

Histomorphological analysis of human cranial bones: Relationship to aging

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Summary. Aging induces alterations in bone microarchitecture, including bone function. This study aimed to identify histological changes in the components of cranial bones of the human skull and to investigate their relationship with aging. In this study, sixty-four fresh cranial remains were examined with an age range of 43-90 years, the crania being randomly selected to investigate the microstructure of the frontal, temporal, parietal, and occipital bones, which were stained using picrosirius red. Our results observed similar histological changes in the frontal, temporal, parietal, and occipital bones. Results of the histomorphological analyses demonstrated that there was an age-related gradual increase in the number of osteons. A distinct increase in the number of osteons in the cortical bone was found, particularly over 60 years. As age increased, the area of the circumferential lamellae adjacent to the periosteum also tended to diminish. A greater lamellar area was found in association with younger individuals, the area declining with age. In some samples from individuals older than 80 years, the outer circumferential lamellae were no longer visible. In addition, there was an association between cortical porosity and numbers, and the enlargement of porosity and age. The size of the pores in the cancellous bone tended to increase as well. This histomorphological study increases the comprehension regarding the relationship between the aging process and the structure of the human cranial bone in adults, and the results can be regarded as alternative data in the determination of age in humans.

Key words: Age, Bone Histology, Osteon, Bone remodeling, Cranial bones

Introduction

Bone is a living and specialized connective tissue that protects the body's essential organs (Bicalho, 2020; Gartner, 2020). The hardness of the bone is achieved by the incorporation of inorganic contents, such as phosphate and carbonate salts of calcium and other minerals, into the extracellular matrix. Bone also has vascular tissue that obtains vital nutrients via the periosteal blood vessels (Mescher, 2018; Flis et al., 2019). Aging can affect bone structure in many ways, including gross macrostructure, dimension, shape, and microstructural composition, all of which impact the function of human bone, especially with regard to histological changes of the bone. Knowledge in this field is crucial for understanding the aging process and its impact on bone structure and function (Thompson, 1980).

Previous reports had demonstrated that with increasing age, significant changes occur in bone thickness and porosity, and also alterations in the size and number of haversian systems (Kerley, 1965; Thompson, 1980). Alterations in the bone with aging involve the process of bone remodeling, known as bone turnover, which is a lifelong process (Frost, 1964, 1969). Continuous bone remodeling, with its associated microstructure changes during life, generates indicators for histological age determination.

The majority of previous studies have focused on aging related changes in various areas, specifically the cortical region of the femur (Kerley, 1965; Ahlqvist and Damsten, 1969; Singh and Gunberg, 1970; Kerley and Ubelaker, 1978; Ericksen, 1991; Watanabe et al., 1998; Goliath et al., 2016; Maggio and Franklin, 2021), tibia (Kerley, 1965; Singh and Gunberg, 1970; Kerley and Ubelaker, 1978), fibula (Kerley, 1965; Kerley and Ubelaker, 1978), humerus (Yoshino et al., 1994; Nor et al., 2014), ribs (Stout and Paine, 1992; Cho et al., 2002; Kim et al., 2007; Absolonova et al., 2013), mandible (Singh and Gunberg, 1970), clavicle (Lee et al., 2014; Sobol et al., 2015; Kranjoti et al., 2020), and iliac bones (Castillo et al., 2012). Early research by

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www.hh.um.es. DOI: 10.14670/HH-25-012



Kerley et al. (1965) established that age-related remodeling occurs in long bones, such as the femur, tibia, and fibula. They quantified the osteon count and Haversian canal features. Their results showed strong correlations with age ($r=0.79-0.97$) and an estimation accuracy within ± 10 years (Kerley, 1965). Another study by Singh and Gunberg (1970), using a histological approach to the mandible, found that the osteon number and lamellae increased, whereas the diameter of the haversian canal decreased with aging, with standard errors of 2.6 years (Singh and Gunberg, 1970). Then, Stout and Paine (1992) and Cho et al. (2002) examined histological age estimation using the rib and clavicle. The finding revealed that total osteon density and relative cortical area provided an accurate age detector (Stout and Paine, 1992; Cho et al., 2002). A recent study on histomorphometry of the sixth rib and clavicle depicted that most of the histological variables were significantly correlated with advancing age. Fragmented osteon numbers and osteon population density provided the strongest values, with an error of 10.7 years of age. Moreover, differences between sexes and populations were identified in osteon measurements (García-Donas et al., 2022). The cortical bone of long bones was investigated most frequently because of its large area and clear definition of the compact bone, and because it is usually well-preserved (Murad and Boddy, 1987; Nor et al., 2014). The majority of previous studies were carried out in the appendicular skeleton; however, in this study, the focus is on the cranial bones.

Human cranial bones are classified as flat bones, composed of three layers of inner and outer compact bones and a mid spongy bone and a structurally strong bone. This compact bone has an important role in protecting the brain and supporting the structure of the head (Drake et al., 2015). Most previous studies on cranial bones focused on cranial sutures (Meindl and Lovejoy, 1985; Dorandeu et al., 2008; Bassed et al., 2010) and other external morphological analyses (McIntyre and Mossey, 2003; Marcus et al., 2008; Kanodia et al., 2012; Urban et al., 2016). The primary emphasis in previous studies on morphological alterations of the skull bone is on pediatrics (Chen et al., 2000; Axelsson et al., 2003; Marcus et al., 2008; Kanodia et al., 2012). The usual rationale for the selection of the cranium is that the skull is most commonly used as biological evidence in every forensic event and is quite well-preserved (Sorg and Haglund, 1996; Kampan et al., 2014). The cranium is also a prominent, easily recognizable bone, which is significantly different from other species (Langley and Tersigni-Tarrant, 2017). However, research focusing on the histological structure of adult skull remains is still rare and limited. Therefore, the aim of this study was to evaluate the age-related histological changes in human cranial bones to enhance our understanding of how the microstructure changes with aging.

Materials and methods

All specimens in this study consisted of human skull bones. Sixty-four bone specimens were obtained from fresh cadavers, with known age, sex, and cause of death, from the Department of Anatomy, Chiang Mai University Faculty of Medicine, Chiang Mai, Thailand. Ethics Committee approval was obtained from the Chiang Mai University School of Medicine (Research ID: FAC-MED-2566-0476, approval date: October 10, 2023). The age range of the samples was 43-90 years. The middle regions of the frontal, parietal, temporal, and occipital bones of each skull were extracted. Specimens with obvious pathological damage to the bone structure detected on gross and histological examination were excluded from the study. After the removal of skin and soft tissues from the skull, the specimens were cut to approximately 2x2 cm in size using an electrical saw. After cutting, all specimens were fixed in 10% formalin for 24 hours and then decalcified using 65% diluted nitric acid for two weeks. The decalcification process was carried out at room temperature. After bone decalcification, the samples were cut into 2-3 μm -thick cross-sections. All these sections were then prepared and stained for histological examination using Picrosirius red staining. Section slides were captured with a light microscope camera (Olympus DP73 digital microscope camera, Tokyo, Japan) using the CellSens standard program, and the histological features were analyzed using Image J software. Histological variables included the number of secondary osteons (N.On), fragmentary osteons (N.Fg.On), total osteon number (T.On), area of haversian canals (HCA), perimeter of haversian canals (HCP), and feret diameter of haversian canals (maximal diameter) (HCD). The study consisted of qualitative and quantitative analyses. Descriptive analysis and Pearson correlation test were used for data interpretation with SPSS version 19.0. The definition of histological variables was the following:

1. Secondary osteon: a haversian canal surrounded by concentric lamellae
2. Fragmentary osteon: an osteon with a complete circumference of the haversian canal
3. Total osteon number: sum of secondary and fragmentary osteons
4. Area of haversian canals: average area of haversian canal of secondary osteon
5. Perimeter of haversian canals: average perimeter of haversian canal of secondary osteon
6. Feret diameter of haversian canals: average maximal diameter of haversian canal of secondary osteon.

Results

Descriptive statistics for the sample and the assessed features can be observed in Table 1. Analysis of histomorphology revealed that the observed histological

changes in the frontal, temporal, parietal, and occipital bones were identical. The basic structures of the different cranial bones were similar. Within the overall samples, it was determined that the cortical bone was composed of two distinct layers, the outer and inner cortical bone layers, separated by the cancellous bone between them. Both gross anatomical and histological examinations indicated that the outer cortical bone layer is markedly thicker than the inner cortical bone layer. The periosteum, surrounding the outermost surfaces of both cortical bones, is composed of fibrous connective tissue rich in collagen fibers. Histologically, under picosirius red staining, the periosteum was stained light pink, which contrasted with the deeper cortical bone. In all cranial bones, the periosteum was quite thick. The layers of outer and inner cortical bones illustrated a similar microstructure, which was characterized by the presence of Haversian systems known as osteons. Fig. 1 shows the cortical bone morphology regarding the Haversian system or osteon of the cranial bones. Table 2 shows the mean values of all variables of the cranial bones in each age group.

There were significant positive correlations with all variables in the frontal, temporal, parietal, and occipital bones, as shown in Table 3. Aging statistically correlated with N.On ($r=0.536-0.642$), N.Fg.On ($0.327-0.504$), T.On ($r=0.544-0.655$), HCP ($r=0.564-0.670$), HCA ($0.488-0.674$), and HCD ($r=0.555-0.658$) (Table 3). The greatest correlation with age can be seen in the HCA of the occipital bone. The N.Fg.On parameter showed the lowest correlation with age. Among all cranial samples, N.ON, N.Fg.On, and T.On show an obvious progressive increase with age, especially over 70 years. Dimensions of haversian canal variables also increased after 60 years of age. The parietal and occipital bones exhibited the highest number of osteons and haversian canal

Table 1. Descriptive statistics of the sample and histological variables.

N=64	Minimum	Maximum	Mean	Std. Dev.
Age	43	90	68.08	13.4
Frontal bone				
N.On	2	14	6.4	2.7
N.Fg.On	0	5	1.3	1.3
T.On	3	16	7.6	3.5
HCP	67.2	180.6	116.9	34.1
HCA	309.0	2301.7	1025.6	547.2
HCD	23.8	76.1	45.5	14.7
Temporal bone				
N.On	3	11	6.44	2.1
N.Fg.On	0	5	1.13	1.5
T.On	3	15	7.50	3.3
HCP	70.0	180.6	113.0	24.7
HCA	349.1	3018.2	1005.6	496.0
HCD	26.1	65.9	42.5	9.5
Parietal bone				
N.On	2	12	6.3	2.5
N.Fg.On	0	5	1.0	1.2
T.On	2	14	7.4	3.3
HCP	57.5	169.5	118.1	23.7
HCA	220.1	2621.1	1089.9	454.6
HCD	21.7	65.9	45.9	9.2
Occipital bone				
N.On	2	12	6.2	2.1
N.Fg.On	0	6	1.3	1.5
T.On	3	15	7.6	3.2
HCP	66.1	185.4	118.3	31.1
HCA	308.7	2409.4	1077.3	575.9
HCD	24.9	73.0	45.8	12.4

Std. Dev., Standard deviation; N.On, the number of secondary osteons; N.Fg.On, the number of fragmentary osteons; T.On, total osteon number; HCA, area of Haversian canals; HCP, perimeter of Haversian canals; HCD, feret diameter of Haversian canals.

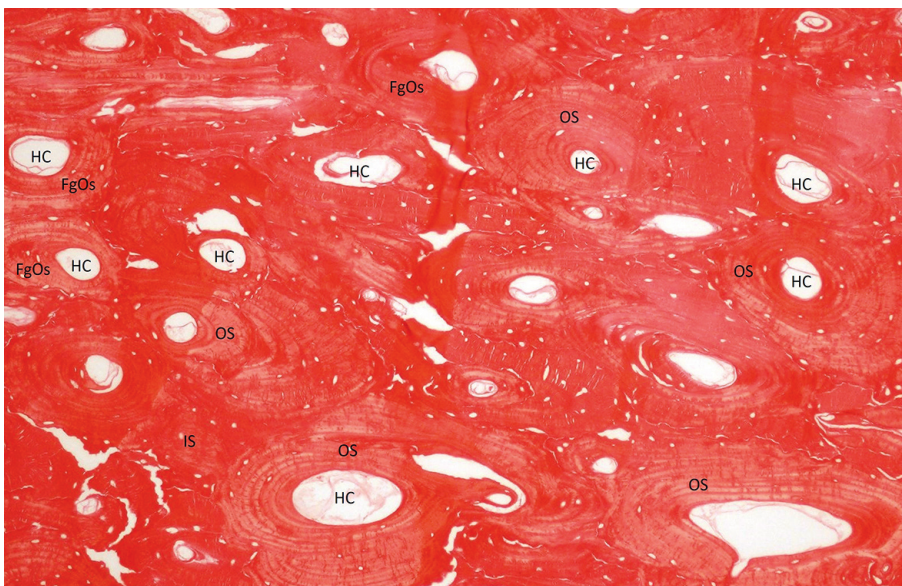


Fig. 1. Decalcified cross-section of the cranial bone showing the cortical morphology with Picosirius red. OS: osteon; FgOs: fragmentary osteon; HC: Haversian canal; IS: interstitial lamellar. Note the concentric lamellar (white rings) surrounding the Haversian canal. x 10.

enlargement in older individuals (>80 years). However, the frontal and temporal bones showed lower values. In the frontal bones, the HCA increasingly rose beyond 60 years, while there was a consistent increase in HCA and HCD in the temporal bone (Table 2).

In individuals aged 43-49 years, histological assessment revealed that two layers of the cortical bone showed solid and dense structures. Picrosirius red staining emphasized that special lamellae of osteons are red with a central Haversian canal (central canal). The outermost border of each osteon is marked by a cement line (also known as the reverse line), which is dark red in color and serves as the primary structural feature distinguishing adjacent osteons, including the interstitial lamellae between osteons. Haversian canals were usually circular or sometimes oval, pale areas that contained blood vessels. In the stained sections, osteocytes were not observed in the lacunae; however, lacuna-canalliculi networks were seen as pale-stained structures in the specialized layers surrounding the osteons. Osteons were rather sparse and scattered in some areas in the cortical bone region, especially in the middle cortical region and near the endosteal surface. A few porosities in the inner cortical bone were found in the regions adjacent to the bone marrow cavity. Additionally, beneath the periosteal region, outer circumferential lamellae were observed. Outer circumferential lamellae were mainly arranged in

a parallel orientation to the periosteum. It was notable that osteons near the periosteal surface were smaller in size than those of the endosteal side, including haversian canals. Areas of interstitial lamellae were large due to a small number of osteons. The structure of cancellous bone was weaker and less dense compared with cortical bone. The primary structural component of cancellous bone. Many cavities were filled with bone marrow containing fat cells. Quantitative results revealed that the dimensions of haversian canals, such as perimeter, area, and diameter, were the smallest in this age group. There were few secondary osteons and hardly any fragmentary osteons (Table 2).

For the age group of 50-59 years, the N.On progressively increased and were sparser. Interestingly, a few small osteons were developing in the circumferential lamellar area adjacent to the periosteum. The osteons in the outer circumferential lamellar were smaller than those of other cortical regions. The average size of Haversian canals was larger in individuals under 50 years of age. The Haversian canals had a more oval shape. Large pores in the cancellous bone were observed. Cortical porosity also increased. In the samples from individuals aged 60-69 years, a distinctive increase in T.On was observed with greater density. The osteons are scattered in groups through the cortical region. Fragmentary osteons were found clearly in this

Table 2. Mean values of histological variables of the cranial bones in each age group.

Age ranges (years)	Variables (Mean $\pm$ SD) (μ m)					
	N.On	N.Fg.On	T.On	HCP	HCA	HCD
Frontal						
40-49	3.17 $\pm$ 0.4	0.33 $\pm$ 0.5	3.50 $\pm$ 0.8	80.38 $\pm$ 15.4	450.27 $\pm$ 145.8	30.68 $\pm$ 6.7
50-59	3.75 $\pm$ 1.0	0.33 $\pm$ 0.7	4.08 $\pm$ 0.7	81.42 $\pm$ 11.7	469.45 $\pm$ 118.0	31.18 $\pm$ 5.2
60-69	6.81 $\pm$ 2.1	1.19 $\pm$ 1.1	8.00 $\pm$ 2.7	116.08 $\pm$ 32.6	1054.66 $\pm$ 546.6	44.20 $\pm$ 13.9
70-79	7.07 $\pm$ 2.0	1.86 $\pm$ 1.4	8.93 $\pm$ 3.1	137.55 $\pm$ 30.0	1339.81 $\pm$ 492.3	54.67 $\pm$ 13.7
Over 80	8.44 $\pm$ 2.7	1.87 $\pm$ 1.4	10.31 $\pm$ 3.2	139.83 $\pm$ 19.0	1354.55 $\pm$ 378.6	55.21 $\pm$ 8.9
Temporal						
40-49	4.83 $\pm$ 1.3	0.17 $\pm$ 0.4	4.67 $\pm$ 1.5	94.90 $\pm$ 14.9	680.65 $\pm$ 229.7	35.14 $\pm$ 5.4
50-59	4.67 $\pm$ 1.0	0.17 $\pm$ 0.4	4.83 $\pm$ 1.1	92.63 $\pm$ 13.4	651.56 $\pm$ 216.1	34.30 $\pm$ 4.5
60-69	5.81 $\pm$ 1.8	0.81 $\pm$ 1.2	6.56 $\pm$ 2.7	106.06 $\pm$ 16.7	864.08 $\pm$ 318.5	40.32 $\pm$ 6.4
70-79	8.64 $\pm$ 1.4	1.79 $\pm$ 1.8	10.36 $\pm$ 2.7	129.42 $\pm$ 27.1	1342.90 $\pm$ 654.6	49.08 $\pm$ 10.3
Over 80	7.06 $\pm$ 1.8	1.94 $\pm$ 1.8	9.00 $\pm$ 3.4	127.46 $\pm$ 21.3	1239.46 $\pm$ 424.3	48.00 $\pm$ 8.5
Parietal						
40-49	2.83 $\pm$ 1.0	0.50 $\pm$ 0.5	3.33 $\pm$ 1.4	83.14 $\pm$ 24.0	485.18 $\pm$ 272.9	33.26 $\pm$ 9.6
50-59	4.83 $\pm$ 0.6	0.50 $\pm$ 0.7	5.33 $\pm$ 0.8	108.77 $\pm$ 6.5	895.93 $\pm$ 96.9	41.80 $\pm$ 3.2
60-69	6.00 $\pm$ 2.4	0.56 $\pm$ 0.6	6.56 $\pm$ 2.9	113.76 $\pm$ 22.9	984.80 $\pm$ 398.4	44.63 $\pm$ 8.8
70-79	6.71 $\pm$ 1.8	1.14 $\pm$ 1.2	7.86 $\pm$ 2.5	123.28 $\pm$ 17.3	1167.67 $\pm$ 318.3	47.72 $\pm$ 7.0
Over 80	8.81 $\pm$ 1.6	1.94 $\pm$ 1.7	10.75 $\pm$ 2.6	137.94 $\pm$ 18.3	1499.35 $\pm$ 466.1	53.29 $\pm$ 7.5
Occipital						
40-49	3.33 $\pm$ 1.0	0.17 $\pm$ 0.1	3.50 $\pm$ 0.8	78.31 $\pm$ 9.3	400.24 $\pm$ 70.3	31.44 $\pm$ 5.1
50-59	5.25 $\pm$ 0.9	0.50 $\pm$ 0.7	5.75 $\pm$ 1.0	100.09 $\pm$ 20.3	709.75 $\pm$ 286.2	38.82 $\pm$ 8.6
60-69	5.75 $\pm$ 1.8	0.81 $\pm$ 0.9	6.56 $\pm$ 2.4	103.51 $\pm$ 18.8	794.53 $\pm$ 302.8	39.33 $\pm$ 7.3
70-79	7.43 $\pm$ 1.6	1.93 $\pm$ 1.7	9.36 $\pm$ 2.6	141.7 $\pm$ 20.3	1525.00 $\pm$ 417.7	53.93 $\pm$ 8.9
Over 80	7.50 $\pm$ 2.2	2.31 $\pm$ 1.8	9.81 $\pm$ 3.2	141.09 $\pm$ 28.7	1497.78 $\pm$ 579.7	55.60 $\pm$ 11.4

Descriptive statistics are expressed as mean with standard deviation (SD). N.On, the number of secondary osteons; N.Fg.On, the number of fragmentary osteons; T.On, total osteon number; HCA, area of Haversian canals; HCP, perimeter of Haversian canals; HCD, feret diameter of Haversian canals.

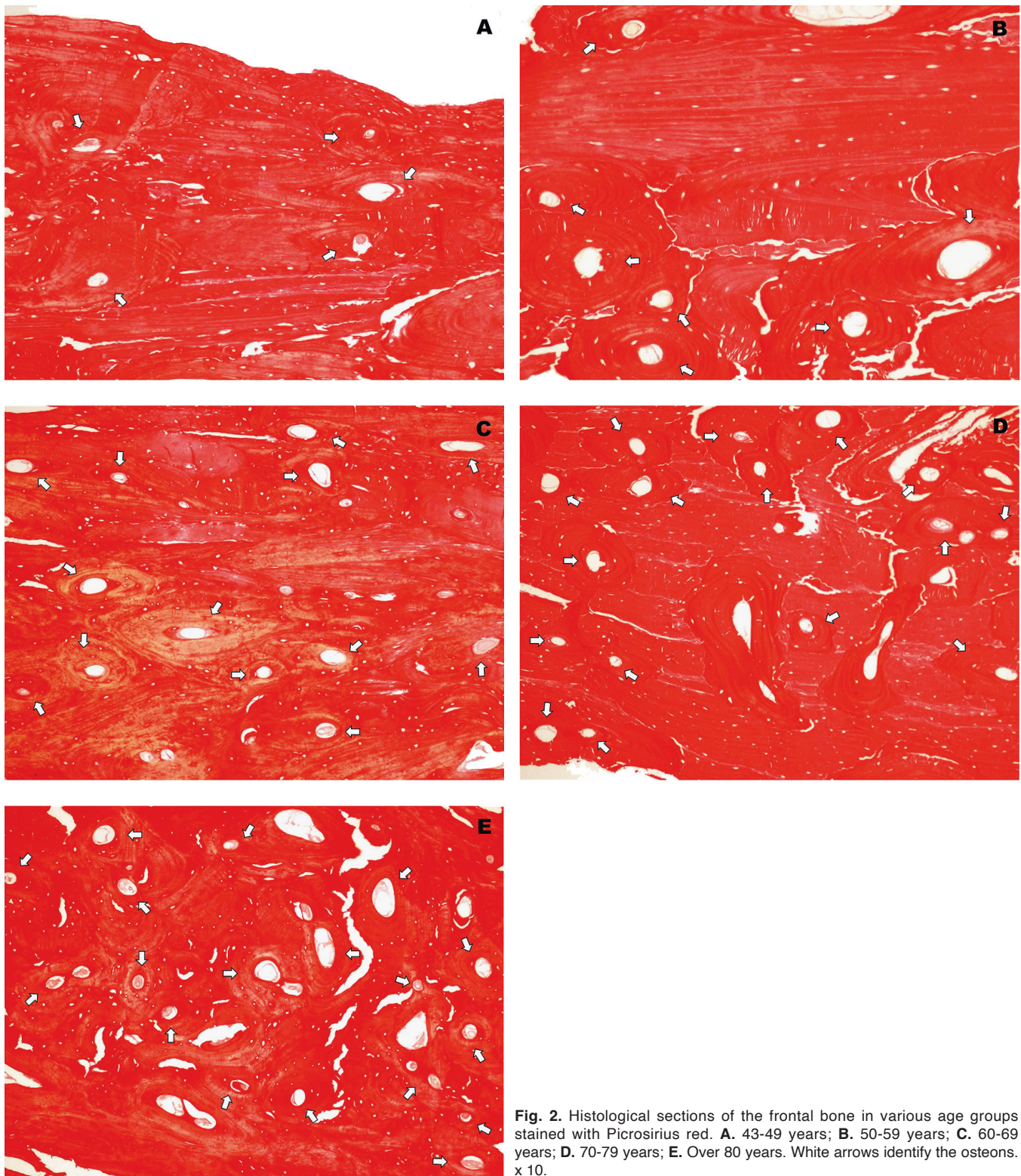


Fig. 2. Histological sections of the frontal bone in various age groups stained with Picrosirius red. **A.** 43-49 years; **B.** 50-59 years; **C.** 60-69 years; **D.** 70-79 years; **E.** Over 80 years. White arrows identify the osteons. x 10.

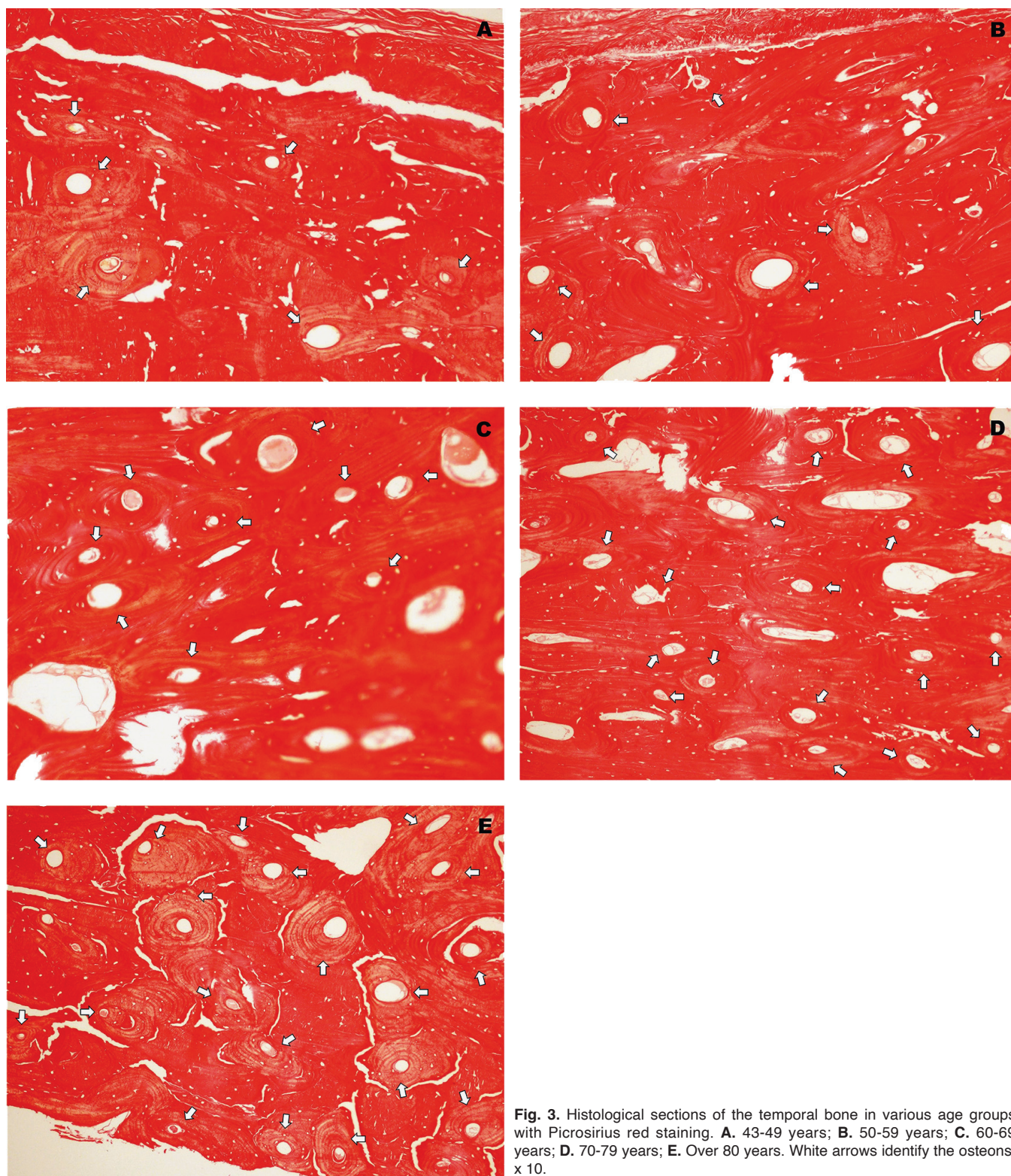


Fig. 3. Histological sections of the temporal bone in various age groups with Picrosirius red staining. **A.** 43-49 years; **B.** 50-59 years; **C.** 60-69 years; **D.** 70-79 years; **E.** Over 80 years. White arrows identify the osteons. x 10.

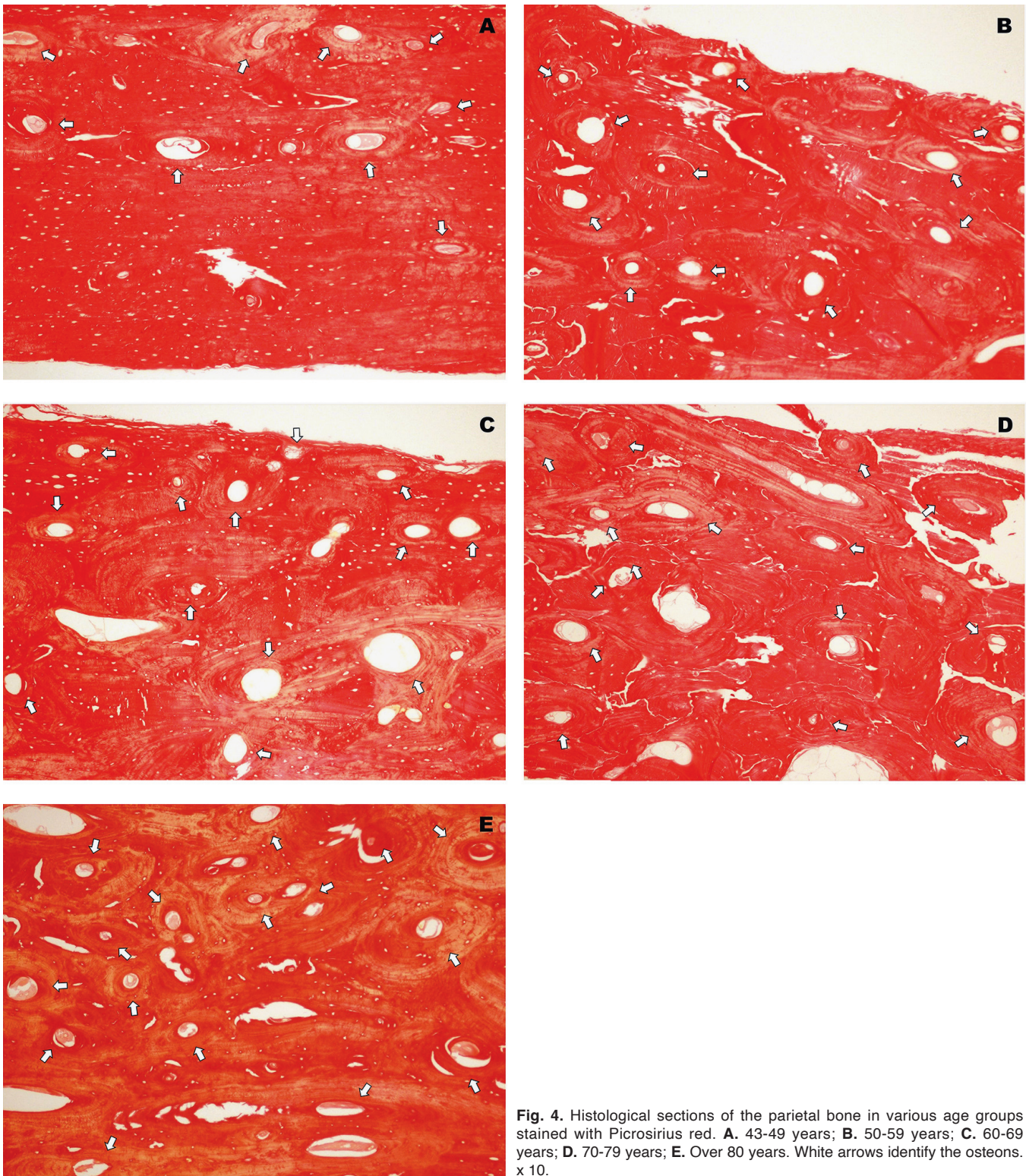


Fig. 4. Histological sections of the parietal bone in various age groups stained with Picrosirius red. **A.** 43-49 years; **B.** 50-59 years; **C.** 60-69 years; **D.** 70-79 years; **E.** Over 80 years. White arrows identify the osteons. x 10.

age period. The regular histological appearance of the outer circumferential lamellae was altered due to the increased number of osteons. Most of the osteons located closer to the endosteal surface, including their Haversian canals, were larger. Porosity was increased compared with that of younger individuals. In cancellous bone, the pores in the bone marrow were more dilated, the connections between bone trabeculae were reduced, and the typical meshwork was less structured. The number of fat cells in the bone marrow was increased.

In sections of specimens aged 70-79 years, a significant increase in N.On and N.Fg.ON, including T.On in the cortical bone, was observed. Numerous osteons were distributed throughout the cortical bone. The peripheral lamellar area was narrowed, and few osteons were observed in this area. The porosity of the cortical bone was increased, and the pores were enlarged. The pores in the bone trabeculae were more prominent. Increased loss of bone trabeculae was also observed. In some cases, the pores of the cortical bone merged with those of the cancellous bone. HCP and HCA were obviously larger, while the mean HCD did not seem very different.

In specimens over 80 years old, the number of secondary, fragmentary, and total osteons increased further, and they were densely distributed throughout the cortical bone, particularly in areas adjacent to the periosteum. The increased number of osteons was more densely packed, with narrow interstitial lamellae. While narrow outer circumferential lamellae were seen in some of these specimens, in others these lamellae were absent, and the area was filled by numerous osteons. HCAs were largest in the frontal and parietal bones. However, the areas were not very different in the temporal and occipital regions. HCDs were slightly different compared with those of younger ages. Moreover, some Volkman's canals were found in the cranial bone, but there were very few. It did not seem to be correlated with age. The number of large pores in the cortical bone area increased, and the porous area increased significantly due to pore coalescence. The bone trabeculae of the cancellous medullary bone were thinned, and the porosity of the area increased significantly. Figures 2-5

show histological changes in osteons of cranial bones in different age groups. Figure 6 shows histological changes associated with aging in the outer circumferential lamellar area of cranial bones. Figure 7 shows the increase in porosity in cortical bone with aging of the cranial section.

Discussion

This study investigates the histological changes in relation to aging in human cranial bone specimens, with a specific focus on frontal, temporal, parietal, and occipital bones. The findings indicate that all four types of cranial bones had similar morphological structures and identical changes related to the aging process. With advancing age, there was a significantly distinctive increase in the number of secondary, fragmentary, and total number of osteons, a decrease in the circumferential lamellar area of the cortical bone and size of the porosity of the cortical bone. Additionally, in cancellous bone, an expansion of the porosity. The prominent finding of this study is the increment in the number of osteons and dimension changes of the Haversian canal with aging in all cranial bones examined. Microstructural changes in osteon numbers and Haversian canal parameters were found to clearly progressively increase over 60 years, indicating an enhanced bone remodeling process and enlargement of Haversian canal sizes with increasing age. The variability of the cranial bones is clearly greater in older age. Although all cranial bones correlated with age, there were different values as a result of different bone remodeling rates among different bones and sites.

A review of the previous literature revealed a lack of definitive studies regarding age-related microstructural changes of osteons and the Haversian canal in human cranial bones. However, when compared to studies on other types of bones, our results were consistent with several previous studies. Most of the following studies were investigated in a quantitative manner, and their results corresponded with our findings regarding osteons. Kerley et al. in 1965 explored the midshafts of the tibia, fibula, and femur sections. After examining osteon morphology, they discovered a substantial correlation between age and the number of osteons in three different bones. A close relationship was found between age and the number of osteons in the fibula ($r=0.922-0.974$), particularly in fragmented osteons. The results for osteon parameters were more pronounced. Consistent with the results of previous researchers (Kerley, 1965), the study found no significant differences in terms of race or gender. Similarly, the results of Singh and Gunberg (1970), who studied the femur, tibia, and mandible, showed that the number of secondary osteons increased with age. They also found the most effective age indicator was the number of osteons in the mandible ($r=0.969$). Moreover, they revealed that the average minimum diameter of the Haversian canal correlated with age (Singh and

Table 3. Coefficient correlations between age and histological variables of the cranial bones.

Sample	Coefficient correlations (r)					
	N.On	N.Fg.On	T.On	HCP	HCA	HCD
Frontal bone	0.610**	0.425**	0.655**	0.655**	0.602**	0.640**
Temporal bone	0.536**	0.409**	0.544**	0.564**	0.488**	0.574**
Parietal bone	0.642**	0.327**	0.611**	0.571**	0.570**	0.555**
Occipital bone	0.539**	0.504**	0.601**	0.670**	0.674**	0.658**

**Correlation is significant at the 0.01 level (2-tailed) in Pearson correlation. N.On, the number of secondary osteons; N.Fg.On, fragmentary osteons; T.On, total osteon number; HCA, area of Haversian canals; HCP, perimeter of Haversian canals; HCD, feret diameter of Haversian canals.

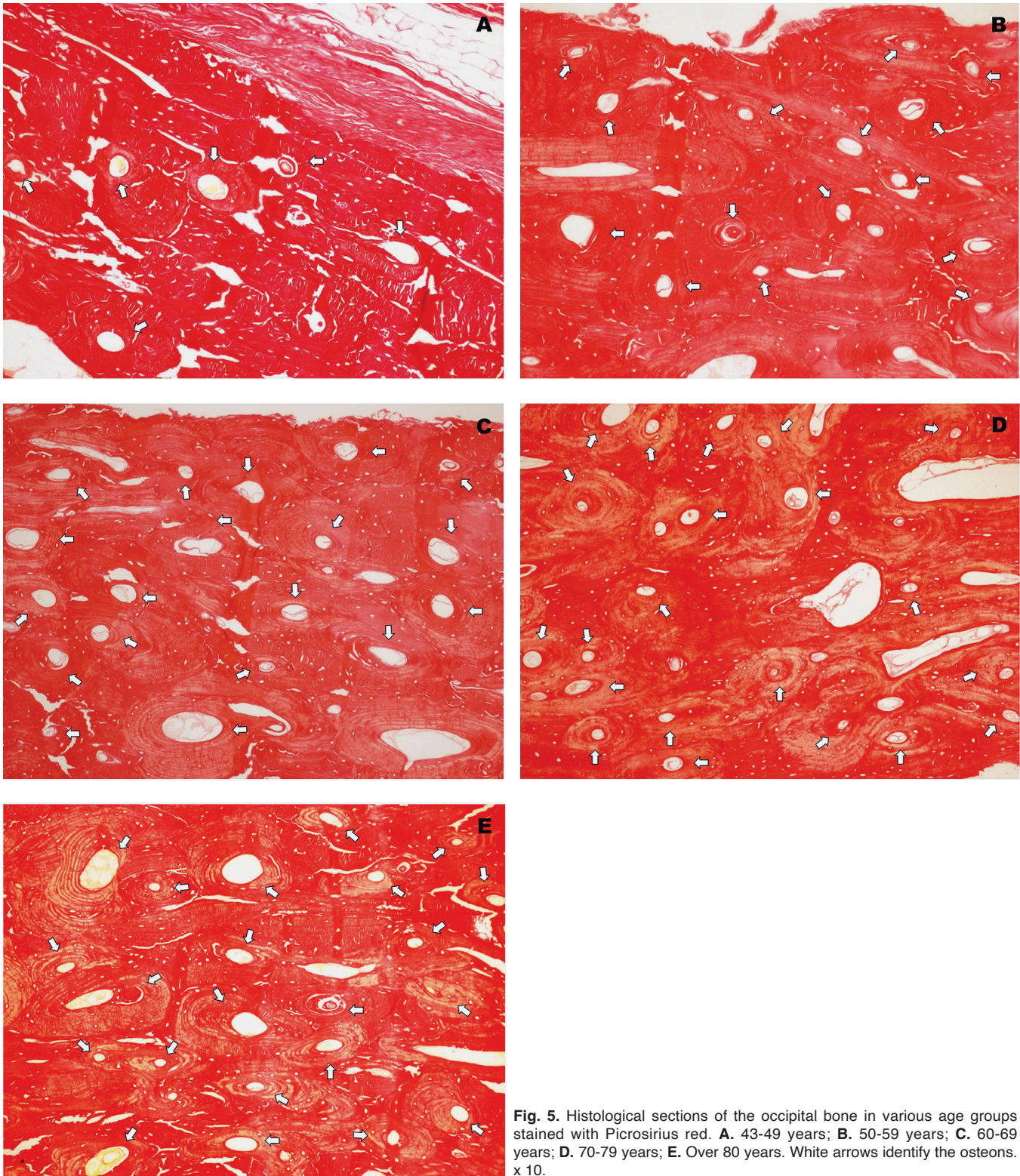


Fig. 5. Histological sections of the occipital bone in various age groups stained with Picrosirius red. **A.** 43-49 years; **B.** 50-59 years; **C.** 60-69 years; **D.** 70-79 years; **E.** Over 80 years. White arrows identify the osteons. x 10.

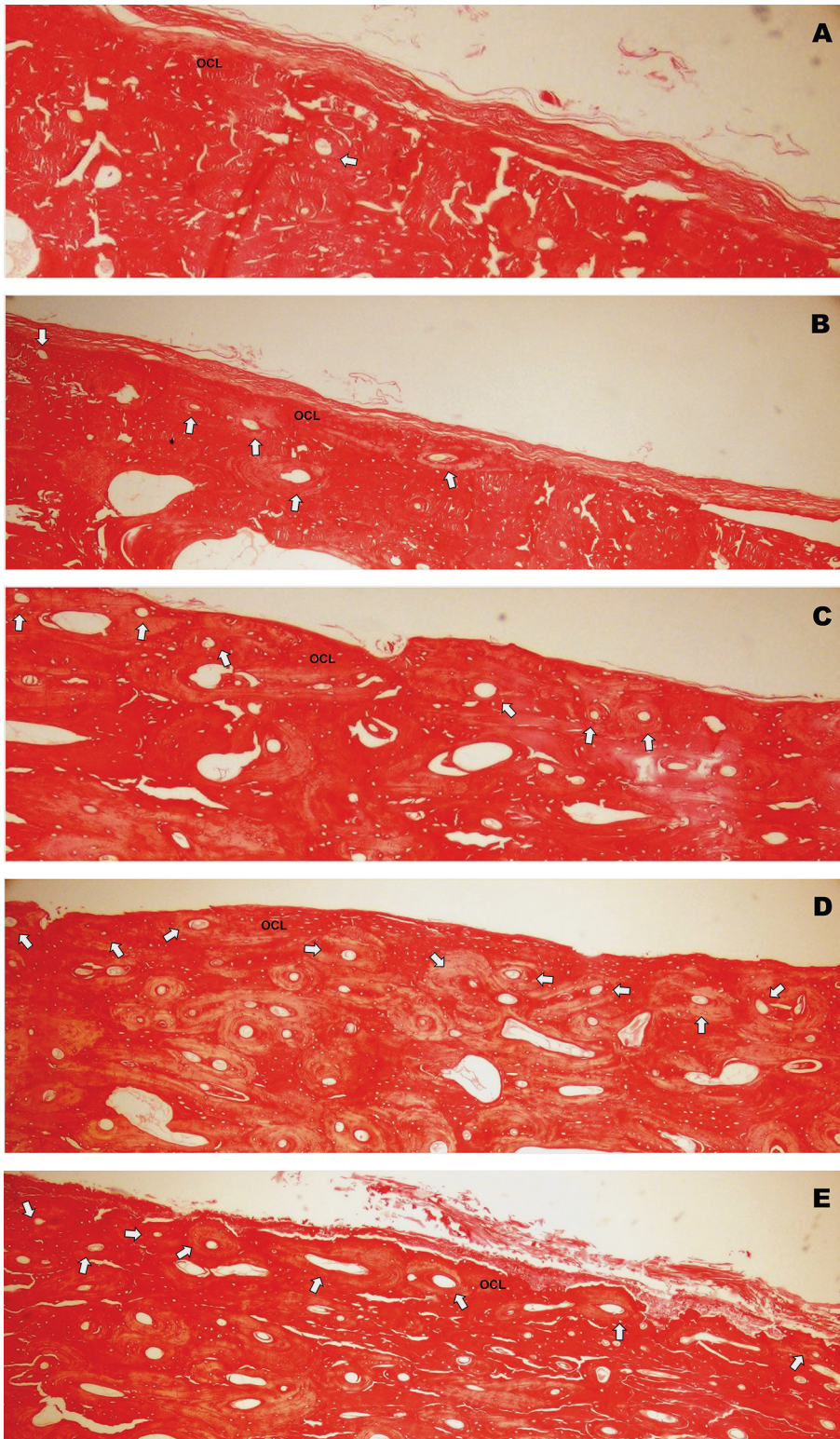


Fig. 6. An example of the outer circumferential lamellar area of the cranial sections to illustrate changes with age. Stained with Picrosirius red. **A.** 43-49 years; **B.** 60-59 years; **C.** 60-69 years; **D.** 70-79 years; **E.** Over 80 years. White arrows identify the osteons in the circumferential lamellar area. OCL: outer circumferential lamellae. x 4.

Gunberg, 1970). Thompson and Galvin, in 1983, carried out a quantitative assessment on tibial sections and reported that the number of osteons also increased with

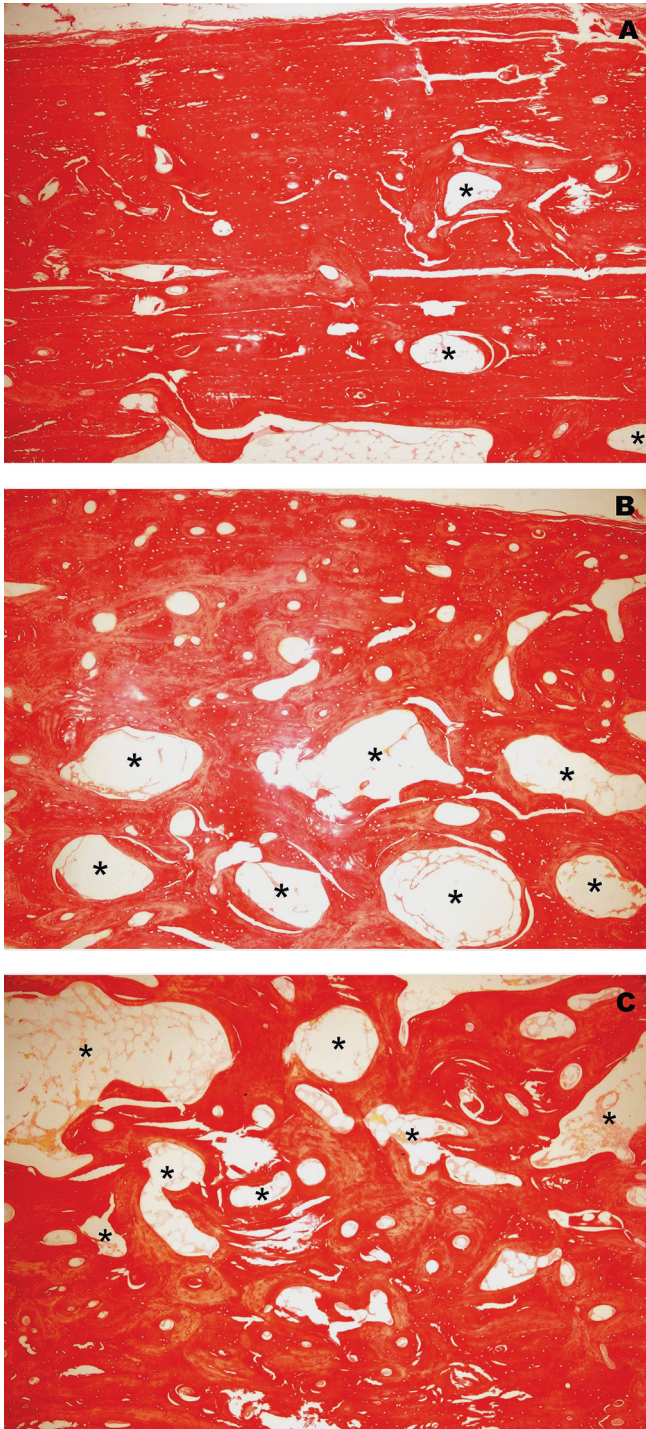


Fig. 7. Histology illustrates the changes in the porosity of the cranial cortical bone with aging. Stained with Picrosirius red. **A.** 43-49 years; **B.** 60-69 years; **C.** Over 80 years. White asterisks identify the porosity in the cortical bone. x 4.

increasing age (Thompson and Galvin, 1983). The study of Nor et al. (2014) analyzed different long bones, such as the humerus, ulna, radius, tibia, femur, and fibula. They reported that the number of secondary and fragmentary osteons changed in relation to aging.

In addition to studies on long bones, the rib and clavicle have also been studied by Stout and Paine (1992) to understand potential age-related histological changes (Stout and Paine, 1992). In their study, they found an increase in T.On in both bone specimens ($r=0.59$). In a previous study on the rib by Kim and colleagues (Kim et al., 2007), the density of osteons significantly increased in association with aging ($r^2=0.803$). The HCA was little correlated with aging. Nevertheless, the authors also indicated that the differences between males and females in the histological structures of their bones, resulting from the effects of menopause, were observed in the changes that occur with aging. Other studies by Lagace (2019) and Kranioti (2020) focused on the femur and clavicle, respectively. The osteon density showed the most significant correlation with advancing age in the femur ($r=0.68$) and ($r=0.505$) in the clavicle (Lagacé et al., 2019; Kranioti et al., 2020). Changes regarding the number of osteons were consistent with our study. Moreover, other studies found a positive increase in the number of secondary and fragmentary osteons ($r=0.49-0.71$) and osteon density ($r^2=0.583$) with increasing age in the clavicle (Lee et al., 2014; Nor et al., 2014). In early 2015, Sobal et al. found positive correlations in the clavicle between age and the number of osteons ($r=0.58$) and mean HCD ($r=0.90$) (Sobal et al., 2015).

Crowder et al. in 2022, studied histomorphometric variables in the ribs and found significant positive correlations between the density of secondary, fragmented osteons, including osteon population density and age; being highest in fragmented osteons ($r=0.711$), followed by total density of osteons ($r=0.626$), and secondary osteons ($r=0.469$) (Crowder et al., 2022). In a very recent report, the sixth rib was employed to identify age in different samples. The researchers (García-Donas et al., 2022) reported that all features of the osteons displayed close relationships to aging, the most predominant being the number of osteons. As mentioned above, the N.Fg.On in our study exhibited less correlation than that of other studies. Fragmented osteons were rarely found under the age of 60 years. However, the notable fragmented osteons were found in individuals aged over 70 years. Compared with the previous report, the correlation values of our study regarding the number of osteons and Haversian canal sizes were similar to those of the rib and clavicle samples. Meanwhile, in long bones like the femur, most correlations were quite high. It implied the different remodeling rates among the different types of bones. Long bones are weight-bearing-type bones, which are subjected to mechanical activities during life, whereas the ribs are subjected to less mechanical force (Cannet et al., 2011). The cranial bone was one of the axial skeleton

that might be less prone to loading stress, similar to the ribs. The cranial samples obtained the highest correlation in Haversian canal dimensions and T.On. The weakest correlation was found in N.Fg.On, indicating that the formation of fragmented osteons was less related to aging. The variations in various types of bones emphasized the importance of site-specific regions. Considering the area of circumferential lamellae, the largest area was detected in samples of individuals aged 43-49 years. The circumferential lamellar area gradually thinned with age and disappeared in some samples over 80 years of age. This suggests that the circumferential lamellar area tends to decrease with age. Because this study did not include samples from age groups under 40, data on histological changes in the circumferential lamellar area in younger groups are lacking. However, a previous study (Kerley, 1965) reported a decrease in circumferential lamellar area with increasing age in the femur, tibia, and fibula. However, no circumferential lamellar area was observed in older individuals. In another study on the femur by Chompoophuenl et al. (2019), the proportion of lamellar bone area decreased with aging. The circumferential area was especially noticeable in childhood. Throughout life, the circumferential lamellar area and the number of osteons increased. In older age, the circumferential lamellar is replaced by osteons (Ingraham, 2004). Similarly, a gradual increase in the number of osteons in the circumferential lamellar region beneath the periosteum was observed in specimens over 50 years of age.

As previously mentioned, age-related histomorphological changes in osteons and surrounding lamellae occur as a result of bone remodeling. Remodeling, also known as bone turnover, is a complex, lifelong process involving new bone formation and resorption to maintain bone strength and bone homeostasis. It occurs continuously from birth to death. The remodeling process consists of several stages: activation of bone cells, resorption of aging bone, and formation or replacement of new bone. The primary role of remodeling is the formation of new bone tissue by the formation of new osteons. The rate of bone turnover slows with aging (Frost, 1964, 1969; Tersigni, 2005; Chang and Liu, 2022).

Nevertheless, when compared with other species, a difference was found in osteon density and Haversian canal dimensions. Zedda et al. studied bone morphometry of the femur and tibia in pigs and wild boars. Both had smaller mean values than those of the current study in humans. Mean HCD was approximately 25-32 μm , and HCP was 73-86 μm . Moreover, the osteon density was 1.6 in pigs and 2.5 in wild boars, indicating fewer osteons compared with human bones (Zedda et al., 2019). Another study was performed in various mammals, such as pigs, cows, sheep, and rabbits, using a digital image tool. They found that the Haversian canal perimeters, area, and maximum diameters were less in pigs, sheep, and rabbits, whereas in cows, there were slightly larger mean HCAs and HCDs compared with

human bones (Martiniakova et al., 2006). Different histomorphometric data among species could increase the understanding of bone remodelling, help to identify human bone from other species, and be useful for bone graft surgery.

In this study, in addition to changes in osteon density in the specimens with aging, cortical pores were also observed in the cortical region, particularly in the region close to the endosteum. These pores seemed to gradually increase with age. Results of a previous study on the effects of aging on iliac cortical bones (Andreasen et al., 2018) also indicated that aging causes an increase in cortical bone porosity. Increased cortical porosity leads to a significant decrease in cortical bone tissue area. The observation of a decrease in cortical bone area with aging in the present study is consistent with the finding by Cho et al. (2002), that cortical bone area decreases significantly with age in their histological study of the sixth rib (Cho et al., 2002). The results of Andronowski and Crowder (2019) revealed that the bone area in the rib midshaft decreases with age, and that the cortical bone area decreases with aging (Andronowski and Crowder, 2019).

Considering cancellous bone, the results of this study demonstrate that pores within trabecula widen with aging, and bone trabeculae loss increases. These findings are consistent with previous studies on the iliac bone (Castillo et al., 2012), which showed that bone volume decreases after age 40. The increase in bone porosity can be assumed to be due to bone remodeling. Because the modeling-remodeling balance on the endosteal side is negative, the pores between trabeculae widen with aging, and trabeculae gradually become thinner over time. This study found that bone trabeculae were thinner in samples from older individuals. Current advances in medical treatment are improving survival rates and life expectancy, extending the average lifespan of the population. Global aging trends indicate that one-quarter of the world's population consists of older individuals. In 2010, the number of older people increased by approximately 524 million and is projected to reach 1.5 billion by 2050, approximately 16% of the population, particularly in developing countries (Health and Services, 2011). The results of this study will be useful for future studies on monitoring and strengthening changes in the skeletal system of older people.

The use of relatively small numbers of bone samples from young individuals in this study limits conclusions about the bone tissue of younger populations. Furthermore, the findings suggest differences in the histological structure and age-related changes in bone tissue between men and women. It is crucial for future studies to consider gender differences in bone microstructure and the impact of menopause on age-related changes in women. The inclusion of more selective staining techniques for bone tissue could also reveal more detailed histological details. The findings of this and similar studies may also contribute to the use of artificial intelligence for age estimation in forensic

medicine. Similar results were obtained from different cranial bone samples; therefore, any of the different skull bones could be used in analyses. It may be suggested that the frontal bone is more suitable as a sample material because it is easier to obtain by dissection.

Conclusion

In summary, the current study examined age-related histological and histomorphometric changes in human skull bones. The significant changes observed in skull bones with increasing age comprised an increase in the number of secondary, fragmentary, total osteons, Haversian canal area, perimeter, and diameter in cortical bone. Furthermore, narrowing of the peripheral lamellar spaces and thinning of medullary bone trabeculae, as well as expansion of porous areas and an increase in the number of pores in the bone marrow, were also observed. The results of this study not only provide useful data on the effects of aging on bone structure but also provide information that can be used in forensic age estimation using skulls.

Acknowledgements. The authors would like to thank the Departments of Anatomy and Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, for providing access to their laboratory and for their support. The authors are also grateful for the support from the Excellence Center in Osteology Research and Training Center (ORTC), with partial support from Chiang Mai University, Thailand.

Funding. This work was supported by a grant from the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand [grant number: FAC-MED-2566-0476].

Author contributions. TG: conceptualization, methodology, investigation, acquisition of data and data analysis, and the writing of the original draft manuscript; PM: conceptualization, supervision, editing and critical revision of the manuscript, and approval of the final version of the article.

Declaration of Competing Interest. The authors declare that there is no conflict of interest.

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Accepted October 31, 2025