

# Novel Osteo Refurbishment Method and Choice of Colouring Materials for Anatomy Specimens

Yeni Kemik Yenileme Yöntemi ve Anatomi Numuneleri için Renklendirme Malzemelerinin Seçimi

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#### ABSTRACT

**Objective:** The introduction of competency-based medical education *curriculum and a shifted focus towards self-directed learning* (SDL) in medical schools across the world has necessitated a reduction of didactic teaching hours. Museum specimens are an indispensable tool in anatomy teaching and can play a pivotal role in fostering and reinforcing SDL. Coloring of specimens makes it attractive and facilitates understanding anatomy. The aims of this study were: (1) weigh up the two commonly used coloring materials in anatomy students towards a Novel Osteo Refurbishment Method (NORM) for restoration of partially damaged osteology specimens.

**Methods:** Undergraduate and postgraduate students of anatomy were recruited in the study. Participants responded to a 5-point Likert scale questionnaire. Obtained data were analyzed using Microsoft Excel version 2019. For a comparison of the two coloring agents Mann-Whitney U test was applied to the responses toward the Likert scale-based questionnaire. P-value <0.05 was considered significant.

**Results:** Out of 318 study participants, 63.7% and 36.3% of the respondents were male and female, respectively. 90.5% were new entrants to university and the remainder 9.5% had a prior university degree. Students found the acrylic paint to be a better coloring agent and they also opined that NORM generated refurbished osteology specimens could serve as a useful self-learning tool. Acrylic paint was found to be more suitable as compared to nail polish with significant p-value in most of the parameters tested.

**Conclusions:** Acrylic colour holds a good place in coloring both specimens and models in anatomy. Nail polish can be an alternative but with some noted disadvantages. The initial experience with NORM yielded encouraging results with potential practical utility.

**Keywords:** Anatomy teaching, competency-based medical education, museum specimen, novel osteo refurbishment method

## ÖΖ

Amaç: Yetkinlik temelli tıp eğitimi müfredatının tanıtılması ve dünya çapındaki tıp fakültelerinde öz-yönetimli öğrenmeye (SDL) doğru yönelim, didaktik öğretim saatlerinin azaltılmasını gerektirmiştir. Müze numuneleri, anatomi öğretiminde vazgeçilmez bir araçtır ve SDL'nin teşvik edilmesi ve pekiştirilmesinde çok önemli bir rol oynayabilir. Numunelerin renklendirilmesi onu çekici kılmakta ve anatominin anlaşılmasını kolaylaştırmaktadır. Bu çalışmanın amaçları, anatomide yaygın olarak kullanılan iki renklendirici malzemeyi, akrilik boya ve ojeyi, değerlendirmek ve anatomi öğrencilerinin kısmen hasarlı osteoloji numunelerinin restorasyonu için yeni kemik yenileme yöntemine (NORM) yönelik algılarını gözden geçirmektir.

**Yöntemler:** Çalışmaya anatomi lisans ve lisansüstü öğrencileri dahil edildiler. Katılımcılar 5'li Likert ölçekli bir anketi yanıtladılar. Elde edilen veriler Microsoft Excel versiyon 2019 kullanılarak analiz edildi. İki renklendirici ajanın karşılaştırılması için Likert ölçeğine dayalı ankete verilen yanıtlara Mann-Whitney U testi uygulandı. P-değeri <0,05 anlamlı kabul edildi.

**Bulgular:** Çalışmadaki 318 katılımcının %63,7'si erkek ve %36,3'ü kadındı. %90,5'i üniversiteye yeni girenlerdi ve geri kalan %9,5'i daha önceden bir üniversite derecesine sahipti. Öğrenciler akrilik boyayı daha iyi bir renklendirici olarak bulmuşlar ve ayrıca NORM tarafından yenilenmiş osteoloji numunelerinin yararlı bir kendi kendine öğrenme aracı olarak kullanılabileceğini düşünmüşlerdir. Test edilen parametrelerin çoğunda, anlamlı bir p-değeri ile, akrilik boya ojeye göre daha uygun bulunmuştur.

**Sonuçlar:** Anatomide hem numunelerin hem de modellerin renklendirilmesinde akrilik boya önemli bir yer tutmaktadır. Oje bir alternatif olabilir, ancak bazı dezavantajları vardır. NORM ile ilk deneyim, potansiyel kullanım kolaylığı ile cesaret verici sonuçlar sunmuştur.

Anahtar kelimeler: Anatomi öğretimi, yetkinlik temelli tıp eğitimi, müze numunesi, yeni kemik yenileme yöntemi

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## INTRODUCTION

The earliest records of human anatomy specimens aggregated in a place such as a natural history museum date back to the 16<sup>th</sup> and 17<sup>th</sup> centuries. (Cole, 1949)<sup>1,2</sup>. Ever since the advent of the medical museum, it has undergone tremendous transformation in its size, design, appearance, and has always proved to be vital in medical education<sup>3</sup>. There has been a gradually increasing disinterest in using medical museums in teaching learning activities due to the ease and convenient access to the web-based learning, but the importance of an integrated blended learning approach cannot be ignored. The rapid paradigm shift was expedited even more during the global covid-19 pandemic. Nevertheless, a good quality human anatomy specimen remains an indispensable part of the anatomy learning<sup>4-6</sup>. An attractive and informative museum is a cornerstone of the anatomy department and a powerful tool for anatomy teaching-learning exercise<sup>7</sup>. This has gained much attention these days as the focus is being more centred toward self-directed learning (SDL). The goal of achieving SDL in the budding medicos at a very early stage of the medical school curriculum is really a difficult task<sup>8</sup>. These young brains and minds are very receptive when they see a real human body part or organ that can ignite more enthusiasm and eagerness for learning the subject. One of the major reasons for using the museum in teaching-learning activity is because, studies show that visual recognition memory provides a major portion of the long-term memory, which is used for retrieving stored information; and as such visual memory has high fidelity, has an impressive capacity to retain images forms, than the echoic or semantic memory alone<sup>9-10</sup>. Therefore, it sounds more practical to capitalize on coloured museum specimens for stimulating the bright brains in an academic course that exclusively deals with human life and suffering.

A useful museum specimen should not only be an eye-catching attractant to the viewer, and at the same time it should be an informative tool also. This purpose is served by specimens and models, which are clean, well labeled, and non-hazardous to the viewers and offer clarity. Often the specimens and models are coloured with different standard colour codes to display the desired structures viz., red for artery, blue for vein, and yellow for nerve etc. Sometimes, contrasting colours are used for differentiating different parts of a particular structure, when no definite colour coding may be followed. Coloring agents were often chosen to highlight the area of interest to be shown to the viewers. While choosing a coloring agent, it should be kept in mind that the colour used is compatible with the commonly used formalin-based preservatives and mounting solutions, non-toxic and non-hazardous to both man and nature. More importantly, the colour should be retained for a longer duration as the preparation of these teaching materials is very laborious and involves a lot of effort and precious time. Furthermore, the coloring agents should impart a colour in the preserved specimen or material that can be well appreciated by the students. Ever since the advent of the anatomy museum, anatomists of the past have tried using different materials and methods to make the specimen look more and more attractive and informative to the viewer. Different coloring agents have been employed and examined, which has its own merits and demerits. Some materials used as coloring agents were albuminous paints, inorganic salts, oil paints, intravascular injection of silicone, epoxy, latex etc. in freshly embalmed cadaveric specimens and in plastinated specimens<sup>11-14</sup>.

Recently, acrylic paints have gained much attention owing to its easy availability, easy to apply and nonexpensive nature. However, commercially available nail polish has also been tried in different stages of wet specimen preparations and in dry bone specimen making with or without adding suitable solvents such as amyl alcohol. Due to the lack of sufficient evidence, it is not clear which option an anatomist should choose. Moreover, a difference in opinions exists among anatomists with respect to the usefulness and durability between the two. In this study, we evaluated and compare the immediate outcome of the two coloring agents with respect to their shared variable properties such as ease of procuring, comfort in coloring, the outcome of coloured wet or hard specimen, stability of wet specimen in the commonly used mounting media and cost effectiveness and most importantly the perception of the viewers (anatomy students).

Preparation of a good museum specimen is a tedious job that demands tremendous planning from the stage of procurement through careful handling to the stage of mounting. This holds true for osteology specimens owing to the complex process involved in its preparation. Additionally, there are associated region-specific ethical and legal issues governing procurement, preparation, and transfer of such specimens. We observed a few old damaged osteology specimens in our gross anatomy lab, which were not appropriate for anatomy teaching. During the very initial stage of conception of the study, it was hypothesized that the readily available laboratory essentials and common stationeries can be a good option to refurbish the damaged and non-demonstrable osteology specimens and make them a valuable asset for the museum. Refurbishing a damaged osteology specimen requires a variable number of flexible materials to work with apart from skillful workmanship. We describe here our institutional experience of using the readily available materials in our gross anatomy laboratory combined with common stationery items in refurbishing the same and bringing them to its optimal elegance.

# **MATERIALS and METHODS**

The aim of this study was to evaluate the feasibility and effectiveness of the Novel Osteo Refurbishment Method (NORM) along with a comparison of two coloring agents (acrylic paint vs. nail paint) commonly used to prepare anatomy specimens and models. We hypothesized that NORM can yield effective osteology specimens for students and acrylic paint will be better for coloring anatomical specimens and models. The study was conducted at the department of anatomy, All India Institute of Medical Sciences, New Delhi. The study protocol was designed; specimens were procured and processed as per the prevailing guidelines of the institute on the use of human cadaver for teaching and research. Also, relevant consent for the use of cadaver in teaching and research was taken at the time of wholebody donation obtained from voluntary donors or their family. The list of the specimens to be assembled and mounted was prepared beforehand. For wet specimens, meticulous dissection of the region was performed in a freshly embalmed cadaver. Some already partially dissected regions were also chosen for the same.

The materials required for the technique are acrylic paints, nail polish, painting brushes of different sizes, and turpentine oil for cleaning the brushes, etc. Once dissected properly, the specimens were kept at room temperature for 24 hours to allow adequate drying so that coloring agents can be applied over the desired structures. At places where small neurovascular structures have to be coloured, small pieces of blotting papers were kept between the structures and the underlying deeper structures, which expedited drying of structures and rendered painting easier without the brush accidentally touching deeper or adjacent structures. The desired shades of the colour were obtained by diluting the acrylic paint or mixing two or more acrylic paints on a commercially available colour mixing palette. As acrylic paint is water soluble, tap water could be used as diluent whenever required. We used red, blue, yellow, green, brown, and white colors. As far as possible colour coding as per the universal standard coding system was followed for artery, vein, nerve, connective tissue, and other anatomical structures. Painted specimens, especially the

wet ones, were air dried and mounted in appropriately sized glass or perspex jars in mounting media containing 4% formaldehyde and sealed with either dibutyl phthalate polystyrene xylene (DPX) or wax.

The same methodology was employed in the other set of specimen preparations using nail polish as the coloring material. Considering the minimal handling expected in these wet specimens to be mounted, durability did not seem to be a major issue and was not investigated. In the immediate follow-up period of 6 months, the specimens did not reveal any deterioration like fading of color and staining of adjacent tissues in the specimen. However, we investigated the viewer's perception of the differently coloured specimens and obtained the same using a pretested proforma, which was to be filled anonymously by the viewer at the end of the demonstration.

Three hundred eighteen medical and paramedical students having the subject anatomy in their syllabus participated in the study. Out of the 318 students, 133 were 1<sup>st</sup> year MBBS students, 150 were 1<sup>st</sup> year B.Sc. paramedical students, and the remaining 35 students were postgraduates MD and M.Sc. students studying human anatomy. The guestionnaires were administered to the participants after they had observed the coloured anatomy gross specimens and refurbished osteology specimens. Students were asked to complete a 5-point Likert scale questionnaire in which strongly disagree, disagree, neutral, agree, and strongly agree were indicated by the numbers 1, 2, 3, 4, and 5. We designed the questionnaire after a rigorous search of the relevant literature. Students were provided with a participant's information sheet describing the aims of the study and the participants' informed consent form before enrollment in the study. Data obtained through written feedback were analyzed. Qualitative comparison between acrylic paint and nail polish as coloring agent was done using parameters such as ease of procurement, comfort of coloring, stability in 4% formaldehyde, cost effectiveness, and viewer's perception. Aggregate feedback about the coloring agents used for coloring specimens were obtained from the participants. The parameters used for taking feedback were clarity and aesthetic look, colour distinction and 3D relations, overall comprehension, educational value, and self-learning approach.

The damaged osteology specimens kept for condemnation and replacement were chosen, which were not suitable for teaching anatomy. A protocol for refurbishing them was devised using the locally available materials in the gross anatomy laboratory combined with common stationeries viz. microporous surgical tape backed by thin highly permeable non-woven paper (commonly referred to as micropore adhesive), dry cotton swabs, cotton gauge, DPX, superglue, acrylic paints of various shades, scissors, blades, sewing needle, threads etc. The term-NORM was coined for our proposed method. The dry bones were refurbished in such a manner so that the morphology and other anatomical features of interest are not lost or exaggerated. Then, these partially refurbished specimens were coloured with acrylic colour. Following drying, these were kept mounted in the museum for display. Only well-prepared specimens retaining normal anatomical features and relations using NORM and the two coloring agents were included for obtaining responses from participants in the pre-tested proforma. Specimens undermining the anatomical features were excluded. Aggregate feedback about the refurbished specimens was obtained from the participants, as stated earlier. Various parameters used for taking feedback were clarity, aesthetic look, and acceptability of the method, anatomical features of bone, relations, overall comprehension, educational value, stability, safety, and self-learning approach.

# **Ethical Consideration**

Ethical clearance was not required from the local institutional ethical committee as the study was conducted on cadavers. However, all norms related to the use of human cadavers in teaching and research were followed strictly as per the institutional guidelines. All the participants had provided written and informed consent to participate in this study; so ethical clearance was not mandated for recruiting the participants that did not involve any intervention.

# **Statistical Analysis**

The obtained responses were analyzed with Microsoft Excel version 2019. Mann-Whitney U test was performed to determine any significant differences in the participant's perceptions between acrylic colour painted and nail polish painted specimens.

# RESULTS

Coloring wet specimens for mounting in formalinbased media could be achieved with both the coloring agents. Whereas the acrylic paint application required only 1-2 coatings on average; >4 coatings of nail paints could bring comparable finishing results. Fewer coatings of nail polish did colour the wet specimens, but in mounting media-filled jars they were not as exquisite as their acrylic painted counterparts (Figure 1). Peeling of colour was also observed in the immediate postmounting follow-up period for nail polish-painted specimens. Acrylic painted specimens and models were found to be better than their nail-painted counterparts



**Figure 1.** Composite image showing the end result of acrylic paint versus nail polish in coloring a specimen of dissected coronary vessels before and after mounting. **A**, **B**, **C**. coloured with the acrylic paints; **D**, **E**, **F**. coloured with nail polish.

when analysed in terms of various parameters as responses of the viewer's perceptions (Tables 1, 2).

The osteology specimens restored with our hypothetical NORM method were shown to be mimicking their non-damaged counterparts (Figure 2). Table 3 highlights the perception of students learning anatomy toward the refurbished osteology specimens. Although there were demographic variables such as gender, ethnicity, and academic background etc., it did not affect the respondent's views because we used a structured Likert scale-based questionnaire for evaluation. All the participants recruited in the study responded to all the questions in the questionnaire and adhered to the study till the end. Out of 318 study participants, 63.7% and 36.3% of the respondents were male and female, respectively. 90.5% were new entrants to university and the remainder 9.5% had a prior university degree. Majority of the students (69%) agreed that museum specimens are an indispensable tool for anatomy learning. However, 7.3% commented that museum specimens, though helpful for anatomy comprehension, are not an absolute requirement for anatomy learning. The response between undergraduate and postgraduate students showed minimal disparities. University-entrants strongly felt that coloured museum specimens will definitely facilitate anatomy learning

Table 1. Qualitative comparison between acrylic paint           and nail polish as a coloring agent.							
Parameters assessed	Acrylic paint	Nail polish					
Ease of procurement	Easy	Easy					
Comfort in coloring	Better	Difficult					
Stability in 4% formalin	Stable	Stable					
Cost effectiveness	Cheaper	Costly					
Viewer's perception	Better	Good					

in the SDL mode. No colour peeling or wearing of refurbishing materials was observed. Overall, students found the restored specimens useful for self-learning. The obtained responses in the 5 point Likert scale regarding the NORM method have been graphically represented in Figure 3.

Acrylic paint was found to be more suitable as compared to nail polish with significant p-value in most of the parameters, except the aesthetic look when tested by Mann-Whitney U test.

## DISCUSSION

Acrylic paints are widely used in contemporary artwork for the eighteenth century. Chemically, it is an emulsion of pigments suspended in a mixture of polymer resins, which also contains components such as plasticizer, binders, fillers<sup>15</sup>. By virtue of its fast drying, easy water solubility and easy-to-use nature, it has become one of the favorite coloring agents. However not much has been investigated about the various physicochemical properties with regard to its use in anatomical specimen coloration. Consequently, more research is warranted to guide anatomists or conservation scientists in other domains to guide them choosing the best coloring agent<sup>16</sup>.

Acrylic paints produced a bright, clear and flexible adhesive paint film, dried relatively slowly but did not peel off or stain during handling after sufficient drying. Commercially available ordinary nail polish is a chemical compound in a polymeric form, the most common polymer being nitrocellulose in a suitable solvent such as ethyl or butyl acetate. Plasticizers stabilize and prevent the polish from cracking, whereas an inorganic or organic pigment imparts the colour to it<sup>17</sup>. Very few studies have investigated the role of nail polish in isolation or

 Table 2. Aggregate feedback about the coloring agents used for coloring specimens obtained from MBBS (1st year), MSc, and MD students studying anatomy.

	Nail polish				Acrylic paint				p-value (Mann- Whitney II tost)		
Questionnaire	Frequency				Frequency						
	SD	D	N	Α	SA	SD	D	N	Α	SA	whichey o test)
Clarity	79	138	50	42	9	46	122	80	54	16	0.00006
Aesthetic look	28	60	71	125	34	25	78	57	113	45	0.37886
Color distinction and 3D relations	75	113	78	41	11	19	70	82	112	35	0.00001
Overall comprehension	82	137	63	30	6	42	147	86	37	6	0.00086
Educational value	63	114	82	46	14	16	72	88	104	38	0.00001
Self-learning approach	25	111	61	92	29	14	47	63	132	62	0.00001

Number of responders in a particular column are shown as frequencies, Mann-Whitney U test was applied, a p-value <0.05 was considered significant SD: Strongly disagree, D: Disagree, N: Neutral, A: Agree, SA: Strongly agree



**Figure 2.** Composite image showing few of the damaged osteology specimens and their refurbished forms. **A**, **B**. Damaged scapula, **C**, **D**. scapula refurbished, **E**. partially refurbished skull viewed from below, **F**. refurbished skull base turned into an useful model showing the blood vessels and cranial nerves at the skull base, **G**, **H**. restoration of the zygomatic arch of the skull and representation of the different cranial sutures, **I**, **J**, **K**. partially refurbished mandible turned into an effective tool showing muscles of mastication and the inferior alveolar nerves and vessels; black arrows indicate sites of the restoration of broken parts of the bone.

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residents) through the 5 point Likert scale-based questionnaire.								
Criteria	SD	D	N	Α	SA			
Clarity	12	34	68	154	50			
Aesthetic look	6	17	35	162	98			
Acceptability of the method	8	29	93	121	61			
Bony features	11	56	88	114	49			
Comprehension	9	44	69	141	55			
Educational value	9	44	86	108	72			
Stability	5	38	63	141	71			
Safety	25	111	61	92	29			
Self-learning approach	16	95	62	112	33			
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Values are shown in percentage.

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SD: Strongly disagree, D: Disagree, N: Neutral, A: Agree, SA: Strongly agree



**Figure 3.** Responses on the 5 point Likert scale regarding refurbished bone specimens prepared by the NORM method. Colour codes in the figure are as follows: dark pink: Strongly disagree, light pink: Disagree, grey: Neutral, light green: Agree, dark green: Strongly agree

NORM: Novel osteo refurbishment method

along with other coloring agents for use in wet and dry specimens in anatomy and obtained variable results<sup>11,18</sup>.

We compared the two coloring agents with respect to several qualitative parameters and analyzed the student's perspective. Although the resultantcoloured specimens using the two agents are pretty much comparable, we could not find enough merit to recommend the use of nail polish in lieu of acrylic paints at present. The acrylic painted specimens and models were found to be significantly superior compared to its nail painted counterparts when the viewer's responses were statistically reviewed and analyzed. Furthermore, concerns over the adverse effects of phthalate and organophosphate plasticizers cannot be ignored. Studies show that endocrine disrupting plasticizer triphenyl phosphate can cause toxicity even through dermal exposure<sup>19,20</sup>. After mounting, we labeled the specimens by an indirect labeling method. The process of indirect labeling displaying the labels in a good quality-coloured photograph of the same mounted specimen, which is kept by the side. Nail polish is claimed to be cost-effective and superior in application by few recent studies, but we found it wasteful in terms of cost and time. Studies have reported transparent nail polish mixed with amyl alcohol to be useful in prolonging the colour durability, especially in direct labeling of specimens<sup>11</sup>, but direct labeling is a difficult task and the created specimen is also not very clear and easy to understand.

Numerous published works on the preparation and mounting of fresh osteology specimens are readily available, but only very few studies have addressed the issue of refurbishment. A refurbishing agent that seems more compatible with refurbishing osteology specimens is plaster of Paris (POP), which is in widespread use among the conservation technologists of different fields. We too tried POP in special circumstances and realized that the use of POP in the refurbishment process requires more skillful labor, and the use of special apparatus in the processing of POP to achieve good results (Figure 2. E and F). Japanese tissue paper has been successfully used in the conservation of osteological specimens<sup>21</sup>. We found that commonly available laboratory accessories along with routine stationeries would be an effective feasible alternative and therefore we utilized the POP, surgical micropore adhesive tape, DPX as sealing agent, cyanoacrylate superglue, acrylic colors etc. Dried paint in the refurbished specimens provides sufficient rigidity to the area that has been damaged. Although moisture and other chemicals might re-damage the refurbished osteology specimens, it would definitely rescue the damaged osteology specimens and bring them to its optimal utility at least for a few years. It may be useful in both resourceful and resource-poor settings; in resource-poor setup, this method may be employed to save some of the partially damaged specimens, and a setup with ample resources can refurbish such items and transfer it to resource-poor set up.

A novel substitute for the refurbishing material can range from a simple sheet of paper available in the gross anatomy lab to a surgical gown. The choice of the material depends on the innovativeness in the mind of the creator and locally available suitable goods. Micropore adhesives, DPX, commercial superglue, and acrylic paints are stable enough after adequate drying and retain their rigidity. Although the chemical inertness of these materials used has a question mark in an environment of high humidity, it was observed that the refurbished osteology specimens did not lose integrity, contour, or colors in the immediate follow-up period of 6 months. The NORM approach we formulated for renovating osteology specimens has several advantages. First, the process required only inexpensive local materials available in the department and thereby avoided outsourcing. The involved process does not require skillful apparatus, labor, or any specialized training.

Furthermore, the specimens can be made more informative and attractive by supplementing it with clinico-pathological, surgical, and radiologic correlations. As it is not an easy job to display all this information, including normal anatomy in a finite space, use of quick response code labeling may also be undertaken, which will enable students and spectators to smartly learn with a simple smart phone or other electronic device. A well coloured, well labeled specimen is an asset for the medical museum in the anatomy department. The threedimensional relationship depicted in such a specimen makes more sense than a real image of the same. It obviously supplements the verbal and written narratives used in teaching.

The effect of long-term preservation beyond 6 months period in formalin was not evaluated. The durability of NORM needs to be evaluated in a greater number of specimens and for a long duration.

## CONCLUSION

Acrylic paint is a relatively non-toxic, stable coloring agent appropriate for coloring anatomical specimens till a more suitable one evolves through ongoing research. It is premature to recommend the routine use of nail polish as a coloring agent except in conditions that demand rapid drying of colors or its use in mixtures. The refurbished specimens with the proposed NORM were good enough for use as visual teaching aids, and this initial experimental experience with the NORM was encouraging. More concrete studies with elaborate methodology considering a greater number of specimens must recommend its practical application in the refurbishment of osteology specimens.

## Ethics

Ethics Committee Approval: No ethical clearance was required from local institutional ethical committee to carry out this study. The present study was carried out on cadavers.

**Informed Consent:** All the cadavers used in this study were donated to the department with written and informed consent for carrying out whole body dissection for education and research purpose.

**Peer-review:** Externally and internally peer-reviewed.

## **Author Contributions**

Surgical and Medical Practices: D.B., R.K., Concept: D.B., R.K., N.R., Design: D.B., R.K., N.R., R.D., Data Collection and/or Processing: D.B., R.K., Analysis and/ or Interpretation: D.B., R.K., N.R., R.D., Literature Search: D.B., R.K., N.R., R.D., Writing: D.B., R.K., N.R., R.D.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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