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Active teaching-learning methodology in the teaching of human anatomy: Preparation of skeletons from cemeterial exhums

Metodologia ativa no ensino da anatomia humana: Preparo de esqueletos provenientes de exumações cemiteriais

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ABSTRACT
Introduction: The study and teaching of human anatomy are important processes to understand how the human body presents itself and relates to other species. Osteology techniques are important for the classification of anatomical variations, scientific research tools, and didactic use, and is performed through the physical, chemical, or biological cleaning of bones for skeletal preservation. Objective: To prepare, conserve, describe and catalog the human bones of the Human Anatomy Laboratory of the Regional University Center of Espírito Santo do Pinhal, UniPinhal. Method: 50 complete human skeletons were donated to the Human Anatomy Laboratory of UniPinhal and went through the process of general and specific cleaning, disinfection, whitening, drying, description, cataloguing in its own database, conservation, and storage. Results: All 50 bones went through all the processes, 30% of which were in good condition, 55% were in poor or poor condition, and 15% were pathological, such as bone malformations, fractures, and vicious bone consolidations. The mean age at death was 64 ± 16 years. The majority were male (67%, n = 33) and white (57%, n = 28). The most common cause of death was septic shock (31%, n = 15) and it is estimated that 40% (n = 20) of deaths were from unnatural causes. A total of 5729 bones were cataloged in the database. Conclusion: The male bones declared as white are the ones with the highest number in the ossuary, and one third were in good condition for use in practical classes, scientific research, anatomical and forensic studies by students of Health courses.

Keywords: Bones, Cemetery, Education, Forensic Medicine.

1 INTRODUCTION

Anatomy is a science that studies the structure of an organism and the relationships between its parts, and it is long based on the process of dissecting the body. The study and teaching of human anatomy are important processes to understand how the human body presents itself and relates to other species1-4. Between the seventeenth and eighteenth centuries, during the Renaissance, the value of anatomical knowledge was observed through scientific advances in the area of health. The first techniques for dissecting cadavers and studying human anatomical specimens were also developed5. With the advances in studies and technologies, the techniques for the study of the human body have been modernized, emerging new techniques for the conservation of human biological material, increasing its durability, texture and visibility of structures with greater details, making the study increasingly didactic.

The discipline of human anatomy is taught in the first years of undergraduate health courses, providing students with knowledge of the architecture of the body that subsequently allows the understanding of disease processes6. The study of human anatomy allows us to understand the basic organization of the human body and the principles of operation of its structures, contributing to the future professional to provide excellent care to their patients, from the correct interpretation of the patient's history, as well as the signs and symptoms, performing an adequate physical examination, the correct approach with minimally invasive techniques of the different regions of the body for diagnostic or therapeutic purposes, as well as the correct interpretation of complementary exams. In addition, it enables future professionals to use the appropriate terminology to describe pathological findings, as well as to prevent errors in interpretation or communication among colleagues7. Thus, incomplete or deficient anatomical knowledge can generate a potential risk of causing harm to the patient, threatening their safety8.

The main method in learning human anatomy is, without a doubt, the practical class, within a specific laboratory. Even with new technologies, synthetic materials and the possibility of using animals, the human body continues to be irreplaceable in the teaching of human anatomy9. The use of cadavers contributes to research aimed at teaching through exhibitions, demonstrations in class or staff training, becoming essential in practical classes of human anatomy, which are important and necessary for a good professional training of students in the health area. Furthermore, as described by Korf et al.[9] Only the cadaver offers the multidimensional aspect and anatomical variations, it is the "first patient": it trains behaviors, respect, lectures on theoretical classes, uses didactic-constructive criteria, confers psychological education, and presents characteristics of the connective tissue, only seen in cadavers.

In Brazil, currently the most viable and legal way to obtain bodies is through donation while still alive, which must be done based on Article 14 of Law 010.406.202 of the Brazilian Civil Code, being allowed the free disposal for scientific purposes of the body in whole or part of it after death. To do so, the donor needs to make a declaration or even a will, drawn up in a notary's office, authorizing the donation by family members, in the declaration the educational institution to which the donor wants his body to be forwarded must be specified. In addition, Law 8.501/92 remains in force, which regularized the use, for teaching and research purposes, of corpses not claimed by public authorities without any documentation and no information regarding the address of relatives or guardians, after the publication in the media of the aforementioned death, within a period of up to 30 days. But even so, the number of cadavers destined for Higher Education Institutions is quite small. For the study of the skeletal system, another possibility, in addition to the dissection of corpses, is the acquisition of human bones from administrative exhumations carried out in cemeteries.

The skeletal system is related to several functions, such as organ protection, production of blood cells (red bone marrow) and storage of lipids (yellow bone marrow), absorption of toxins and heavy metals, in addition to supporting the soft tissues of the human body, being tendon insertion points for skeletal striated muscles, a factor that allows joint movements through levers[10,11]. To this end, it is of great importance to study the bones in depth, their particularities such as bone accidents, presented as protrusions and depressions. The practical study of these accidents is of great clinical and functional importance, where it is possible to have the real perception of textures, topographic correlations, in addition to the sensory experience in learning.

Bones are composed of about 60-70% mineral content, and in this way, the bones do not undergo *post-mortem* change. Osteology techniques have been used for a long time to facilitate the fixation of theoretical knowledge and the association with morphology and physiology, improving teaching and facilitating student interaction, making it easier to assimilate theory with practice. These techniques are also important for the classification of anatomical variations, scientific research tools, and didactic use12. Osteotechnics aims at the conservation of anatomical specimens as a didactic tool for practical study in human anatomy disciplines, and is performed through the physical, chemical or biological cleaning of bones for skeletal preservation[13,14].

Thus, the objectives of this study were to present a practical and conceptual view on the assembly of an Ossuary together with an Osteotechnics Laboratory in Higher Education Institutions, making it possible to know the donation procedures, the methods of preparation, conservation and analysis of the anatomical characteristics that should be considered during the study of bones. In the future, it will be possible to learn about the variations of anatomical accidents and the correlation of these variations with the origin of the bones, ancestry, gender and even a probable cause of death.

2 METHOD

This is a descriptive study seeking to carry out the preparation, conservation and cataloguing of human bones from the Human Anatomy Laboratory of the Regional University Center of Espírito Santo do Pinhal, UniPinhal. Fifty complete human skeletons were requested and later donated by the General Technical Services (SETEC) of the city of Campinas/SP. These human skeletons come from the administrative exhumations carried out in the general blocks of the Nossa Senhora da Conceição Park Cemetery, also in the city of Campinas/SP. For scientific research purposes, these human skeletons have been identified with information such as declared color, sex, age, and cause of death.

All the bones received were included in the Human Anatomy Laboratory of UniPinhal. However, for didactic purposes, the bones were classified as good in a state of conservation –

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without structural damage detrimental to the study (used both in practical classes and in research), medium state of conservation – with little structural damage, and poor state of conservation – with several structural damages, and the bones of the last two categories were stored separately to possibly be used in research. The excluded waste, such as the deceased's belongings, hair, among others, were properly separated, packaged and exhumed.

2.1 ETHICAL ASPECTS

This study complies with the guidelines proposed in CONEP resolution 466/12, which regulates research involving human beings, and was submitted to the Research Ethics Committee of FACERES, through Plataforma Brasil, and approved under Certificate of Presentation for Ethical Appreciation (CAAE) No. 43536721.5.0000.8083, with Opinion No. 024/202.

2.2 PROCEDURES

2.2.1 Donation of bones

Initially, an official letter was sent to SETEC requesting the donation of 50 complete human skeletons from administrative exhumations, for exclusive didactic use at the Human Anatomy Laboratory of UniPinhal. Information such as declared color, gender, age, and cause of death was also requested, in accordance with ethical and administrative issues, for use in scientific research. The request was promptly authorized by SETEC, and with all the documentation regulated, within a period of six months the bones were received at the Osteotechnical Laboratory of UniPinhal.

For the removal of the skeletons from the cemetery, the only requirements on the part of SETEC were the presence of a registered employee of UniPinhal in an official vehicle, in addition to the donation of 50 resistant 80L bags in black or blue, to replace those that were storing the skeletons, since in each bag were the bones referring to a single individual and were numbered according to the internal records of the cemetery and sealed (Figure 1).

Figure 1. Reception of the bones at the Osteotechnical Laboratory of UniPinhal. In A, storage of the 50 bones inside plastic bags; B, detail of the numbering of each bag according to the internal records of the cemetery.

Source: Personal archive.

The entire process of preparation, conservation and cataloguing of human bones is shown in Figure 2 and described below:

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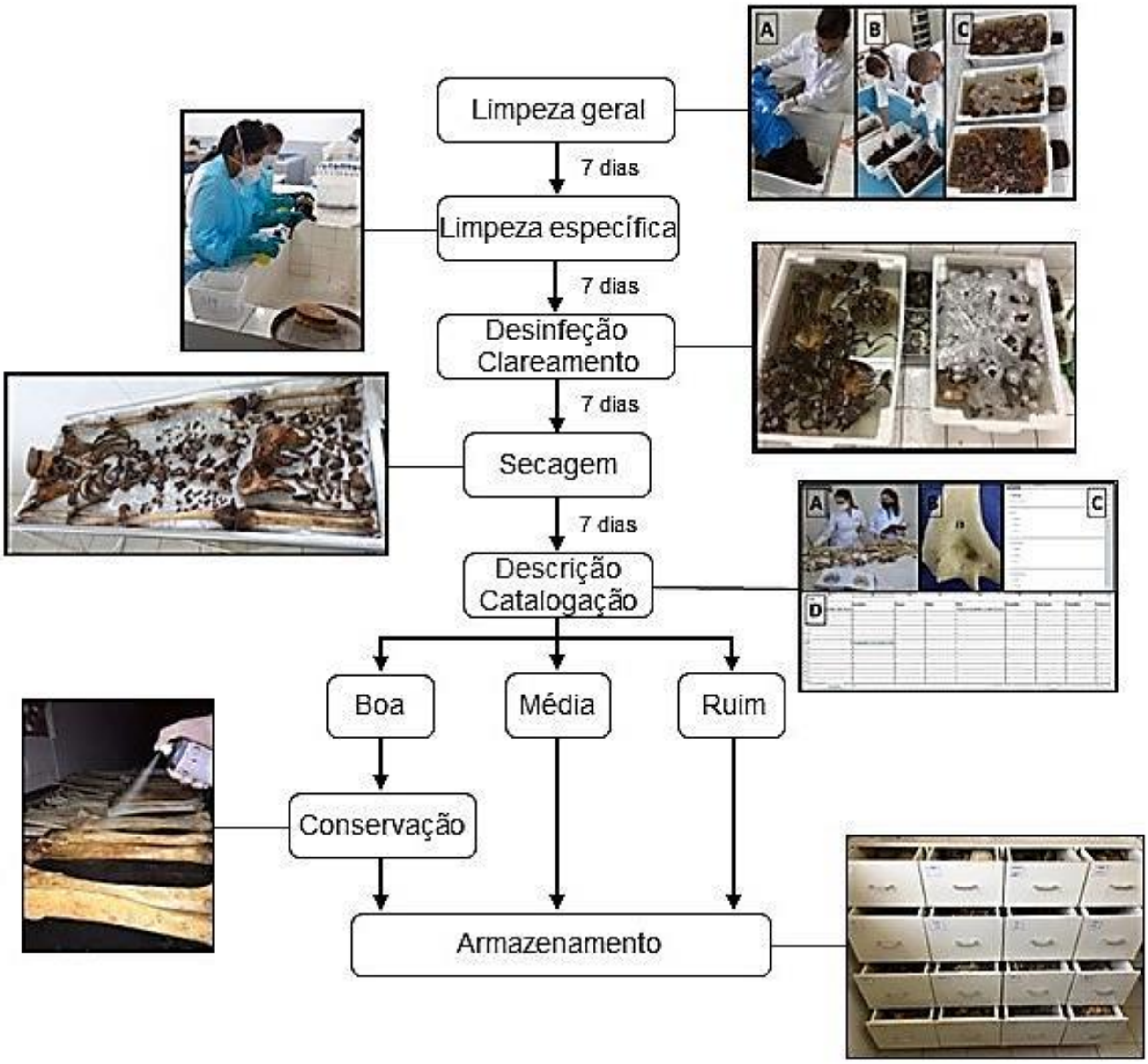
Figure 2: Flowchart of the process of preparation, conservation and cataloguing of human bones. In A, opening the bags; B, separation of biological material from others; C, packaging the bones in plastic containers to be soaked for seven days in a solution of water with detergent; Specific cleaning process: Individual washing of each bone with brush and detergent; Disinfection and whitening process: Packaging of the bones in plastic containers to be soaked for seven days in a 15% Hydrogen Peroxide (H202) solution dissolved in water, for disinfection and whitening of the pieces; Drying process: Exposure of the bones for the drying process for seven days, after washing in running water for complete removal of chemical residues; Description and cataloguing process: A. Checking the presence of all bones and their characteristics. B. Individual numbering of each bone, according to the bone belonging to it with a black permanent pen. C. Electronic form for typing and cataloguing bones. D. Spreadsheet generated after cataloging; Conservation process with application of colorless varnish on bones classified as "good" to be used in practical classes and research; Storage of bones classified as "good" in drawers appropriate for use in practical classes and research.



Source: Personal archive.

For general cleaning, the bags were opened one by one, carefully, trying to preserve all the bones. Each bone was completely handled without contact with the other bones, allowing for subsequent complete identification. A thick separation of biological material from the others, such as soil, hair and objects, was carried out. Finally, the bones were placed in plastic containers, and the smaller bones were kept in a separate container from the larger ones, and remained submerged for seven days in a solution of water with detergent (Figure 2). Subsequently, a specific cleaning was performed, where all the bones were individually and carefully washed by hand with a brush and detergent (Figure 2).

After specific cleaning, the bones were again placed in a plastic container, with the smaller and larger bones separated, and submerged in a 15% Hydrogen Peroxide (H202) solution dissolved in water for seven days (Figure 2). All procedures contributed to the disinfection and whitening of the pieces. When removed from the previous stage, the bones were washed in running water for approximately 40 minutes for complete removal of chemical residues. Subsequently, they were exposed on a flat, dry and airy surface for drying for seven days (Figure 2).

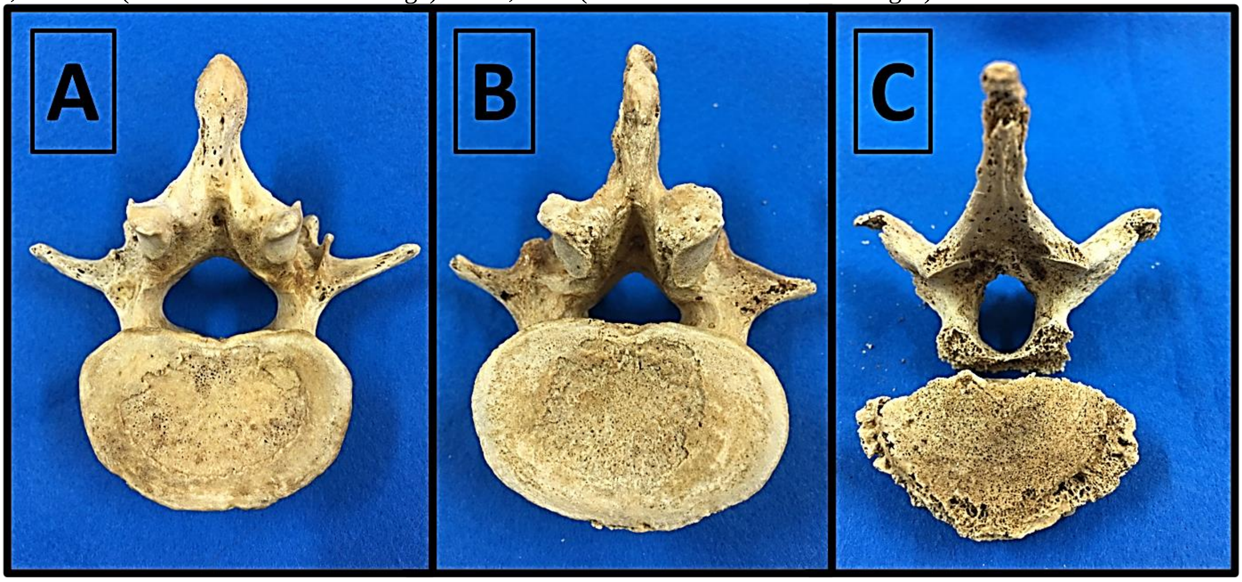
2.2.2 Description and Cataloguing

An electronic form was developed in the Google Forms application containing specific fields for each bone of the human body, identified for each bone, allowing the archiving of the singularities of each bone, such as consolidated *ante-mortem* fractures and prostheses, or other pathological findings, such as bone malformations or vicious bone consolidations. Each bone was analyzed individually, numbered with a black permanent pen, cataloged and described in the database, allowing the counting and analysis of alterations (Figure 2). With this, it was possible to generate a descriptive report of the total quantity and quality of the bones stored in the ossuary of the Human Anatomy Laboratory of UniPinhal, stratified by the total bones and by each topographic region of the human body, in order to facilitate the registration, storage and analysis of the collected data.

2.2.3 Conservation and storage

Initially, the bones were classified according to their state of conservation as good, medium and poor (Figure 3).

Figure 3. Classification of bones according to their state of conservation. In A, Good (no harmful structural damage); B, Medium (with little structural damage) and C, Poor (with several structural damages).



Source: Personal archive.

The good bones underwent a conservation procedure with the application of colorless spray varnish (Spray Premium, Universo Tintas®), where the bones were placed on a plastic surface and the varnish was applied at a distance of 10 cm, to avoid accumulation of the product on its surface (Figure 2). This step has the function of sealing the bone and favoring its preservation. After applying the varnish, the bones were left in room air for the varnish to dry completely. Subsequently, the bones were separated according to type and stored in drawers suitable for use in practical classes and research (Figure 2). The ribs, vertebrae, bones of the hands and feet were stored together in plastic bags numbered according to the bone (Figure 4). The bones classified as medium and poor did not go through the process of conservation with varnish and were packed in plastic boxes identified inside a specific cabinet, for possible use in research.

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Figure 4. Storage of bones belonging to the same bone. In A, bones of the hands and feet; B, ribs and C and D, vertebrae.

Source: Personal archive.

2.3 DATA ANALYSIS

All the information on the bones was organized and tabulated in such a way as to allow the quantitative analysis of the data through simple descriptive statistics.

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3 RESULTS

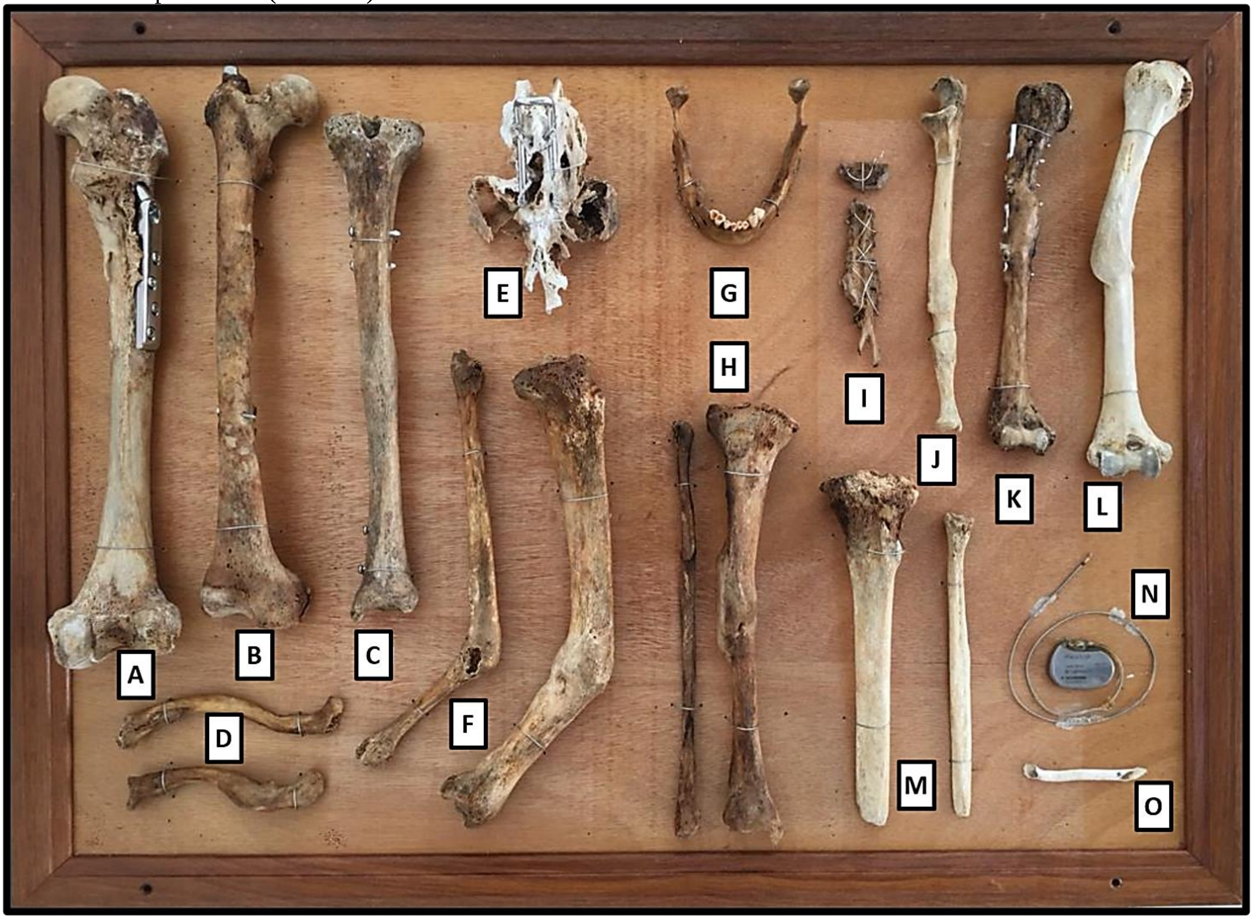
All 50 bones went through all the processes of preparation, cataloguing and storage. Thirty percent of the bones were in good condition and will be used in practical classes and scientific research. Fifty-five percent were in medium or poor condition, and were therefore stored separately for possible use in scientific research. The remaining 15% of the bones presented pathological findings, such as bone malformations, fractures and vicious bone consolidations.

Only one bone could not be received information from the death certificate. Among the 49 bones with information, it was observed that the mean age at death was 64 ± 16 years, with the youngest being 25 years old and the oldest 94 years old. The majority were male (67%, n = 33) and white (57%, n = 28). The most common causes of death were: septic shock (31%, n = 15), respiratory failure (10%, n = 5) and trauma (10%, n = 5); and it is estimated that 40% (n = 20) of these deaths were of non-natural cause (or external cause, such as that resulting from injury caused by violence – homicide, suicide, accident or suspicious death) due to the presence of section by the Griesinger plane15, indicating the performance of an autopsy probably to diagnose the causes of death.

Each bone was analyzed individually and described in the database, stratified by the total number of bones and by each topographic region of the human body. The total number of bones cataloged was 5729, of which 1421 were from the axial skeleton and 4308 from the appendicular skeleton. The distal phalanges were the most absent. It was possible to completely remove the ossicles of the middle ear in seven skulls.

A synthetic cardiovascular prosthesis and a cardiac pacemaker were found, as well as several fractures (consolidated or not) - mainly in the femur. A total of eleven bones of the upper and lower limbs presented fractures, and seven underwent surgical interventions using orthopedic materials, such as intramedullary nails, self-compression plates and screws. Two bones had multiple fractures, one of them with five fractured bones. These bones constituted an Orthopedic Framework, making it feasible to use them continuously and routinely in the activities of the Human Anatomy Laboratory, such as practical classes and scientific research (Figure 5).

Figure 5. Orthopedic frame assembled with bones with pathological findings and prostheses. A. Right Femur (Bone 21): Osteosynthesis with "Dynamic Hip Screw (DHS = dynamic hip screw)" is observed; B. Right Femur (Bone 22): Osteosynthesis with "locked intramedullary nail" is observed; C. Left Tibia (Bone 11): Osteosynthesis with "locked intramedullary nail" is observed; D. Right and Left Clavicles (Bone 1): In the Left Clavicle there is a consolidated fracture (bone callus) – Observe a difference in size between them; E. Sacrum (Bone 47): Stem (arthrodesis) is observed; F. Tibia and Right Fibula (Bone 41): Observe consolidated fracture (bone callus); G. Mandible (Bone 1): Osteotomy with a "reconstruction plate" is observed; H. Tibia and Right Fibula (Bone 22): Observe consolidated fracture (bone callus); I. Sternum (Bone 12): Sternotomy with "steel wire cerclage" is observed; J. Right Ulna (Bone 1): Observed consolidated fracture (bone callus); K. Right Humerus (Bone 22): Osteosynthesis with "self-compression plate and screws" is observed; L. Right Humerus (Bone 17): Observed consolidated fracture (bone callus); M. Tibia and Left Fibula (Bone 42): Observe amputation of the distal epiphysis; N. Cardiac pacemaker (Bone 2); O. Endovascular prosthesis (Bone 35).



Source: Personal archive.

4 DISCUSSION

Using the appropriate osteotechniques, it was possible to integrate the 50 bones donated in the Ossuary of the Human Anatomy Laboratory of UniPinhal. These bones will be used for practical classes, scientific research, anatomical and forensic studies by students of Health courses, including Biomedicine, Physical Education, Nursing, Pharmacy, Physiotherapy and Nutrition. Few institutions have an ossuary of this size, with all the information recorded/cataloged and organized to facilitate the studies of professors, students and researchers. All the work developed with these bones was carried out by undergraduate students, who were assisted by trainee students who took turns weekly, in addition to the supervision of a professor. This allowed an interaction between students from different Health courses, as well as very valuable discussions for the teaching and learning process, making it a unique experience for all students and teachers.

The process of donating and receiving the bones was simple and cost approximately 10 times less than the acquisition of a disarticulated and complete synthetic anatomical model. This cost referred to the 50 plastic bags used to replace the cemetery and the transportation of the skeletons to the institution in an official vehicle. In addition, only the study of biological material allows the complete visualization of multidimensional aspects and the presence of anatomical variations, not seen in synthetic models. Other than that, the handling of biological/human material offers students the experience of respect and professional ethics.

One of the drawbacks of using bones received from cemeteries is related to the quality of the material received, which often already presents an advanced state of decomposition and, consequently, becomes extremely fragile, requiring care in the cleaning and whitening process, as the use of chemical products in this process can lead to aggression and loss of material14. In addition, most of the time the bones present marked alterations in their surfaces, or even destroyed parts, especially in the most fragile bones, such as those that constitute the nasal cavities13. Of the 50 skeletons received, 30% of the bones were fully used. However, for the purposes of future research, all the material was stored, and there was no discarding of biological structures.

Each stage of the preparation of these bones was carried out within a period of seven days, since the meetings between professors and students in the Osteotechnics Laboratory took place only once a week. These steps included: 1. general cleaning; 2. Specific cleaning, disinfection and whitening; 3. Drying, description, cataloguing, conservation and storage. Therefore, for each bone, 21 days were needed for the entire process. After some adjustments in the techniques to be used, it was possible to optimize the time by reconciling several processes simultaneously (three bones per week), such as washing, bleaching and drying, resulting in a constant (weekly) work period for six months. It is noteworthy that the seven-day period in each process was due to the routine of the laboratory, professors and students, however, some processes may take less time, according to the techniques published by Rodrigues13 and Oxley, Barros and Fazan14.

The work began with the opening of only one bag of bones at a time, performing all procedures individually, anticipating to achieve a positive result in the practical techniques. In this first stage, the students had contact with the belongings of the deceased, giving them the experience of dealing with the remains of a person who, like us, had a life story, showing the importance of ethics and respect for that material. Inside the bags it was also possible to find soil, and some bones still had soft tissues, and consequently the presence of insects, carrying out the natural process of maceration. The insects were exterminated with the use of common insecticides, and all the soil and objects found were packed in specific bags and later exhumed.

In the first stage, of general cleaning, we tried to use ammonia, aiming to promote a satisfactory result in the cleaning of the bones, since it is an alkaline agent, increasing the activity of surfactants, would help to remove fat. However, it was observed that this agent deteriorated the bones, as a consequence, this product was no longer used in the washing processes, opting only for the solution of water with detergent.

The specific washing of each bone with a brush and detergent was the most laborious and consequently the one that required the most time, and the presence of the trainee students was essential. During this individual washing of the bones, the students observed the characteristics of each bone and already pointed out the presence of anatomical variations or bone malformations, always with the help of atlases and anatomy books, being an important stage for learning.

For the disinfection and whitening process, H202 was used, being the main chemical agent that brought benefit in the washes, being a powerful fighting agent against anaerobic organisms (such as bacteria). In the stage where the bones were soaked for seven days, an average of 1.5 L of H202 was used, dissolved in 25 L of water. Purposely some bones were more or less whitened in order to show the characteristics of the bones as real as possible. In a general sum, 75 L of the product was needed. After this step, each bone was again cleaned by a specific manual in running water to remove possible biological and/or chemical residues.

In room air, these bones were exposed on a surface for complete drying, and later the bones were analyzed individually for the description of their characteristics, numbering and cataloguing in the database. One of the limitations in relation to the cataloguing of the bones was the non-standardization of the numbering in specific places of each bone, which makes it difficult to quickly locate the numbers.

After cataloguing, the bones went through the conservation process. The spray varnish application technique was used. However, there was difficulty in relation to the penetration of the sealant into the biological material, which was absorbed almost instantaneously, leading to an unsatisfactory result, especially in parts that already presented some state of deterioration (classified as medium or poor). The best strategy for this process would be to immerse the bones in a tank with varnish, by immersion, however, it was not a viable process for the laboratory. Thus, only the bones classified as in good condition were varnished and it was decided to perform a new procedure after complete drying, resulting in two coats. Even so, it is suggested that maintenance be carried out at intervals of at least one year to ensure the longevity of the material, because as it is used, it can suffer friction and wear, leading to external biological loss13,14.

After a conservation procedure with the use of varnish, the "good" bones were placed in a specific cabinet for use in practical classes, during monitoring and in scientific research. The "average" and "bad" bones were packed in plastic boxes and are available for comparison use and scientific research. The use of this material will be of paramount importance in the training of health professionals, corroborating the study by Martelli et al.[15] portraying that health professionals act throughout the exercise of their profession on the health of other human beings, and it is unacceptable that one of these professionals carries out their activities with outdated knowledge in human anatomy in their area of expertise.

5 CONCLUSION

The male bones and the white bones are the ones with the highest number in the ossuary, and one third were in good condition for use in practical classes and future research, facilitating the students' learning in the identification of alterations/pathologies associated with the human skeleton.

The experience obtained throughout the process of assembling the ossuary went beyond the use of osteotechnics, as it allowed students and teachers to add knowledge far beyond the anatomical. The work with these bones will still yield many fruits, and the production of didactic material (such as the assembly of articulated skeletons) and scientific research (such as the description of anatomical variations) will be incessant.

In addition, the study of biological specimens is indispensable to the teaching-learning process in the study of human anatomy, and the handling of these specimens strengthens the humanization of future health professionals, reflecting in their conduct with patients.

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