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REVIEW ARTICLE

Age Estimation from Clavicle by Histomorphometry Method: A Review

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ABSTRAK

Kaedah histomorfometri yang merupakan kajian histologi secara kuantitatif adalah kaedah berguna untuk meramal umur semasa kematian pada tinggalan rangka zaman kini atau purba dengan mengukur morfologi osteon. Kaedah ini mengurangkan berat sebelah individu dan aras pengalaman untuk meramal umur,berguna untuk membina model 'paleodemographic' dan pengecaman forensik apabila fragmen rangka dijumpai. Kebanyakan bahagian rangka yang dikaji melalui kaedah histomorfometri adalah tulang femur, tibia, rusuk dan selangka. Tulang selangka kurang dikaji melainkan dalam populasi Kaukasia. Lagipun, tulang selangka sangat menarik kerana ia tulang yang tidak menahan beban, kerap dijumpai sebagai tulang lengkap dan mempunyai perkembangan berbeza dari tulang panjang yang lain. Oleh itu, artikel ulasan ini mengetengahkan ramalan umur melalui kaedah histomorfometri tulang selangka. Artikel ulasan ini menerangkan kaedah histomorfometri, tulang selangka, remodeling tulang dan aplikasi forensik ke atas tulang selangka melalui kaedah histomorfometri.

Kata kunci: umur, tulang selangka, ramalan, sains forensik, histomorfometri

ABSTRACT

Histomorphometry method, the quantitative study of histology, is a useful method to estimate age of death in the present and ancient skeletal remains by measurable morphology of osteon. This method, which reduces individual bias and difference in the level of experience for estimation of age, is useful for constructing paleodemographic models and forensic identifications when adult fragmentary skeletal remains are encountered. Most parts of skeletons that are studied by histomorphometry method are femur, tibia, rib, and clavicle. Clavicle bone has

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been poorly studied except for Caucasian populations. Moreover, clavicle is a very interesting bone because it is a non-weight bearing bone, is usually found as entire bone and has different bone development from other long bones. Thus, the interest of this review article is on the age estimation by histomorphometry method of clavicle. This review article also describes the histomorphometry method, clavicle bone, bone remodeling, and forensic application of clavicle with histomorphometry method.

Keywords: age, clavicle, estimation, forensic science, histomorphometry

INTRODUCTION

Estimation of age at death of human is one of the four steps of biological identification (White et al. 2011). Documentation of age at death from unknown skeletons is very important because this data determines information about reason of death regardless of passed time after death (Kerley 1965). The investigation of estimation of chronologic age in cadaver, living human and skeletal remains has several methods (Ohtani & Yamamoto 2005; Ritz-Timme et al. 2000; Waite et al. 1999; Williams 2001) such as visual morphological method (Buckberry & Chamberlain 2002; Hanihara & Suzuki 1978; Listi & Manhein 2012; Stewart 1958; Watanabe & Terazawa 2006), histomorphometry method (Calixto et al. 2015; Lee et al. 2014; Stout & Paine 1992), CT-scan (El Morsi et al. 2015; Kellinghaus et al. 2010; Mühler et al. 2006; Tangmose et al. 2013), radiography (Bhise et al. 2012; Mateen et al. 2013; Pardeep et al. 2010), MRI (Tangmose et al. 2013; Tangmose et al. 2014; Vieth et al. 2014) and racemization of aspartic acid (Hare & Abelson 1968; Helfman & Bada 1975; Man et al. 1983; Masters et al. 1978; Yekkala et al. 2006). Radiology and visual morphological methods of dental and development of skeleton do not appropriate for adults (Ball 2002) because these methods have wide range of age (Ball 2002; Frost 1987; Ritz-Timme et al. 2000), large standard error of estimation (Frost 1987) and more closer biological age than chronological age (Ball 2002; Ohtani et al. 2002; Ritz-Timme et al. 2000).

In contrast, the histomorphometry method has been developed for the estimation of age at death (Ericksen 1991; Kerley 1965; Singh & Gunberg 1970). The histomorphometry method is a quantitative determination by using measurement of osteon morphology. Furthermore, age-estimating research based on various population groups with different bones has been extended. The sternal end of the fourth rib and anterior cortex of the femur were tested for use to as samples (Han et al. 2009; Kim et al. 2007) whereas, the previous study that conducted on clavicle, only focussed on Caucasian population (Stout & Paine 1992). Hence, we decided to review the histomorphometry method in clavicles.

HISTOMORPHOMETRY METHOD – A BRIEF HISTORY OF TIME

Histomorphometry, the quantitative study of histology, is a useful method to estimate age at death in the present and the ancient skeletal remains. This method has been used by physical anthropologists to determine skeletal age at death, health status and degree of preservation in modern (Kerley 1965) and archaeological (Ericksen 1991). This method reduces individual bias, reduces the level of experience for estimation of age (Frost 1987), is useful in constructing paleodemographic models and for forensic identifications when adult fragmentary skeletal remains are encountered (Stout & Paine 1992). Histological methods also provide smaller reported error of estimate values than morphological techniques. All histological aging methods are based upon the fact that the bone tissue is replaced or turned over throughout individual's life. Living bone is a dynamic tissue that is constantly changing to meet daily demands.

In 1965, Kerley (1965) is the first researcher that determined age estimation method in human cortical bone from the microscopic examination by using cross-sections of long bone diaphysis. This method implies osteons counting, old osteons fragments and non-haversian canals and evaluation of the percentage of circumferential lamellar bone remaining in four circular visual fields located at the periosteal margin of the cortex. Several histological methods have been developed for estimation age at death in skeletons (Ericksen 1991; Kerley 1965; Kerley & Ubelaker 1978; Singh & Gunberg 1970; Stout & Paine 1992). The histological methods for age estimation get acceptant for useful in forensic and physical anthropology because these methods have greater accuracy of age estimation for older skeletal remains that age over 50 yrs than the conventional gross morphological methods.

Most current age estimation by histological methods depend on increasing the number of osteons and their fragments, osteon population density (OPD), with age (Stout & Paine 1992). In 1992, Stout and Paine (Stout & Paine 1992) described a method for estimating skeletal age from histology of the rib and clavicle, which may be more available for thin-sectioning since they are not typically used for standard anthropological estimations. Moreover, non-remodelled bone can predict relationship with advancing age (Maat et al. 2006). Young people have greater amount of lamellar (non-remodeled) bone and non-haversian canals than their older people. Increasing age, the non-remodeled bone in young persons is replaced with mature Haversian systems including osteons, lamellae and fragments of osteons. Thus, histomorphometric factors such as the percentage of non-remodeled bone (Maat et al. 2006) and the total number of osteons (Kerley 1965) present strong correlations with age (Kerley 1965; Singh & Gunberg 1970).

Several histological methods for age estimation have been developed in archeological and forensic skeletal remains (Crescimanno & Stout 2012;

Keough et al. 2009; Kerley 1965; Lee et al. 2014) because these methods increase the number of intact and fragmentary osteons in the identified fields or per measured area with age. It is important to use the measurement of osteons for estimate age at death in elderly individuals who present high osteon densities. Numerous measurements of osteons have been studied in recent years (Britz et al. 2009; Crescimanno & Stout 2012; Dominguez & Crowder 2012; Han et al. 2009; Keough et al. 2009; Lee et al. 2014), and have been reported to differ with age. An age relate to decreasing of osteon size in human has been repeatedly observed in several researchers (Pfeiffer 1998; Singh & Gunberg 1970; Thompson 1980) and nonhuman primates, macaques, has been found the decrease in osteon size like a human (Burr 1992; Havill 2004).

However, several researchers attended to the relationship between osteon size and age, but few studies attended to the relationship between osteon shape and age. Moreover, osteon circularity (On.Cr) are studied. The results are limited by qualitative strain effect (Skedros et al. 1994) and location in cortex (Pfeiffer 1998). Currey (1964) reported that the osteons of elderly individuals are nearly circular shape, but younger individuals have more irregularly shape of osteons. Another study, Britz and colleagues (Britz et al. 2009) found that circularity of osteons of femur increased with age. Moreover, circularity of osteons has been used like a variable for evaluate species identification due to human osteons have less circular than non-human species (Crescimanno & Stout 2012; Dominguez & Crowder 2012). Moreover, Narasaki (1990) applied Thompson's core technique to femoral samples of modern Japanese cadavers for testing the reliability and for establishing the Japanese population equations. In his study, the multiple correlation coefficients were not high, compared with other methods (Kerley 1965; Singh & Gunberg 1970; Stout & Paine 1992). It is claimed that age estimation equations for specific population should be established.

ANATOMY OF CLAVICLE AND ITS FUNCTIONS

The human clavicle (collar bone) is a paired-long bone and only set in horizontal plane (Moore et al. 2011) that starts from base of neck to the shoulder and locates on first rib (Scheuer et al. 2000). The shaft of clavicle has S-curved bone (Moore et al. 2011; Scheuer et al. 2000; White et al. 2011) that has medial end (sternal end) and lateral end (acromial end) (Moore et al. 2011; White et al. 2011). The medial half of clavicle is anteriorly convex and the lateral half is anteriorly concave (Moore et al. 2011). Medial end has rounded and diverged shaped like a trumpet and lateral end has flatten shaped in superoinferior (White et al. 2011). Medial end of clavicle articulates with clavicular notch of the manubrium of the sternum at sternoclavicular (SC) joint and lateral end of clavicle articulates with acromial process of scapular at acromioclavicular (AC) joint (Moore et al. 2011; Scheuer et al. 2000; White et al. 2011).

The clavicle has several functions: (i) it is rigid base for muscle attachments, (ii) it is holding for glenohumeral joint and it increases range of motion of shoulder joint, (iii) it increases power of arm-trunk mechanism and (iv) it provides a protection for major vessels at the base of neck and vessels and nerves passing to the upper limb (Moseley 1968). Moreover, the roles of clavicle are transmission load along its long axis from upper extremity to the thorax (Harrington et al. 1993).

The clavicle has several clear benefits. First, the size determines the relatively high ratio of compact bone to spongy bone, so there is less possibility of damage to the clavicle during a fracture. Second, the bone is likely to be found entire preferably than in fragments. Third, the clavicle is not a weight-bearing bone, so few factors, including daily lifestyle, can affect the bone remodeling rate over time. Finally, the clavicle does not influence other anthropological tests (Stout & Paine 1992).

DEVELOPMENT OF CLAVICLE

In the human embryo, the clavicle and the mandible are the first bones that begin to ossification. Both bones begin in membrane and follow by secondarily develop growth cartilages. The development of bones and joints at prenatal period have been reviewed by Gardner (Gardner 1956). Human development of embryonic period was separated into 23 stages (the first 7 to 8 weeks of human embryonic development). A number of external and internal characteristics, shape and size, degree of differentiation and organization of several tissue and organs are used for identify each stages of human embryonic development. Thus, it can discuss and compare differentiation and growth in each embryo stage.

The early part of prenatal life, primary ossification in the human skeleton has been investigated by the best method that is histological examination of serial sections. Since ovulation, the human embryonic period composes of the first 7 to 8 weeks of development. At the end of this period, differentiation is almost completed, and the bones and the joints have a form and an preparation characteristic of adult (Gardner 1968).

The clavicle, like most membrane bones, initiates to form during the embryonic period. Firstly, the fibrocellular proliferation, blastema, which extends from the locality of the scapula in downward, forward and medially direction to the midline region. The blastema which forms clavicle seperates from the blastema which forms all other bones of the upper limb and sternum. The clavicle blastema forms in stages 17, 18 or 19 that form about five postovulatory weeks (O'Rahilly & Gardner 1972).

Soon after the blastema appears, an organic matrix is formed by blastema and is mineralized nearly as soon as it forms and bonds the connective tissue fibers together. This process shows intramembranous ossification. The bone forms continuously to grow like the cells that surround it become osteoblasts which continue to proliferate, forming fibers and

matrix. During stages 18, 19 or 20 (about 5 post-ovulatory weeks on), the clavicle ossification usually begins like 2 separate centers, a medial and a lateral (O'Rahilly & Gardner 1972). The two centers of clavicle ossification are preceded by hypertrophy of the blastema cells in the beginning ossification areas and by increasing the amount of intercellular matrix. The two centers of clavicle ossification unify to form a long mass of bone that is more solid than trabeculae bone like other membrane bones (Andersen 1963; Gardner 1956; Hanson 1920).

Osteoblasts are the first cells in the two centers of ossification. These cells are formed by the cell that surround the centers. However, the cells on the acromial aspect of the lateral center and the cells on the sternum aspect of the medial center remain chondrogenous. The cartilage forming cells of the lateral center combine with densely packed mesenchymal cells area in which the forming of acromioclavicular joint. Similarly, the cartilage forming cells on the end of the medial center combine with densely packed mesenchymal cells are in which the forming of sternoclavicular joint. In the stages 20 to 21, the chondrogenous cells on lateral and medial ends of the clavicle have formed cartilage that are larger cells and less intercellular matrix than the hyaline cartilage and the chondrogenous activity at the sternal end of clavicle is more noticeable. The forming of blood, vascular elements, osteoblasts and osteoclasts are formed by immediate differentiation of incomplete cells in stages 20 to 21 too. The bone is destroyed, but a marrow cavity begins to be formed. Later, the clavicle grows like a long bone of the cartilage type. It increased in diameter by periosteal that is intramembranous ossification and increased in length through the activity of cartilaginous ends (Gardner 1968). By the end of the embryonic period, stage 23 (7 to 8 postovulatory weeks), the clavicle is surely S-shaped and is alike the form and relationships of adult. It has reached both the acromion and the sternum, usually has an early marrow cavity and grows like a typical cartilage bone (Gardner 1968).

The human foetal period, prenatal period, is started from the end of the 2nd month until term. The bones and joints are characterized by growth, maturation, remodeling and reconstruction process for a maintain its characteristic shape of the bone. In the normal skeleton, the increasing growth in personal bones bear accurate relationship to those skeletons as a whole. Ligaments become more collagenous, appearing of synovial fat pads, bursa development, tendinous attachments to bone move to adapt to growth and vascular epiphyseal cartilages (Gardner 1968).

Soon after the beginning of foetal period, the central invasive process in the clavicle reaches to the cartilaginous ends and cartilage cells calcify and form growth zones like the diaphysis aspects of epiphyseal plates. The clavicular growth zone, however, unusually shows the arrangement of zones like in other long bones. After beginning of epiphyseal ossification, articular cartilage form the growth zones. The hypertrophy of cartilage cells tends to be arranged irregularly

more than longitudinal columns. The invaded cells from the marrow destroy the hypertrophied cartilage cells and place down bone around the fraction of calcified cartilage matrix masses. The majority forming of endochondral trabeculae is destroyed almost rapidly, so the relatively few are found in the marrow space (Gardner, 1968).

After beginning of bone remodeling, the pattern of growth of the clavicle is like other long bones. The bony surface of the shaft that near the growth zone is removed and the remnants of endrochondral can be found in the compacta. The remodeling in clavicle, however, is not noticeable like other bones because the diameters at the levels of the growth zones are not much greater than the center of the shaft. Throughout the foetal period, the clavicle is markedly S-shaped and has a thick, trabeculae compacta and a small marrow space (Gardner 1968).

The foetal period, the clavicle is vascularized by peripheral vessels. Hence, this vascularization begins formerly any occurring of secondary or epiphyseal ossification. Finally, the perichondrium which caps each cartilage becomes fibrocartilage and serves as articular cartilage. Corrigan (1960) described the neonatal clavicle that is usually only one development of epiphyseal center. The epiphyseal center of sternal end appears during adolescence and fuses with the shaft by the 3rd decade. The epiphyseal center of acromial end appears during adolescence and quickly fuses with the shaft.

THE PROCESS OF CLAVICLE OSSIFICATION

Bone information has two different processes, intramembranous or endochondral ossification, that derived from primitive mesenchymal tissue (Karsenty et al. 2009; Kronenberg 2003). Intramembranous ossification initiates from primitive foetal mesenchyme that differentiate to osteoblasts and forming bone. On the other hand, endochondral ossification needs the formation of a cartilage model, or anlagen, in a process known as chondrogenesis, followed by bone formation. These processes encircle a series of events that start from differentiation of mesenchymal cell to chondrocytes. Then, cells proliferate and change their phenotype by increasing in volume as hypertrophy. Afterward, hypertrophic chondrocytes synthesize a calcified matrix before they have apoptosis. Finally, osteoblasts enter this mineralized scaffold for further mineral deposition and tissue maintenance (Ballock & O'Keefe 2003; Stevens et al. 1999; Zuscik et al. 2008).

The clavicle is intramembranous ossification that is not preceded by hyaline cartilage model but bone develops from the condensation of mesenchyme that forms an ossification center. The mesenchymal cells differentiate into osteoblasts, which produce the surrounding osteoid matrix. Many ossification centers are formed, anastomose and produce a network of spongy bone that composes of thin rod, plates and spines called trabeculae. The hemopoietic tissue located between trabeculae. The osteoblasts are

encircled by bone in the lacunae and become osteophyte. The osteocytes are trapped in the lacunae and they form a complex cell-to-cell connection through canaliculi. The other bones that are formed by intramembranous ossification are mandible, maxilla, and the most flat bones of skull (Telser et al. 2007).

The clavicle is a long bone, but it is difference from other long bones. The clavicle is the first bone that starts process of bone ossification but it is the last bone that completely fuses (Gardner 1968; Kumar et al. 1989; Ogata & Uhthoff 1990). The clavicle has not medullary cavity like other long bones and it is the first foetal bone to get ossification by only membranous ossification like other long bones (Kumar et al. 1989; Ogden et al. 1979; Scheuer & Blach 2004). The clavicle of human has been reported to complete fusing this bone that approximate 25 years old (Ogata & Uhthoff 1990). The ossification initially starts with two primary ossification centers from medial end and lateral end that fuse together in shaft of clavicle during 5th and 6th foetal week (Kumar et al. 1989; Moore et al. 2011; Ogden et al. 1979; Scheuer & Blach 2004). In 5th week, ossification center of clavicle presents primary center of ossification; medial and lateral centers; at shaft of clavicle and fuse in 6th week of development of embryo (Kumar et al. 1989; Ogden et al. 1979; Scheuer & Blach 2004). Then, secondary ossification begins develop at sternal end and acromial end from mesenchymal tissue. These cartilaginous masses permit growth by endochondral ossification. Endochondral ossification of medial cartilage mass at sternal end of clavicle maintains longitudinal growth of clavicle and intramembranous ossification of periosteum increase diameter of clavicle (Gardner 1968; Glenister 1976; Ogden et al. 1979). The secondary cartilage ossification shows configuration of epiphysis with different histological areas: reserve zone I, proliferation II, hypertrophy III, calcification IV, and vascular invasion V (Brighton 1984). Secondary ossification centers of longitudinal growth of clavicle show different pattern from other major long bones of the limb (Gardner, 1968; Ogata & Uhthoff 1990). Moreover, the ossification process of clavicle is slow maturation (Scheuer & Blach 2004). A secondary ossification center at the sternal end begins to fuse with the shaft of clavicle between age 18 and 25 years old and completely fused at age between 25 and 31 years old. The acromial end of clavicle may appear smaller scale-like epiphysis and it is not a fracture of clavicle. Failure of fusion of two ossification centers of clavicle cause bony defect of clavicle (Moore et al. 2011). Therefore, the clavicle is the first bone ossification and the last fused epiphysis bone.

The morphology of adult human clavicle (S-curve bone) is finished early in foetal period before birth (Black & Scheuer 1996). Length of clavicle in males and females create 80% of total length of clavicle in 12 and 9 years old, respectively (McGraw et al. 2009). The skeletal growth of clavicle has longer period, so clavicle may be response to several mechanical loading and shear stress. The various factors that may

affect asymmetry clavicle feature are several mechanical loading, asymmetric vascularization, lateralized behavior, activity-induced changes or more stress loadings of the dominant hand side of the clavicle (Mays et al. 1999).

BONE REMODELING

The bone remodeling is a livelong process (Kini & Nandeesh 2012) that removes the part of old bone and replaces it with the newly bone including the matrix (Fernandez-Tresquerres- Hernández-Gil et al. 2006). In normal activity, this process repairs the microscopic damage of the bone and prevents accumulation of damage in the bone (Martin 1998; Turner 1998) Bone remodeling react to mechanical loading. Moreover, this process is important to bone strength preservation and mineral homeostasis (Kini & Nandeesh 2012). Bone remodeling cycle of normal bone, osteoclasts (bone resorption) and osteoblasts (bone formation) work respectively together (Martin et al. 1998). The average remodeling process is about 2 to 8 months, majority time is bone formation. The third decade of life has the maximum bone mass and balance of bone remodeling process until the 50 years old. Afterward, bone mass begins to decrease and more resorption process especially perimenopausal and early postmenopausal women (Kini & Nandeesh 2012).

REMODELING PHASES

Bone remodeling can be separate to the following six phases that are quiescent, activation, resorption, reversal, formation, and mineralization. The first step is activation followed by resorption, reversal and the last step is mineralization. This process is distributed randomly, but it targeted to repair areas (Burr 2002; Fernandez-Tresquerres-Hernández-Gil et al. 2006).

Firstly, the bone cells are lining in endosteal membrane that is the rest state (quiescent phase) follow by resorption of membrane lead to bone cells retraction (activation phase). Then, osteoclasts begin to dissolve the mineral matrix and decompose the osteoid matrix for resorb the bone (resorption phase). The resorption of osteoclasts creates irregular scalloped cavities on the trabecular bone surface, called Howship's lacunae, or cylindrical Haversian canals in cortical bone. This phase of each bone remodeling cycle takes the time about 2–4 weeks. Afterwards, the bone resorption transition to bone formation (reversal phase) and osteoblast come into replace osteoclasts for bone formation (formation phase). The last phase, osteoid matrix is mineralized (mineralization phase) that occurs after osteoid deposition 30 days and complete at 90 and 130 days after osteoid deposition for trabeculae and cortical bone, respectively. This cycle begins the quiescent phase again. Finally, the amount of bone formation and bone resorption should equal when completed cycle of bone remodeling.

APPLICATION IN FORENSIC

For clavicle, the study of Stout and Paine in (1992) was the first study

in clavicle. This study studied age estimation by histomorphometry of mid-shaft of left clavicle and middle third of the left sixth rib in whites, black, and unknown ethnics from 40 autopsy cases. The slides had 75µm thicknesses and were analyzed by microscopic analysis. The researchers measured cortical area, intact osteon density (p^i), fragmentary osteon density (p^f) and total visible osteon density ($p^i + p^f$). Age-predicting model for clavicle used total visible osteon density ($p^i + p^f$) as the independent variable. The results showed the differences between actual age and predicted age range from -8.1 to +20.6 years for the clavicle, -2.7 to +9 years for the rib, and -2.5 to +14.5years for the combined rib and clavicle formula. The mean difference were 1.1 (± 3.57) year for clavicle, 3.4 (± 1.06) year for rib, and 2.6 (± 2.20) year for combined rib and clavicle formula. The r-square value of age-predicting model (r^2) were 0.699, 0.7211, and 0.7762, respectively. The benefit of clavicle and rib is available bone when the long bone cannot use for age estimation. The histimorphometry method can occur error from sampling sample so this study used two entire cross-sections of clavicle and rib and read every field per section or alternative reading of each field for avoid error. Moreover, the benefit of this procedure is ability to adjust location of microscopic field for reading any section. The combined clavicle and rib formula was recommended because this formula had slightly high standard error and r^2. Therefore, the method of clavicle and rib should be appropriate for demographic studies and forensic identification.

In 2014, Lee and co-workers (Lee et al. 2014) studied age estimation by histomorphometry of clavicle in Korean. The study used right clavicle from 46 dissected cadavers and cut from the sternal end of the right clavicle (length 3 cm) and made two sequential slides (100-ìm thickness). The researchers measured ratio of the relative cortical area (RCA), osteon population density (OPD), and mean osteon area (OA) were microscopic measured and counted by using an image analysis program (Image J). The results showed negative correlation in RCA and OA and positive correlation in OPD from simple linear regression. The highest correlation between estimated age ($R^2 = 0.583$) was OPD follow by RCA ($R^2 = 0.274$) and OA ($R^2 = 0.100$). The OPD and RCA were selected for the age-predicting equation from the multiple regression analysis using the stepwise method ($R^2 = 0.628$). Difference of sex, RCA showed significantly different between sexes ($p = 0.000$). From the results, RCA should be measured by separate sexes. However, previous studies reported no significant relationship between bone composition and sex (Kerley 1965; Ericksen 1991), so this study do not separate data into male and female parts for equation, although sex was studied in RCA measurement. For OA, this study showed moderately unrelated with estimated age that contrast to the study of Osborne and co-workers (Osborne et al. 2004). OA was insufficient for reflect the remodeling rate. In further study, the researcher suggested that unremodeled area should be applied for age estimation by

histomorphometry. Comparing result, this study had regression correlation result (R^2 = 0.628) as well as Stout and Paine in 1992 (R^2 = 0.699). However, difference of population group had different results. Equation for age estimation by histomorphometry in clavicle can be used for especially each population.

In 2015, Sobol and co-workers (Sobol et al. 2015) studied age estimation by histomorphometry of clavicle from fresh cadavers from autopsy in Poland. The study used fragments of shafts of left clavicles taken from 39 males and 25 females. They measured clavicle length (CL), clavicle width (CW), clavicle thickness (CT), number of osteons in the field of vision (ON), number of osteons with the Haversian canal of more than 70 μm (HC > 70 μm), average diameter of the Haversian canals (avg. ØHC), area occupied by interstitial lamellae (ILA %), area occupied by osteons (OA %), area occupied by fragments-remnants of osteons remain as irregular arcs of lamellar fragments (OFA %), average thickness of outer circumferential lamellae (avg. OCL, μm), and the relation of osteons with the Haversian canal of more than 70 μm in diameter to the total number of osteons (HC > 70 μm, %). The age of the bone remains was estimated using linear regression equation. The results showed the shaft of clavicle was the best field for analysis of bone tissue because shaft of the clavicle was most distant from the muscle and ligament attachment (Ingraham 2004) and affected by only subclavian muscle. This area showed the number of osteons with a large diameter (HC > 70 μm) increased with age because osteoclast activity of the resorption of osteon's lamellae that had highest correlation (R^2 = 0.87).

CONCLUSION

The histomorphometry methods can estimate age at death in skeletal remains based on morphology of osteon. Using different area of the same bone, different populations, including different procedures for section and analysis showed different results. Clavicle is the one of interesting bones for age estimation by using histomorphometry method because this bone is non-weight bearing bone that has only subclavian muscle affected mechanical loading. Moreover, it had less possibility of damage from fracture, found entire bone, and had few studies. Thus, the histomorphometry method from clavicle is very useful method for age estimation.

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