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Colorful bones – Can be histology useful for forensic anthropology in the digital era?

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SUMMARY

Methods of forensic anthropology are typically used in the identification process of partially or fully skeletonized human remains. Usually, the first step is to determine whether the examined material is human or animal. It may be easy in case of intact bone due to macroscopic differences between human and animal bone but in case of fragmented or burned remains, it might not be that clear and morphognostic methods of forensic anthropology (examination of bone by the naked eye) cannot be used. The same problem might arise in age at death estimation whereas the post-mortem modifications might change the appearance of bone or diminish the changes related to aging. The solution to these challenging situations could be a histomorphological examination of bone, which can be also very helpful in obtaining the medical history or history of trauma.

Keywords: bone microstructure – histological examination of bone – forensic anthropology – the age-at-death determination from the bone – identification from bone fragments.

Môže byť použitie histologických metód vo forenznej antropológii prínosné v digitálnej dobe?

SÚHRN

Metódy forenznej antropológie sa v praxi najčastejšie využívajú pri identifikácii pozostatkov, ktoré sú čiastočne alebo úplne skeletizované. Prvým nevyhnutným krokom je spravidla určenie, či je vyšetrovaný materiál ľudského pôvodu. Určenie druhovej príslušnosti môže byť vzhľadom na anatomické odlišnosti jednoduché, avšak pri zlomkovom alebo inak poškodenom materiáli nemusí byť vyšetrenie voľným okom postačujúce. Obmedzené použitie morfognostických metód sa tiež vyskytuje pri určovaní veku, nakoľko veku primerané degeneratívne zmeny sa rozkladným procesom môžu stierať. Uľahčenie riešenia týchto komplikovaných situácií sa ponúka v histologickom vyšetrení kostného tkaniva.

Kľúčové slová: vnútorná stavba kosti – histologické vyšetrenie kostí – forenzná antropológia – určovanie veku v čase smrti z kostí – identifikácia z úlomkov kostí.

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Human skeleton usually consists of 206 bones, including the patellæ and auditory ossicles (1). Bone tissue is one of the hardest tissues in the human body and is the second most resistant tissue (following the cartilage). Bones represent the framework for locomotive apparatus, protect soft tissues and internal organs within the cranial, thoracic and abdominal cavity. Bone tissue is also a storage of calcium, phosphates and other ions inevitable for maintaining the hemostasis (2). Bones of human skeleton consist of lamellar bone. Another type of bone, fibrillar (or woven) bone, is the first bone tissue in embryonic development, in adults it appears in the fracture healing process and in the tendon insertion sites (2). In some cases, the woven bone might indicate neoplasm or infectious disease (3). Woven bone has different histological features, for example, there are no layering nor Haversian systems. The intercellular non-mineralized matrix consists of type I collagen bundles with branched osteocytes with numerous processes (4). Plexiform bone is typically found in medium-sized animals (non-human mammals) common in our area (for example pig or sheep) (5). It can occasionally occur in children during a rapid growth spurt (6).

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Lamellar bone

Microscopic structure of bone is framed by periosteum and endosteum. Periosteum forms an osteogenic layer on the bone surface containing collagen fibers, fibroblasts, and blood vessels. Endosteum located inside the bone consists of osteoprogenitor cells, osteoblasts, and collagen fibers. Periosteum and endosteum are connected together by perforating (Sharpey) fibers (2).

In the cross-section of human lamellar bone, two main types of lamellar bone are visible – cortical (compact) and trabecular (cancellous) bone. Trabecular bone is formed into a three-dimensional net comprised of trabeculae or spicules of various thickness and length. These are covered by endosteum. Trabecular bone is internal bone and it is located in flat and irregular bones and in the ends of long bones (2). Cortical bone is located beneath periosteum of bones, it creates a dense layer covering the spongy bone (7). Cortical bone comprises of osteons (also called Haversian canals or systems), cylindrically shaped units surrounding central canal (8). The central canal contains blood vessels, nerves and endosteum and communicates with one another via Volkmann canals (2). Osteon is complex of concentric lamellae, which consist of an intercellular matrix with collagen fibers bind together with amorphous matter incrusted by mineral compounds. Mineral component refers to 65% of bone tissue in the form of apatite mineral salts (mostly hydroxyapatite), citrate and carbonate ions, magnesium, and natrium ions (4). Due to decalcification, mineral compound dissolves, the bone becomes soft but its microscopic structure remains well-pre-

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served. That is important to keep in mind when choosing the method of bone processing (3).

Osteoblasts are cells producing an organic component of bone matrix, e.g. type I collagen and osteonectin. They are responsible for calcification of bone matrix which they produce and are located exclusively at the surface of bone matrix (2).

Osteoclasts are multinuclear gigantic cells of irregular shape and size. This type of cells appears during growth or bone remodeling. Cavities in the matrix in areas where bone undergo resorption are called Howship lacunae (2). An average number of nuclei in osteoclasts varies from 12 to 20, sometimes it is multiplied to 100. Proteolytic enzymes in osteoclast dissolve an organic compound of the intercellular matrix which leads to bone resorption (1).

Bone processing

Due to the hardness of bone tissue, special techniques of processing need to be used. It involves prior decalcification, which significantly alters the structural integrity of the bone. Decalcification using acid solutions (typically formic acid) causes the loss or denaturation of many organic components and damages the ultrastructure of the tissue (6). When slides of bone are cut in the form of thin transparent tables, bone cells are not preserved. This specimen can be used for examination of channels in Haversian system, which appear black on the cross-section. In case the aim of observation is the organic matter of bone tissue and bone cells, inorganic compounds are dissolved in a solution of nitric acid, ethylenediaminetetraacetic acid (EDTA) solution or other chelating agents. The samples are then fixed, cut, and stained (7). Mallory trichrome, hematoxylin and eosine and toluidine blue are routinely used for the study of bone in paraffin sections. Methylene blue/basic fuchsine and other metachromatic stains can be used to view entire long bones with epiphysis due to its ability to distinct growth plates. According to An and Martin (6), a common problem in bone slides preparation is the artifactual separation of osteoblasts and osteoclasts away from the bone surface, which prevents its use for quantitative histomorphometry. Methyl methacrylate (MMA) and glycol methacrylate provide better tissue preservation and detail with osteoblasts and osteoclasts in relation to the bone surface (6). In forensic anthropology, the histomorphology assessment of bone tissue in the dry bone is of great significance (8).

Histomorphologic examination in forensic anthropology

In adults, the bone remodeling process plays a major role in determining age at death from bones. Remodeling is continual resorption of old osteons, in other words, renewal of old lamellar bone, which takes place throughout life (9). Osteoclasts remove old bone, form small cavities, then osteoblasts develop, line the wall of cavities and begin to form a new osteon with concentric layers of bone (lamellae) and future osteocytes trapped within (1). Histomorphologic analysis of tissues from various parts of the skeleton (14, 15, 16, 17, 18, 19) proved the relationship between the age at death and osteons per unit in cross section. A number of intact and fragmentary osteons per space unit is count for every bone (based on minimum 2 sections from each bone) and the result is used in regression formula for age determination. All these methods are quantitative and depend on osteon remodeling and osteon populations. This method was used in the 1960s for the first time and sections from the diaphysis of femur, fibula, and tibia were studied (15). Pfeiffer et al. (16) came to a conclusion that histological profile of bone depends on the area of sample collection, as the histomorphologic determinants of occipital bone were less reliable compared to long bones (17). It is also possible to use burned skeletal remains for age determination using histological methods. By comparison of the microstructure of bone (Haversian canal diameter, central canal diameter, difference between one another, area of Haversian canal, area of central canal and difference between one another) fresh and burned it was found out that under influence of high temperature all the valuables were smaller (20). Harsányi (21) claims that if the temperature is higher than 700°C there is no chance to differentiate microstructure of compact bone. Despite these findings, Stloukal considers histological examination of burned bone for age-at-death determination the optimal option, accepting the lack of macroscopic morphological structures (9).

Although histological methods for age-at-death determination are well documented in the literature, the microscopical bone examination is most commonly used in cases when no other methods can be applied. These are, for example, too fragmented or burned skeletal remains (2, 20, 21, 22). To distinguish human from a non-human bone in fragmented skeletal remains, the diameter of osteon as well as the central canal is considered being reliable diagnostic measurement to use. Femora and tibiae of common species of living animals (mammals and birds) are frequently analyzed (5, 8, 20, 23, 24, 25). The results of studies show that values of measured structural units depend on the skeletal part and age of the subject (5, 23, 24, 26).

Bone cells ratio can be used in practice for establishing a medical history of deceased from skeletal remains. High osteoclasts versus osteoblasts ratio suggests a deficit in nutrition, infectious disease, and the presence of many stress factors during life (22). The disproportion between the thickness of compact bone and size of the medullary cavity can be a sign of osteoporosis as well as type I diabetes mellitus (27). The study conducted on bones from amputated limbs revealed that the side affected by amputation generally exhibited fewer intact secondary osteons than the unaffected side (28). Bone tissue of patients with chronic venous insufficiency and leg ulcers showed linear, woven and lamellar diaphyseal periosteal reaction, spiculated bone formation, the thinness of the cortical bone as well as areas of central sclerosis (29). A periostosis consisting of an extreme periosteal ossification might be a reaction to trauma, periosteal and soft tissues hematomas, venous stasis, toxic factors or blood diseases. Ossified periostitis limited to the metaphysis could be the sign of leukemia or congenital syphilis (30).

Signs of trauma are the possible aim of interest in post-mortem microscopic bone examination. Cross section of bone tissue stained hematoxylin-eosine possibly shows signs of bleeding in the site of bone which was broken 14 days prior to death. Based on results of a pilot study by Cattaneo et al. it might be suggested that vital reactions are detectable also in processed bone tissue (bone specimen after maceration and decalcification) shortly after death (31).

CONCLUSION

Histological methods are routinely used in forensic medicine. Rarely overlooked, nonetheless, they might be beneficial also in the investigation of osseous material. The histomorphological examination is considered being crucial in the identification process of charred bodies or fragmented skeletal remains. Despite the age-at-death determination, further information about the health condition of the deceased can be obtained. Based on the results of the pilot study, also the identification of perimortem trauma seems possible. The presence of the histology laboratory at forensic medicine departments makes these methods widely available and applicable.

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A disadvantage of histomorphological examination of bone tissue lies in hardness of the tissue, thus it might be more time consuming and sometimes not commonly used equipment is required. Otherwise, it can be performed at workplaces with no access to computed tomography or magnetic resonance imaging. Also, an only small fragment of bone can be processed to a specimen which is subsequently investigated. The histological examination might answer crucial questions which working with skeletal remains might bring.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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