *et al*., 2016).

The collected data were entered in MS Excel 2016 and were converted into Statistical Package for the Social Sciences (SPSS), version 11.5 for statistical analysis. Levine's test was used to evaluate difference in mean cleanness score of bones cleaned by the two methods, with a level of significance set at $p \leq 0.05$.

RESULTS

Table I shows the score of bones cleaned by antiformin solution method on various days of study. Only small amount of tissue could be removed (cleanness score, S=1) from all the bones except scapula, where appreciable amount of tissue was removed (S=2) on day one, but on the second day all the scapula were digested at its different parts and were not included in the study further. Though appreciable amount of tissue were removed (S=2) from most area of all bones on day two, significant amount of tissue was still present over the site of muscle attachment, obscuring the visibility of the bone except for patella where still only a small amount of tissue could be removed (S=1). On day three some of tissue could be seen (S=3) on the all bones at the site of muscles attachment except fibula, where we could feel the tissue (S=4) whereas on day four some tissue was seen (S=3) only on femur, tibia & humerus and over the others, tissue could be felt (S=4) except for patella where appreciable amount of tissue was removed (S=2) for the first time. Completely clean (S=5) fibula & radius were retrieved on day five; tissue could be seen (S=3) over patella and felt (S=4) over all other bones. Completely clean (S=5) ulna bones were obtained on day six and there was no change noted over patella. In the notch/ fossae of the femur, tibia & humerus few tissue could still be felt (S=4) on day six and seven; over the patella too tissue could be felt (S=4) on seventh day which were noted to be completely clean (S=5) on twelfth day after drying them for four consecutive days.

Table II shows the score of bones cleaned by laundry detergent method on various days of study. By LD method θ on day one and day two only small amount of tissue could be removed (S=1) from the bones. Appreciable amount of tissue could be removed (S=2) from all bones on day three

Table I Score of bones cleaned by 10% Antiformin solution (10%AFS) method on various days of study.

Bones	Number of bones	Score of cleanness using the scale of 0 to 5							
		Day one	Day two	Day three	Day four	Day five	Day six	Day seven	Day Twelve
Femur	4	1	2	3	3	4	4	4	5
Tibia	4	1	2	3	3	4	4	4	5
Fibula	4	1	2	4	4	5	5	5	5
Humerus	4	1	2	3	3	4	4	4	5
Radius	4	1	2	3	4	5	5	5	5
Ulna	4	1	2	3	4	4	5	5	5
Patella	4r	1	1	1	2	3	3	4	5
Scapula	4	2	-----	-----	-------	-----	-----	-----	-----

Table II Score of bones cleaned laundry detergent (LD) method on various days of study.

Bones	Number of bones	Score of cleanness using the scale of 0 to 5							
		Day one	Day two	Day three	Day four	Day five	Day six	Day seven	Day Twelve
Femur	4	1	1	2	3	3	3	3	3
Tibia	4	1	1	2	3	4	4	4	4
Fibula	4	1	1	2	3	4	4	4	4
Humerus	4	1	1	2	3	4	4	4	4
Radius	4	1	1	2	3	4	4	4	4
Ulna	4	1	1	2	3	4	4	4	4
Patella	4	1	1	1	2	3	4	4	4
Scapula	4	1	1	2	3	3	4	4	4

except on patella. On day four, minimal amount of tissue could be seen (3) on all bones at the site of attachment of muscles except patella where appreciable amount of tissue was removed (S=2) for the first time. Some tissue was still seen (S=3) on femur, patella & scapula at the site of attachment of muscles & over the remaining bones tissue could be felt (S=4) on the fifth day. On the next two consecutive days few tissue could be still seen (S=3) only on femur at the site of muscle attachment & over the other bones minimal amount of tissue could be felt (S=4). Even after drying the bones for 4 consecutive days, dried tissue could be seen (S=3) on the femur and felt (S=4) over all the remaining bones.

The mean score of bones cleaned by two employed methods, when compared, found that there was no significant difference ($p>0.05$) at 95 % CI (Table III). Comparison of the score of each bone by the two methods employed in the study is presented in Figures 1 to 7.

Table III Comparison of mean cleaning score of bones cleaned by 10% AFS & LD methods.

Bone	10%AFS (mean ± SD)	LD (mean ± SD)	P value
Femur	3.25 ±1.282	2.38 ±0.916	0.139
Tibia	3.25 ±1.282	2.88 ±1.356	0.579
Fibula	3.88 ±1.553	2.88 ±1.356	0.192
Humerus	3.25 ±1.282	2.88 ±1.356	0.579
Radius	3.75 ±1.581	2.88 ±1.356	0.255
Ulna	3.63 ±1.506	2.88 ±1.356	0.313
Patella	2.50 ±1.512	2.50 ±1.414	1.000

During the study while cleaning the bones by 10 % AFS & LD method, few bones were noted to have mild avulsion & some even broke down. Superficial flakes could be seen on the surface of all the bones except over patella and few bones were noted to have formation of small aperture by 10 % AFS. After drying the bones under sunlight, longitudinal cracks were noted on few bones cleaned by both methods (Figs. 8 and 9). Though no significant difference was noted, study found that the bones were cleaner by using 10 % AFS method & LD method was found to be more suitable for cleaning of flat bones as the scapula did not get digested till the end of the study (Fig. 10).

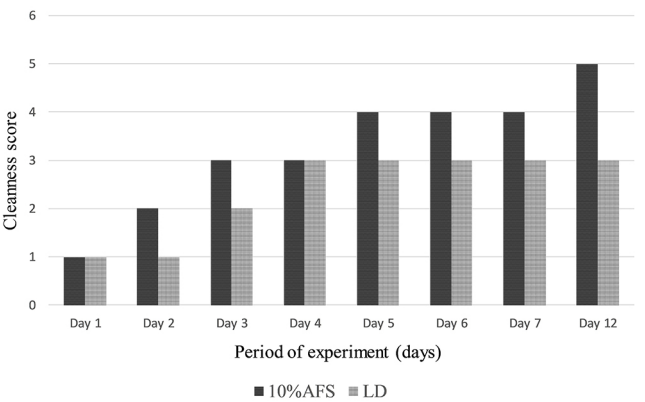

Fig. 1 Comparison for the cleanness score (0-5) of Femur over the period of experiment by 10% antiformin solution (10%AFS) & Laundry detergent (LD) method.

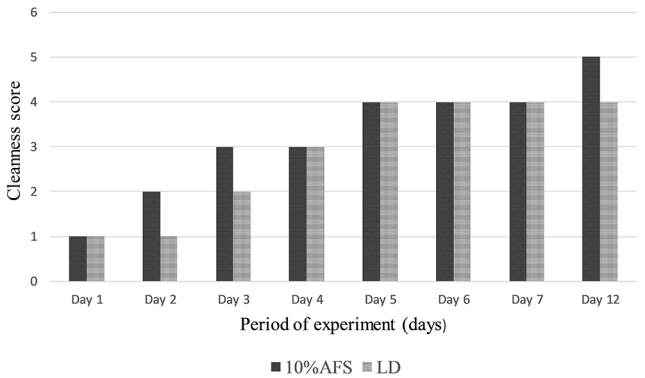

Fig. 2 Comparison for the cleanness score (0-5) of Tibia over the period of experiment by 10% antiformin solution (10%AFS) & Laundry detergent (LD) method.

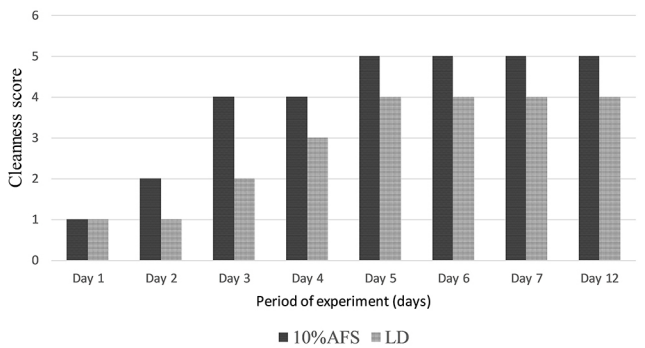

Fig. 3 Comparison for the cleanness score (0-5) of Fibula over the period of experiment by 10% antiformin solution (10%AFS) & Laundry detergent (LD) method.

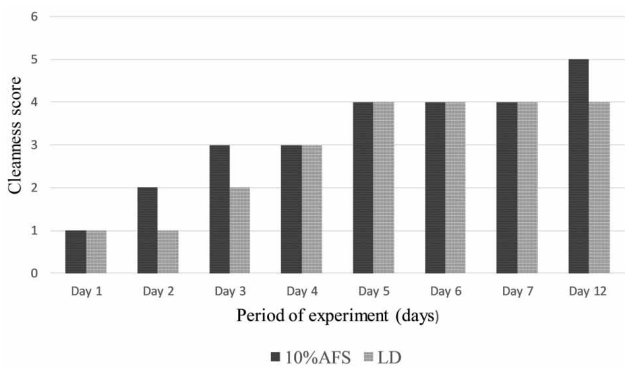

Fig. 4 Comparison for the cleanness score (0-5) of Humerus over the period of experiment by 10% antiformin solution (10%AFS) & Laundry detergent (LD) method.

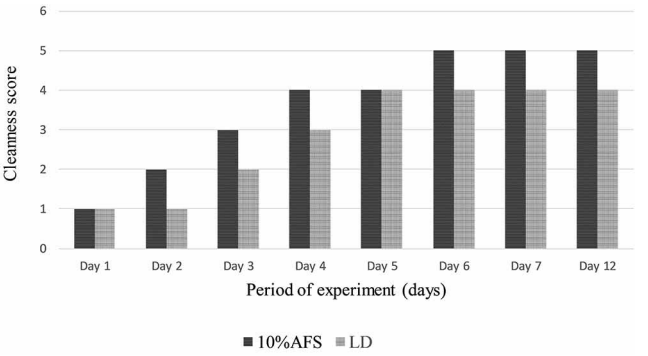

Fig. 5 Comparison for the cleanness score (0-5) of Radius over the period of experiment by 10% antiformin solution (10%AFS) & Laundry detergent (LD) method.

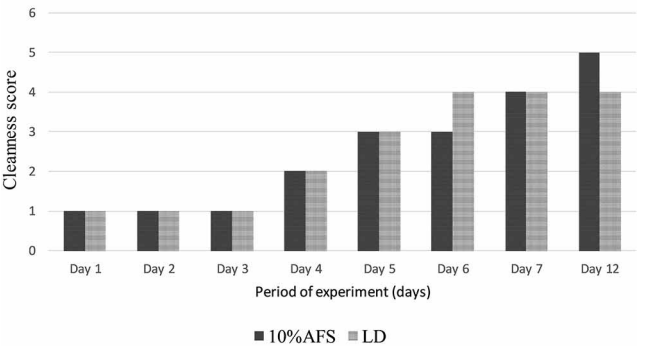

Fig. 6 Comparison for the cleanness score (0-5) of Ulna over the period of experiment by 10% antiformin solution (10%AFS) & Laundry detergent (LD) method.

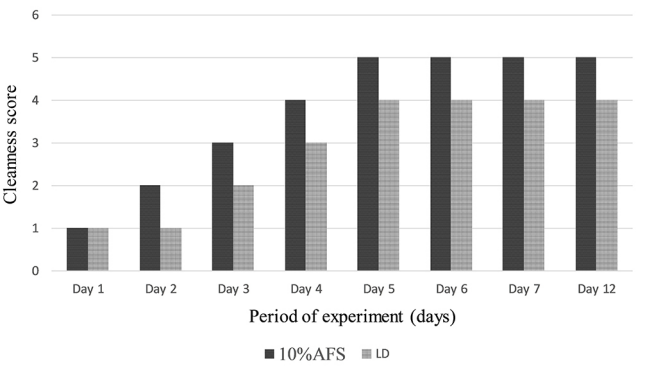

Fig. 7 Comparison for the cleanness score (0-5) of Patella over the period of experiment by 10% antiformin solution (10%AFS) & Laundry detergent (LD) method.

DISCUSSION

In Nepal, there has been an increase in the number of medical college by twenty folds in the last forty years which has increased the demand of the human bones for teaching learning activity. As tradition methods of bone retrieval are tedious, this study evaluated an alternative approach to procure clean bones from embalmed cadaver. The result of our

study shows that the mean cleanness score of the bones cleaned by 10 % AFS method were not significantly different than the mean cleanness score of the bones cleaned by LD method. Therefore, denying the research hypothesis that there is difference in cleanness of bone while procuring clean bone from embalmed cadaver by LD method & 10 % AFS method. Nevertheless, bones were cleaner by using 10 % antiformin method. The bones obtained using laundry detergent method had smooth surface and was found to be more suitable for cleaning of flat bones.

Fig. 9. Deformities noted on bones by use of laundry detergent method. A. Broken bones; B. Longitudinal cracks over the bones. Avulsion noted on the head of femur. Muscle tissue visible at the site of attachments.

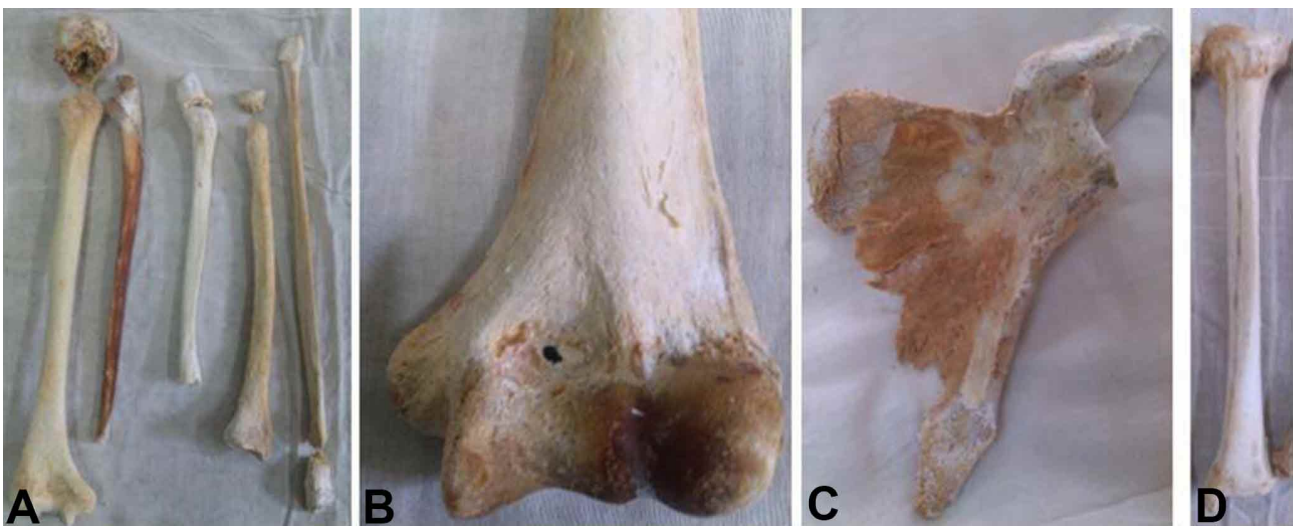

Fig. 8. Deformities noted on bones cleaned by 10 % Antiformin solution Method . A. Broken bones; B. Formation of aperture at coronoid fossa and cortical flakes noted on humerus; C. Digestion of scapula; D. Longitudinal crack on tibia.

Fig. 10. Comparatively cleaner bones retrieved by 10% AFS Method (A) than those cleaned by LD method (B). Flat bones (scapula) retrieved by use of LD method (B).

Bones are firmly attached to flesh which is made of protein, flesh can be removed without damaging the bone by artificially softening the protein (Soon *et al*., 2015). The cross-linking of collagen causes the anchoring of muscle and connective tissue to bone. Embalming process that utilizes formaldehyde increases the cross links within and between collagen fibers, increasing the difficulty of cleaning bone in embalmed cadavers when compared to un-embalmed cadaver (Motz *et al*., 2008). Fixation process makes soft tissue resistant to maceration. Tissue fixation & aggressive maceration to procure bones adversely affect the quality of the retrieved bone (Ator *et al*., 1993). In this study we have used alkaline solution as it breaks down collagen and other proteins (Steadman *et al*., 2006).

Paranjape *et al*. (2017) in their study have stated that immersion of cadaveric bones in solution of washing soda leads to saponification of muscles, tendons, ligaments and other soft tissue attached to bones which can be easily scraped off and also helps in degreasing of bones that prevents oozing of fat from bone marrow and thus prevents bones from getting dirty and sticky.

Vicki Abrams Motz in his study have found that Sodium hydroxide, the most basic macerating agent had the greatest effect in removing flesh from fixed tissue. Detergent is also basic in nature and can hydrolyze the collagen amide bonds rendering it as an effective agent in the maceration of embalmed cadavers. Mixture of detergent and sodium carbonate was almost as effective as the antiformin in removing flesh from fixed tissue whereas detergent alone was less effective when compared to antiformin (Motz *et al*., 2008). Bartels and Meyer used detergents to macerate small vertebrates. The maceration solution produced excellent results when applied to fresh or deep-frozen specimens and ethanol-fixed material but the result with formalin-fixed specimens were unsatisfying (Ajayi & Edjomariegwe, 2016).

Motz *et al*. (2008) found that there was an increase in effectiveness of antiformin from iced (1.6 +/-1.5) to 20 ºC, but no improvement beyond that for 70 ºC. Decomposition of soft tissue is delayed in embalmed bodies due to presence of formalin but boiling leads to enhancement of soft tissue decomposition due to decrease in formalin after boiling. Boiling the bones in the detergent solution further speeds up the process of saponification of soft tissue (Modi *et al*., 2014). In this study, the solution used in LD method was maintained at temperature of 600 ºC whereas solution used in 10 %AFS method was maintained at room temperature. However, still the study found that bones were cleaner by 10 %AFS method which suggests that antiformin solution is more effective compared to LD method.

Snyder, Burdi, and Gaul have mentioned antiformin as an efficient method of skeletal preparation because clean specimens can be obtained within an hour from any type of material, including formalin preserved materials (Thompson, 2015), but in our study it took days to procure the bone.

The disadvantage of the chemical method (Sodium hydroxide) is digestion and cracking of bones if the concentration used is high and prompt action is not taken by checking the bones immersed in solution at frequent interval (Onwuama *et al*., 2012; Ajayi & Edjomariegwe, 2016). Prolonged exposure to any chemical can result in brittle, fragile bones and loss of cortical detail. The use of sodium hypochlorite causes the extensive flaking of the cortical surface, surface flaking hinders the detail study of more delicate skeletal structures (Steadman *et al*., 2006). Snyder *et al*., utilized antiformin solution as a maceration method and noted that the solution may actively dissolve bone (Mairs *et al*., 2004). In this study too, while cleaning the bones with 10 % AFS method, few bones were avulsed, broken, appeared to have an aperture, crack in the bone & all the scapula were digested. Flakes were noted on the surface of all bones as well, except patella which may be because of the fact that it is a sesamoid bone that is wrapped within the fibers of the tendon that have decreased the direct exposure time of the bone to the solution until the fibres were macerated by 10 % AFS method.

Collagen and protein matrix forms the organic part of the bone & the hard outer covering is formed by hydroxyapatite (calcium phosphate) mineral. Deterioration of bone can occur if any of these components is removed. When exposed to heat and temperature fluctuations, collagen deteriorates which makes bone more brittle and fragile (McDonald, 2006). While cleaning the bones with LD method, few bones were avulsed, broken and upon drying cracks on the bones were noted. Boiling can cause bones to scorch, split and crack, especially if boiling is too rapid or extensive (Steadman *et al*., 2006)

Avulsion & Breaking of bones may occur if the specimens used are preserved in formalin since very long time, making the bones brittle and fragile (Paranjape *et al*., 2017). In our study few bones were broken during the process of cleaning by use of the both employed methods. The fish bones macerated in a heated enzymatic detergent solution became extremely brittle after several years (Steadman *et al*., 2006) which could be due to continuation of enzymatic action on collagen even after termination of maceration.

Meris *et al*., cleaned the skeletal remains of the two forensic cases by using various laundry detergents solutions at 60 degree for two days and found that enzymatic action

of the detergent was capable of being able to remove adipose and muscle tissue and the loosening of ligaments, tendons, and periosteum that can be removed completely with minimal effort through the use of a brush or pad or a longer period in solution in contrast to our study where even after seven days of treatment in detergent solution at 60 degree and drying them for four days in the sunlight fibres of dried tissue could be still felt over all the bones (Mairs *et al*., 2004), the difference is most likely due to the use of formalin fixed cadaver in this study.

Hydrogen peroxide and enzymatic laundry detergent can also alter the cortical surface and leave bones chalky (Steadman *et al*., 2006), but the bone obtained in our study using LD method & use of hydrogen peroxide had smooth surface which could be due to the difference in bones exposure time in the solution.

Painting with turpentine increases the shelf life of bones by keeping the pests away and also preventing them from further decomposition (Paranjape *et al*., 2017). Wood polishing product give the bone more attractive look as well as keep the chips of bones bonded together bones by acting as a fixative (Aggarwal *et al*., 2016).

With our study we were able to procure better cleaned bones by 10 % antiformin solution method but this procedure caused the flaking of cortical surface. With LDmethod bones procured had smooth surface and were more suitable for flat bones but bones were not as clean as procured by antiformin solution method. As the temperature, concentration of the solution, duration of the maceration, duration of the preserved cadaver has influence on the quality of the retrieved bone. Further studies need to be carried out by making alteration in one or more of the above mentioned factors to obtain the desired quality of the bones.

The study has not evaluated the cost of the employed methods; which method was more comfortable to work with in terms of odor produced by the maceration methods. The influence of the applied methods of maceration has not been employed on all varieties of bones as short bone, miniature long bones, irregular bones are not included in the study.

In conclusion, though this study in not able to determine and suggest the method to procure clean bones, as the study has found that there is no difference in the bones cleaned by Laundry detergent and 10 % antiformin solution methods. However, bones were found to be cleaner by using 10 % antiformin method and bones obtained by using laundry detergent method had smooth surface as well as more suitable for flat bones. Thus, the method employed to procure clean bone should be selected based on the type of bone. Additionally, this study has established a foundation for further research on the employed maceration methods by modulating the various factors like temperature, concentration of the solution, duration of the maceration, duration of the preserved cadaver as each have influence on the quality of the retrieved bone.

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RESUMEN: Los métodos tradicionales de recuperación de huesos de cadáveres embalsamados no pueden satisfacer la demanda de las facultades de medicina, ya que consumen mucho tiempo y son tediosos de realizar, por tanto es necesario evaluar un enfoque alternativo que incluya el uso de detergente de lavandería. El propósito del estudio fue comparar y establecer el método más eficaz entre el detergente para la ropa y los métodos de solución de antiformina al 10 % para obtener huesos limpios. Fueron utilizados 32 huesos del lado derecho que se incluyeron en los criterios de inclusión obtenidos de los cuatro cadáveres embalsamados. Los huesos se trataron con detergente de lavandería y los del lado izquierdo con métodos de solución de antiformina al 10 %. Los huesos tratados se evaluaron respecto a su limpieza utilizando una escala de 0 a 5. Las puntuaciones media de limpieza de los huesos tratados con el método de detergente no fueron significativamente diferentes en un intervalo de confianza del 95 % de las puntuaciones medias obtenidas respecto a la limpieza de los huesos tratados con antiformina al 10 %. El estudio determinó que, aunque no hay hubo diferencia significativa en la puntuación media de la limpieza de los huesos tratados por los dos métodos, se observó que utilizando el método de solución de antiformina al 10 %, la limpieza de los huesos era mejor, sin embargo, los huesos planos presentaban una superficie más lisa cuando se usó el método de detergente de lavandería.

PALABRAS CLAVE: Limpieza ósea; Cadáver embalsamado; Detergente de lavandería ; Solución de antiformina.

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